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The Evaluation of the Antidiarrheal Properties of Ethanolic Rhizome Extract of *Zingiber zerumbet* (Zingiberaceae)

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APPROVAL SHEET

This thesis entitled:

The Evaluation of the Antidiarrheal Properties of Ethanolic Rhizome Extract of *Zingiber zerumbet* (Zingiberaceae)

prepared and submitted by Raudha A., Johayber M., Sophia M., Princess N.R., has been examined, and is recommended for approval and acceptance as partial fulfillment of the requirements for the degree Bachelor of Science in Pharmacy.

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CERTIFICATE OF ORIGINALITY

We hereby declare that this submission is our own work and that, to the best of our knowledge and belief, it contains no materials previously published or written by another person nor material to which to a substantial extent has been accepted for award of any other degree or diploma of a university or other institute of higher learning, except where due acknowledgement is made in the text.

We also declare that the intellectual content of this thesis is the product of our work, even though we have received assistance from others on style, presentation, and language expression.

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ABSTRACT

Diarrhea was a prevalent condition that affected millions globally each year, representing a significant public health challenge. It often led to acute dehydration, with severe complications particularly impacting vulnerable populations such as the elderly and young children. The condition remained a leading cause of morbidity and mortality, especially in resource-limited settings.

***Zingiber zerumbet*, commonly referred to as shampoo ginger, was a member of the Zingiberaceae family, closely related to *Z. officinale* (ginger). Traditionally used in medicine, *Z. zerumbet* was believed to possess antidiarrheal potential due to its rich phytochemical profile, which included anti-inflammatory and antimicrobial compounds. Research on *Z. officinale* has demonstrated its efficacy in managing diarrhea through mechanisms such as reducing intestinal motility and addressing microbial pathogens. It was hypothesized that *Z. zerumbet* may exert similar effects, potentially mitigating excessive intestinal secretions and resolving microbial factors contributing to diarrhea.**

This study investigated the antidiarrheal activity of ethanolic rhizome extract of *Zingiber zerumbet* using castor oil-induced diarrhea and gastrointestinal motility tests in mice. The extract was administered at 25%, 50%, and 75% of the LD_{50}, with loperamide as the positive control and PNSS with 2% Tween 80 as the negative control. Evaluated parameters included stool frequency, consistency, and intestinal transit using the charcoal meal method.

The extract was consistently prepared through ethanol maceration and demonstrated a favorable safety profile, with no signs of toxicity at the administered doses. Pharmacological assessments revealed significant reduction of diarrhea frequency, improvement of stool consistency, and inhibition of gastrointestinal motility compared to controls. These outcomes led to the rejection of the null hypotheses and supported the alternative, confirming a dose-dependent antidiarrheal effect comparable to loperamide. The findings underscore the potential of *Z. zerumbet* as a safe and effective natural antidiarrheal agent, warranting further exploration for therapeutic use.

Keywords: *Zingiber zerumbet, Antidiarrheal, Ethanolic Extract, Rhizome, Castor Oil-Induced Test, Gastrointestinal Motility Test.*

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CHAPTER ONE
INTRODUCTION

Diarrhea is a gastro condition that usually consists of frequent, loose, watery- like feces. These alarming symptoms bring a serious health concern globally, particularly concerning low-income nations. In addition, this disease develops when the intestines fail to absorb fluids sufficiently or secrete excessive amounts, which leads to increased bowel movements (often more than three times daily) and reduced stool consistency. The disturbances lead to rapid dehydration and electrolyte imbalances, which present a high health risk. Diarrhea has an influence closely associated with bacteria, fungi, and viruses that involve the gut microbiota (Li et al. 2021).

Diarrhea is a disease that affects millions of people in the world every year. Unfortunately, it is one of today's critical public health concerns. This usually causes acute dehydration, and complications are widespread among populations who are in danger, like the elderly and children. The World Health Organization estimates that diarrhea is the third leading cause of death globally, with approximately 443,832 lives lost annually (World Health Organization 2024). Diarrhea is also a severe public health concern in the Philippines. According to records from the Department of Health, 50,058 cases of diarrheal diseases occurred annually from 2010 to 2019. In 2019 alone, 71,774 cases of heavy bleeding diarrhea represented almost 85% of the waterborne diseases. The statistics emphasize the need for effective public health initiatives and interventions (Philippine Statistics Authority 2021).

The plant known as shampoo ginger, scientifically known as *Zingiber zerumbet* (Zingiberaceae), is native to subtropical areas such as Southeast Asia and the Pacific Islands (Chan et al. 2023). In *Z. zerumbet*, its inflorescence has a slimy substance that is rich in surfactants and therefore used as shampoo, while the floral buds are used as vegetables and the leaves are also used in therapies for joint pains. Its rhizome has long been used in traditional medicine to cure conditions including inflammation, stomach cramps, bacterial infection, flatulence and diarrhea which are usually prepared by maceration, infusion of fresh rhizome, and even tinctures (Chavan et al. 2023). The rhizome powder prepared by tea is used to treat stomach disease as well. The study carried out on the rhizome extract of *Z. zerumbet* shows that it contains a significant amount of zerumbone, a phytochemical element classified as a terpenoid. Additionally, it contains alkaloids, flavonoids, and saponins which may contribute to antidiarrheal effects (Assiry et al. 2023). It also has polyphenolic compounds and essential oils containing multiple terpenes, which add to its medicinal benefits (Ramzan et al. 2023). Considering the aforementioned findings, this study, evaluating the anti-diarrheal properties of *Z. zerumbet* (Zingiberaceae) presents a huge challenge, however, it holds great potential for addressing this issue and improving health outcomes on a global scale.

Z. zerumbet, which belongs to the same Zingiberaceae family as *Z. officinale*, may hold promise as an anti-diarrheal agent. This antidiarrheal potential arises from its phytochemical profile, which contains compounds with scientifically recognized antidiarrheal, anti-inflammatory and antimicrobial properties. Much like *Z. officinale*, which has demonstrated effectiveness in reducing diarrhea symptoms through mechanisms such as inhibition of intestinal motility and antimicrobial action, *Z. zerumbet* might exert similar effects. By potentially reducing excessive intestinal secretions and addressing microbial factors that contribute to diarrhea, *Z. zerumbet* could offer valuable therapeutic benefits for managing this condition. Rhizomes of ginger have long been used in traditional medicine to treat various ailments, including gastrointestinal issues like diarrhea. This remedy has been valued for its effectiveness in alleviating digestive disturbances and promoting gastrointestinal health (Giacosa et al. 2015; Haniadka et al. 2013).

- *Background of the Study*

Diarrhea is a frequent gastrointestinal problem, usually characterized by loose, watery stools caused by various factors, including infections (viral, bacterial, or parasitic), food sensitivities, or specific medications. Although most instances clear up without intervention, diarrhea can pose risks, particularly for at-risk populations such as young children, the elderly, and individuals with weakened immune systems, as it can lead to significant dehydration and imbalances in electrolytes (Gotfried 2024).

Z. zerumbet (Zingiberaceae) possessed antioxidant, anti-inflammatory, and antibacterial properties that had been shown in previous studies (Adriana et al. 2016), yet only a small number of studies have precisely examined its antidiarrheal properties. Given the plant's phytochemical profile such as alkaloids, flavonoids, saponins and terpenoids which are known compounds to contribute antidiarrheal activity (Tiwari et al. 2011), the ethanolic extract of *Z. zerumbet* rhizomes might presented a strong candidate for antidiarrheal therapy.

This study aimed to evaluate the antidiarrheal potential activity of the ethanolic rhizome extract of *Z. zerumbet* through two models: gastrointestinal motility test and castor oil-induced diarrhea, investigating its effects on gastrointestinal motility and intestinal fluid secretion. By analyzing the results, this study aimed to provide scientific validation for the traditional use of *Z. zerumbet* and highlight its potential as a cost- effective, natural remedy for managing diarrhea.

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➢ *Statement of the Problem*

Plants had been a significant source since time immemorial in the arena of traditional medicine in the treatment of a wide variety of disease conditions, including diarrhea. *Z. zerumbet* was a perennial herb belonging to the family Zingiberaceae and was reported to be highly regarded for its medicinal properties. Its anti-inflammatory, antimicrobial, and antioxidant activities had been widely documented in several studies. The antidiarrheal study of *Z. zerumbet* and, more precisely, the ethanolic extract of its rhizome, had not been performed so far. Because of this knowledge gap, further research in this context was highly required to explore its potential for therapeutic use against diarrhea.

➢ *Conceptual Framework*

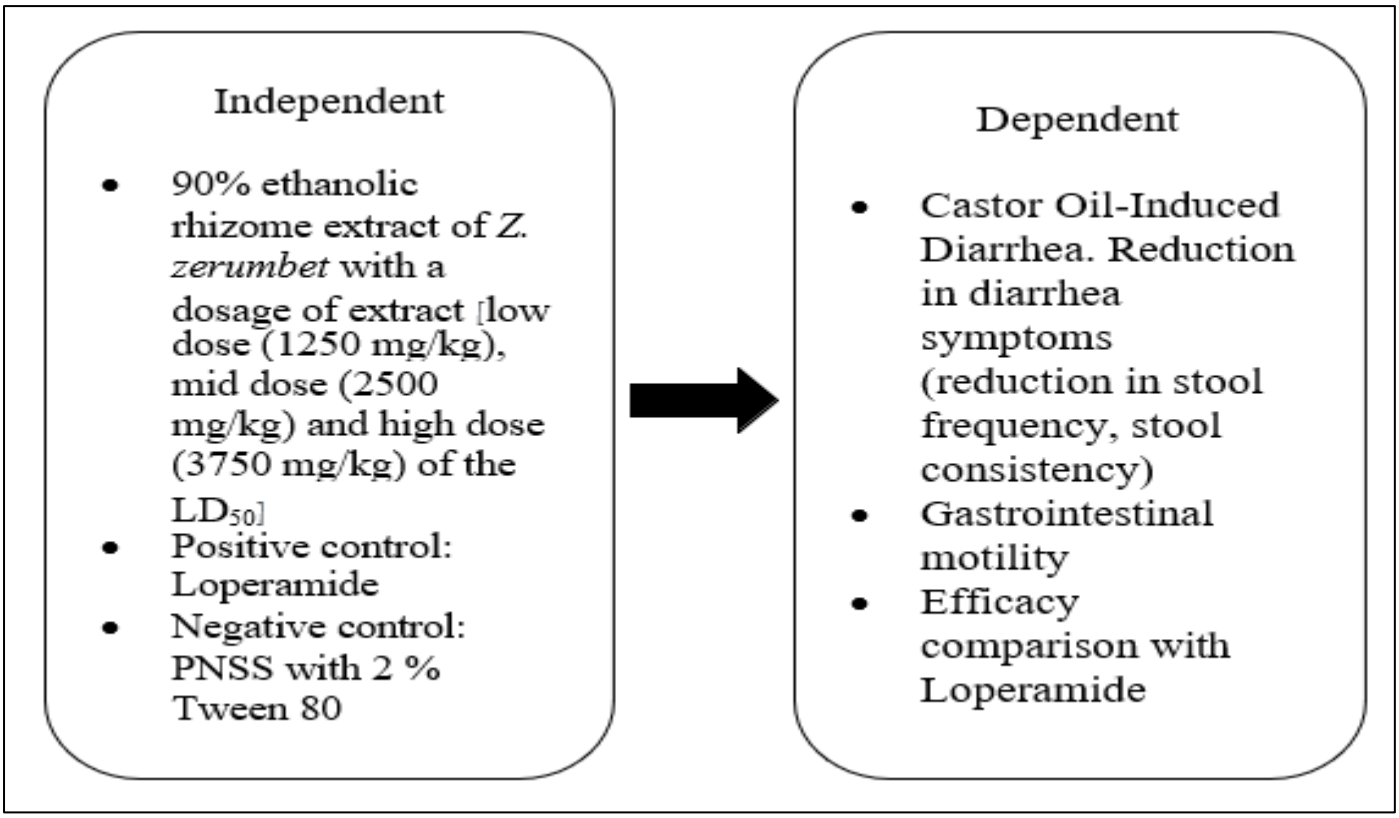

Fig 1 Conceptual Framework

Independent variables of the study include 90% ethanolic rhizome extract of *Z. zerumbet* with a dosage of extract low dose (1250 mg/kg), mid dose (2500 mg/kg) and high dose (3750 mg/kg) of the LD_{50}, positive control which was loperamide, and negative control was PNSS with 2% of Tween 80. The castor-oil induced diarrhea and the gastrointestinal motility test were the independent variables of the 90% ethanolic rhizome extract of *Z. zerumbet* with a dosage of extract (low dose (1250 mg/kg), mid dose (2500 mg/kg) and high dose (3750 mg/kg) of the LD_{50}).

➢ *Purpose and Objective of the Study*

The purpose of this study was to scientifically evaluate the antidiarrheal properties of *Z. zerumbet*. *Z. zerumbet* had been used to treat ailments such as indigestion, inflammation, and diarrhea. However, despite its traditional use, there is limited scientific research that specifically investigated the anti-diarrheal properties of the plant. Given the increasing demand for natural remedies with fewer side effects and their growing popularity. This study seeks to fill the gap by evaluating its potential anti- diarrheal activities using the gastrointestinal test and castor oil-induced method. By doing so, the researchers hope to provide a scientific basis for the use of *Z. zerumbet* as a natural alternative to synthetic antidiarrheal drugs, contributing to both scientific research and public health efforts aimed at combating diarrhea.

➢ *Objectives*

This study aimed to evaluate the antidiarrheal properties of the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae). Particularly, the study aimed:

- To determine the percentage yield of the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae).
- To investigate the phytochemical constituents responsible for the antidiarrheal activity of the ethanolic rhizome extract *Z. zerumbet* (Zingiberaceae).

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- To determine the LD**50** (median lethal dose), the minimum toxic dose and the different concentration of the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae).
- To evaluate the dose and time-dependent antidiarrheal activity of the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae) using a castor oil-induced diarrhea model in Swiss albino mice.
- To assess the effect of the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae) on gastrointestinal motility using charcoal meal test in Swiss albino mice.
- To compare if there is a significant difference in the antidiarrheal efficacy of the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae) against the standard antidiarrheal drug, loperamide.

➢ *Research Hypothesis*

- **H_1:** The ethanolic rhizome extract of *Z. zerumbet* significantly reduced the frequency of diarrhea induced by castor oil and slowed down gastrointestinal transit time as measured by the gastrointestinal motility test, compared to loperamide.
- **H_{01}:** The ethanolic rhizome extract of *Z. zerumbet* did not significantly reduce the frequency of diarrhea induced by castor oil or slow down gastrointestinal transit time as measured by the gastrointestinal motility test, compared to loperamide.
- **H_2:** The ethanolic rhizome extract of *Z. zerumbet* significantly improved stool consistency in castor oil-induced diarrhea and reduced intestinal motility as measured by the gastrointestinal motility test, compared to loperamide.
- **H_{02}:** The ethanolic rhizome extract of *Z. zerumbet* did not significantly improve
- stool consistency in castor oil-induced diarrhea or/and reduce intestinal motility as measured by the gastrointestinal motility test, compared to loperamide.

➢ *Significance of the Study*

This study would like to investigate the antidiarrheal potential of *Z. zerumbet* ethanolic rhizome extract, aimed at discovering the potential for diarrhea. The following sectors might benefit from the possible result of this study:

- *To the Pharmaceutical Industry:*

This study may help to discover a new source of medicine for diarrhea. The findings may lead to safer and more sustainable therapeutic solutions for the treatment of diarrheal disorders. Additionally, the medicinal characteristics of *Z. zerumbet* indicates that its ethanolic extract could be utilized in the formulation of various pharmaceutical products, broadening its potential application in multiple therapeutic areas beyond the treatment of diarrhea.

- *To the Clinical Field:*

The results of this study may broaden the range of empirically supported herbal remedies for diarrhea management and gastrointestinal issues. The findings may potentially have an impact on medicinal recommendations.

- *To the Academic Institutions:*

This study may advance the understanding of *Z. zerumbet's* pharmacological potential, in particular in gastrointestinal health.

It may offer an adequate foundation for additional scholarly investigation and may be helpful in creating a study that focuses on the investigation and use of natural product-oriented therapeutics.

- *Adventist Medical Center College (AMCC):*

The results of the study may provide significant improvements to AMCC's portfolio of studies, thereby promoting the institution's goal of increasing the library of knowledge about alternative medicine.

➢ *Scope and Limitations of the Study*

The scope of this research study was used to evaluate the antidiarrheal properties of the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae), and it focused more specifically on assessing the extract's potential using the two methods which were the castor oil-induced diarrhea and the gastrointestinal motility test in Swiss albino mice. For this study, a 90% ethanol solvent was used for the maceration to completely extract the phytochemicals of the rhizome that is responsible for its antidiarrheal potential, which excludes other plant parts and extraction methods, as this research remained underexplored.

The selection of the castor oil-induced diarrhea method was based on well- established protocols, with slight modifications. These modifications were defined as adjustments made to standard procedures to ensure they are more suitable for the specific goals and conditions of this study. In addition, this approach was assessed variables such as the frequency of defecation and consistency of the stool on Swiss albino mice of either sex, with loperamide serving as the standard of treatment for comparison (Mussarat et al. 2022). Besides, the motility test of the gastrointestinal tract measured the distance that the charcoal meal could move through the gastrointestinal tract using loperamide as a standard for comparison. In addition, it has provided reliability and relevance to the objectives of this study.

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Limitations of the study include that other plant parts and extraction methods had not been considered, and consideration of some other promising in vitro assays may give a wider picture of the antidiarrheal properties of the plant. Reliance on the Castor Oil-Induced Diarrhea method and the Gastrointestinal Motility Test limited the generalization to specific experimental conditions. Besides, variability in the biological responses of the test subjects is another uncontrollable variable factor that may affect the internal validity of the results. Finally, time and resource constraints can also influence the choice of methods and the exclusion of alternative approaches.

➢ *Definition of Key Terms*

- *Anti-Diarrhea:*
  This referred to the ability of the ethanolic rhizome extract of *Z. zerumbet* to reduce or alleviate diarrhea by effectively decreasing diarrhea symptoms.

- *Castor Oil-Induced Diarrhea:*
  This referred to the experimental model in Swiss albino mice that was used to evaluate the antidiarrheal activity of *Z. zerumbet* rhizome by administering castor oil, which irritated the intestines and increased motility.

- *Ethanol:*
  This referred to the extracting solvent to be used to extract the ethanolic extract of the *Z. zerumbet* rhizome.

- *Extract:*
  This referred to the material that had been obtained from the rhizome of *Z. zerumbet* using ethanol, which was studied to assess its potential antidiarrheal effects.

- *Gastrointestinal Motility Test:*
  This referred to the test that measured the movement of food through the digestive tract by administering a charcoal meal after castor oil administered, to reduce diarrhea by assessing the distance traveled by the charcoal in the intestines.

- *Rhizome Extract:*
  This referred to the extract that was obtained from the rhizome of *Z. zerumbet* using ethanol as the solvent.

- *Rhizome:*
  This referred to the underground stem of the *Z. zerumbet* from which the ethanolic extract was extracted.

- *Zingiber zerumbet:*
  This referred to the plant of interest to be used in the study to evaluate its potential antidiarrheal activity.

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CHAPTER TWO
REVIEW OF RELATED LITERATURE AND STUDIES

This chapter presents the relevant literature and studies that provide a foundation for this research. The sources referenced focus on different theories, concepts, and approaches that evaluate the potential of *Zingiber zerumbet's* ethanolic rhizome extract as an antidiarrheal agent. Moreover, it offers a wider perspective on its therapeutic significance within the Zingiberaceae family. The aim of this review is to provide navigation for the study by presenting findings that support and/or offer alternative viewpoints on the current study.

A. Zingiber zerumbet

➢ *Morphological Description*

Z. zerumbet, bitter ginger, or shampoo ginger, is a perennial natural herb native to tropical and subtropical regions of Asia (Chan et al. 2023). It typically reaches upto 1 to 2 meters in height with erect stems that could be very sturdy. The leaves are narrow, elongated, and lanceolate, reaching up to 30 cm. This plant has a peculiar pine cone- shaped inflorescence, composed of green or reddish imbricate bracts when growing. Flowers are small, tubular, green, and turn red when matured and arise from the bracts. Very often, they exude scented saps traditionally used as shampoo in nature. The rhizome is described as thick, aromatic, and yellowish. It propagates vegetatively by producing sprouts from rhizome fragments (Koga et al. 2016). An image of *Z. zerumbet* will be shown in Figure 2.

Fig 2 Morphological characteristics of *Z. zerumbet* inflorescence (a), rhizome (b), leaves (c), leaves and inflorescences (d), of *Z. zerumbet* (Shahrul 2022)

➢ *Taxonomy of Z. zerumbet*

The taxonomic classification of *Z. zerumbet* (L.) Sm. according to Natural Resources Convention Service of the United States Department of Agriculture is as follows:

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Superdivision:** Spermatophyta
- **Division:** Magnoliophyta
- **Class:** Liliopsida
- **Order:**Zingiberales
- **Family:** Zingiberaceae
- **Genus:** *Zingiber*
- **Species:** *Z. zerumbet*

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➢ *Ethnobotanical uses and Pharmacologic Uses of Z. zerumbet*

As presented in the following table, the data documents the traditional medicinal uses and published experimental studies of *Z. zerumbet.* Table 1 summarizes the gathered information on *Z. zerumbet* ethnomedicinal uses specifically in its rhizome.

Table 1 Ethnobotanical uses of *Z. zerumbet*

Species	Study Title	Result/Conclusion
Z. zerumbet	Antisecretory, Gastroprotective, Antioxidant and Anti- Helicobacter Pylori Activity of Zerumbone from *Z. zerumbet* (L.) Smith	In Malaysia, *Z. zerumbet* is locally called "lempoyang" and the rhizomes of the plantare widely used as traditional medicine for the treatment of peptic ulcers, stomach ache, diarrhea and as an anti-inflammatory (Sidahmed et al. 2015).
Z. zerumbet	*Z. zerumbet* (L.) Smith: A Review of Its Ethnomedicinal, Chemical, and Pharmacological Uses	Rhizomes of *Z. zerumbet* (RZZ) traditional botanical medicine include the treatment of diarrhea and pain reliever. The study explores the traditional uses of RZZ in different countries. In Malaysia, fresh rhizomes are used to alleviate stomach ache, abdominal pain, lesions, and anorexia, whereas boiled rhizomes are used to treat helminthic infection. In Thai medicine, it was reportedly employed as an antiflatulent remedy to alleviate gas or bloating in the stomach or intestines. In China, the rhizomes are macerated in alcohol and employed as a tonic, depurative, or even as a stimulant. It is also an anti-inflammatory adjuvant to abdominal pain, sprains, and fever. This study identifies that traditionally, RZZ is employed in managing digestive, inflammation, pain, and dermatological disorders (Yob et al. 2011).
Z. zerumbet	*Z. zerumbet*: Pharmacological Values Of Zerumbone And The Extraction Technology Evolution	Traditionally, *Z. zerumbet* rhizome has been extensively used in the everyday lives of the elderly to cure stomach problems, including stomach cramps, bloating, poisoning, colic pain, and diarrhea (Ahmad et al. 2023).

Additionally, the table below presents the published experimental findings of the pharmacological activities of *Z. zerumbet* such as the anxiolytic, antinociceptive, immunosuppressive effects, and antimicrobial among others. Table 2 summarizes the result of the pharmacological activities of *Z. zerumbet.*

Table 2 Pharmacological Activities of *Z. zerumbet*

Species	Study Title	Result/Conclusion
Z. zerumbet	Anxiolytic Activity of Ethanolic Extract of Three Species of Indonesian Lempuyang (*Z. zerumbet*, *Z. aromaticum*, and *Z. americans*)	In the study, the three species of *Z. zerumbet* significantly reduced anxiety responses in the maze, open field, and hole board tests, with moderate doses consistently showing positive results for anxiolytic activity ($p < 0.05$). The highest anxiolytic activity was provided by a moderate dose of *Z. americans*, 8.5 mg/20 gbb, through three applied methods (Widyastiwi et al. 2022).
Z. zerumbet	Phytochemical analysis and antinociceptive activity of bitter ginger (*Z. zerumbet*) cultivated in Manaus/Amazonas	The study highlights the potent antinociceptive activity of sesquiterpenes, zerumbone from *Z. zerumbet* rhizomes in various neurogenic-induced nociception models in mice and rats. Thus, intraperitoneal. and oral administration of zerumbone in a dose of 150 to 1,500 mg produced a dose-dependent inhibition of the acetic acid- induced abdominal writhing in a highly significant manner compared to Fentanest or dehydrobenzperidol at 20µg/kg (Pinheiro et al. 2019).
Z. zerumbet	Immunosuppressive effects of the standardized extract of *Z. zerumbet* on innate immune responses in Wistar rats	*Z. zerumbet* significantly decreased neutrophil migration, integrin CD11b/CD18 expression, phagocytic activity, and reactive oxygen species production in a concentration-dependent manner. *Z. zerumbet* extract strongly inhibits innate immune responses and can potentially develop into an effective immunosuppressive agent (Ghazalee et al. 2019).
Z. zerumbet	*Z. zerumbet* (L.) Smith: A Review of Its Ethnomedicinal, Chemical, and Pharmacological Uses	The study presents that the rhizome of *Z. zerumbet* has shown anti-inflammatory, antioxidant, antimicrobial, and antiallergic activities, among others, which are described below in various forms, dosages, and concentrations (Yob et al. 2011):

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		The aqueous and methanol extract of *Z. zerumbet* prevented the release of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) in LPS-induced RAW 264.7 macrophages, indicating anti- inflammatory potential. The total antioxidant activity (TAC) of *Z. zerumbet* is 18 mg/100 g, and the total polyphenol content (TPC) is 130 mg/100 g, which indicates moderate antioxidant activity. Antibacterial activity of ethanol extract from the rhizomes of *Z. zerumbet* (EEZZ) and other extracts exhibited antimicrobial activity against *Staphylococcus aureus* and MRSA, showing inhibition zones of 9.5 mm and 9.8mm, respectively. EEZZ and AEZZ inhibited the release of β- hexosaminidase by 8.4% to 53.7% and 10.9% to 59.1%, respectively, with moderate antiallergic activity. Ketotifen Fumarate was used as a standard comparison with an IC50 of 20.2 μg/mL. The chloroform extract exhibited activity against *Giardia intestinalis*, with an IC50 of 69.02 μg/mL, using Metronidazole as the comparison standard at an IC50 of 0.48 μg/mL.

➢ *Phytochemical Constituents*

The phytochemical test for the 90% ethanolic rhizome extract from *Z. zerumbet* shows positive results for terpenoids, saponin, cardiac glycoside, flavonoids, alkaloids, and zerumbone. Additionally, by utilizing HPLC methods, the standard secondary metabolites, such as benzoic acid, gallic acid, sinapic acid, caffeic acid, chlorogenic acid, kaempferol, quercetin, and myricetin, were also determined (Ramzan and Zeshan 2023). Table 3 discusses the mechanism of actions of phytochemical constituents with antidiarrheal activity as described by Tiwari et al. (2011).

Table 3 Phytochemical Constituents with Antidiarrheal Activity.

Phytochemicals	**Study Title**	**Mechanism of Action**	**Relevant/ related Information**
Alkaloids	Evaluation of Anti- Diarrheal Activity of 80% Methanol Extracts of *Vernonia amygdalina* Delile (Asteraceae) Leaves in Mice	Alkaloids decrease the levels of production of nitrates due to its antioxidant properties. The Production of nitrates in excess amounts in the intestines could lead to an inflammatory response which could contribute to diarrhea. Additionally, it inhibits intestinal motility.	The research discusses that reduced water and electrolyte secretion into the small intestine was observed which may be due to the secondary metabolites such as alkaloids (Gudeta et al. 2020).
Flavonoids	Assessment of phytochemicals, antioxidant properties, and *in vivo* antidiarrheal activity of date palm (*Phoenix dactylifera* L.)	Flavonoids increase the Resistance of intestinal mucosa, hence assisting in the reduction of excessive fluid secretion into the intestine.	The result of the study reported that flavonoids such as quercetin produce an inhibitory action on gastrointestinal motility and secretions in a dose- dependent manner (Kharal et al. 2023).
Saponins	Exploring the Antidiarrheal Properties of Papaya Leaf: Insights *In Vivo* Study in Mice-Model and *In Silico* Analysis at M3 Muscarinic Acetylcholine Receptor Interaction	It inhibits histamine release *in vitro*, thereby reducing inflammation in the gut and subsequently preventing excessive secretion of fluids and mucus.	The study shows saponins having antibacterial and anthelmintic activities (Saptarini et al. 2024).
Steroids	Evaluation of the Antidiarrheal Activity of Hydromethanol Crude Extracts of *R. chalepensis* and *V. amygdalina* in Mice	Improve intestinal absorption of sodium and water, which may result in firmer stools and less frequent diarrhea.	The study reports that steroids like phytosterols have a capacity to inhibit the production of prostaglandins which has a crucial role in the stimulation of intestinal secretions (Degu et al. 2020).
Tannins	Anti-Diarrheal Activity of Ethanol and Chloroform Seed Extract of *Cola nitida* in Experimentally Induced Diarrhea	Tannins decrease intestinal transit, slowing it down and Giving the intestines more time to absorb water, thus	The research mentions that tannins form precipitates with proteins in the small intestine form tannates, which will make the mucosa

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		lessening watery stools. It also exerts an astringent action that helps normalize stool consistency.	resistant to any chemical change and therefore reduce peristalsis and secretion (Doe et al. 2019).
Terpenoids	Methanolic Crude Extract of *Hagenia abyssinica* Possesses Significant Antidiarrheal Effect: Evidence for *In Vivo* Antidiarrheal Activity	Terpenoids inhibit release of Autocoids and prostaglandins thereby averting secretion and peristalsis induced by castor oil.	Terpenoids prevent the release of autocoids and prostaglandins and consequently prevent secretion and peristalsis caused by castor oil (Kifle et al. 2021).

➢ *Members of Zingiberaceae with Antidiarrheal Activity*

The table below shows the members of Zingiberaceae that are concluded to exhibit antidiarrheal activity according to published experimental studies. Table 4 shows the family of *Z. zerumbet* with antidiarrheal activity.

Table 4 Family of *Z. zerumbet* with Antidiarrheal Activity.

Study title	Member of Zingiberaceae	Phytochemicals	Citation
Anti-diarrheal effects of Methanol extract of *Curcuma longa*	*C. longa*	Curcuminoids, Zingiberene, Phenolics, Flavonoids	Mirza et al. 2017
Antidiarrheal effect of *Alpinia oxyphylla* Miq. (Zingiberaceae) in experimental mice and its possible mechanism of action	*A.oxyphylla*	Flavonoids, Terpenoids, Phenolics, Tannins, Alkaloids	Wang et al. 2015
Study Of Antidiarrhoeal Activity Of Two Medicinal Plants Of Bangladesh In Castor-Oil Induced Diarrhoea	*Kaempferia galanga*	Flavonoids, Terpenoids	Dash et al. 2014
Antidiarrhoeal activity of *Z. officinale* (Rosc.)	*Z. officinale*	Zingerone, Flavonoids, Terpenoids, Phenolics	Daswani et al. 2010

B. Diarrhea

Diarrhea is a gastrointestinal tract infection wherein defecation is frequent for more than three times a day in which the consistency of the stool may be liquid or semi- solid, with or without, mucus, and blood, due to an imbalance of absorption, water, and electrolyte secretion. In developing countries, diarrheal disease is the world's leading cause of morbidity and mortality (Fadilah and Kurniawan 2022). According to Rosseels (2018), an increase in stool fluidity or volume, change of consistency, and bowel movement are frequently observed in diarrhea. In 2019, diarrhea led to the deaths of 370,000 children under the age of five, presenting a significant global health challenge. Dehydration is the main danger of diarrhea, as it causes the loss of essential water and electrolytes such as sodium, chloride, potassium, and bicarbonate, through various pathways during episodes. This issue is pervasive in developing nations, where diarrheal infections are widespread, and children under three, typically experience around three episodes annually, worsening malnutrition and increasing susceptibility to future illnesses (Liu et al. 2024).

There are three types of diarrhea: (1) acute watery and bloody diarrhea, (2) persistent diarrhea, and (3) chronic diarrhea (WHO 2024). Diarrhea can be classified as either acute or chronic and infectious or non-infectious, depending on the duration and nature of the symptoms. Most cases result from viral infections, and the condition typically resolves independently.

The beginning of three or more loose or watery bowel movements per day, for 14 days, or less characterizes acute diarrhea due to infection. If the episode extends beyond 14 days, it is termed chronic or persistent diarrhea, which tends to be non- infectious (Nemeth and Pfleghaar 2022). Infectious causes typically underlie acute diarrhea. Viruses, such as norovirus, rotavirus, or adenovirus, are frequently responsible for episodes of acute diarrhea. Dysentery or also called acute bloody diarrhea, which involves blood and possibly mucus, indicates a more invasive form of diarrhea. The bacteria most commonly responsible for bacterial diarrhea are *Escherichia coli* (prevalent globally), *Shigella*, *Salmonella*, *Campylobacter* (common in children), *Yersinia*, and *Clostridium* spp (Akhondi and Simonsen 2023).

Meanwhile, chronic diarrhea is frequently caused by non-infectious factors, although it can also result from chronic infections. Chronic diarrhea can result from bacterial infection with *Clostridiodes difficile*, or protozoans such as *Giardia*, *Entamoeba*, *Cryptosporidium*, or *Isospora*. Those at higher risk of chronic diarrhea from these organisms include young children, the elderly, individuals with weakened immune systems, or people who have traveled internationally. Acute diarrhea can become more severe due to bacterial infection. The table below outlines and summarizes diarrhea into three main clinical types based on its characteristics, causes, and duration (Jacob 2022).

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Table 5 Clinical Types of Diarrheas and their Characteristics.

Type	Characteristics	Causes	Duration
Acute Diarrhea	Watery stools with potential abdominal cramps, nausea, or fever.	Viral or bacterial infections (e.g., norovirus, rotavirus, *E. coli*), food poisoning, or medication side effects.	Last less Than 2 weeks
Persistent Diarrhea	Prolonged loose stools; symptoms may include weight loss and malnutrition	Often due to unresolved infections, inflammatory conditions, or malabsorption syndromes.	Lasts 2-4 weeks
Chronic Diarrhea	Frequent, loose Stools often accompanied by fatigue, nutrient deficiencies, or systemic symptoms.	Chronic conditions like irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), or celiac disease.	Lasts more than 4 weeks

The mechanisms that cause diarrhea include osmotic, secretory, inflammatory, and altered motility factors. Osmotic diarrhea occurs when an unabsorbed substance pulls water from the blood into the intestines due to osmotic gradients. Secretory diarrhea results from disrupted electrolyte transport and is more often caused by decreased absorption rather than increased secretion. Inflammatory diseases can lead to diarrhea with exudative, secretory, or osmotic components. Changes in the intestine or colon movement can affect fluid absorption by increasing or decreasing the exposure of luminal content to the intestinal absorptive surface. However, from a pathophysiologic standpoint, no single cause of diarrhea is completely unifactorial (Sweetser 2012). Figure 3 below summarizes the mechanism and pathophysiology of diarrhea.

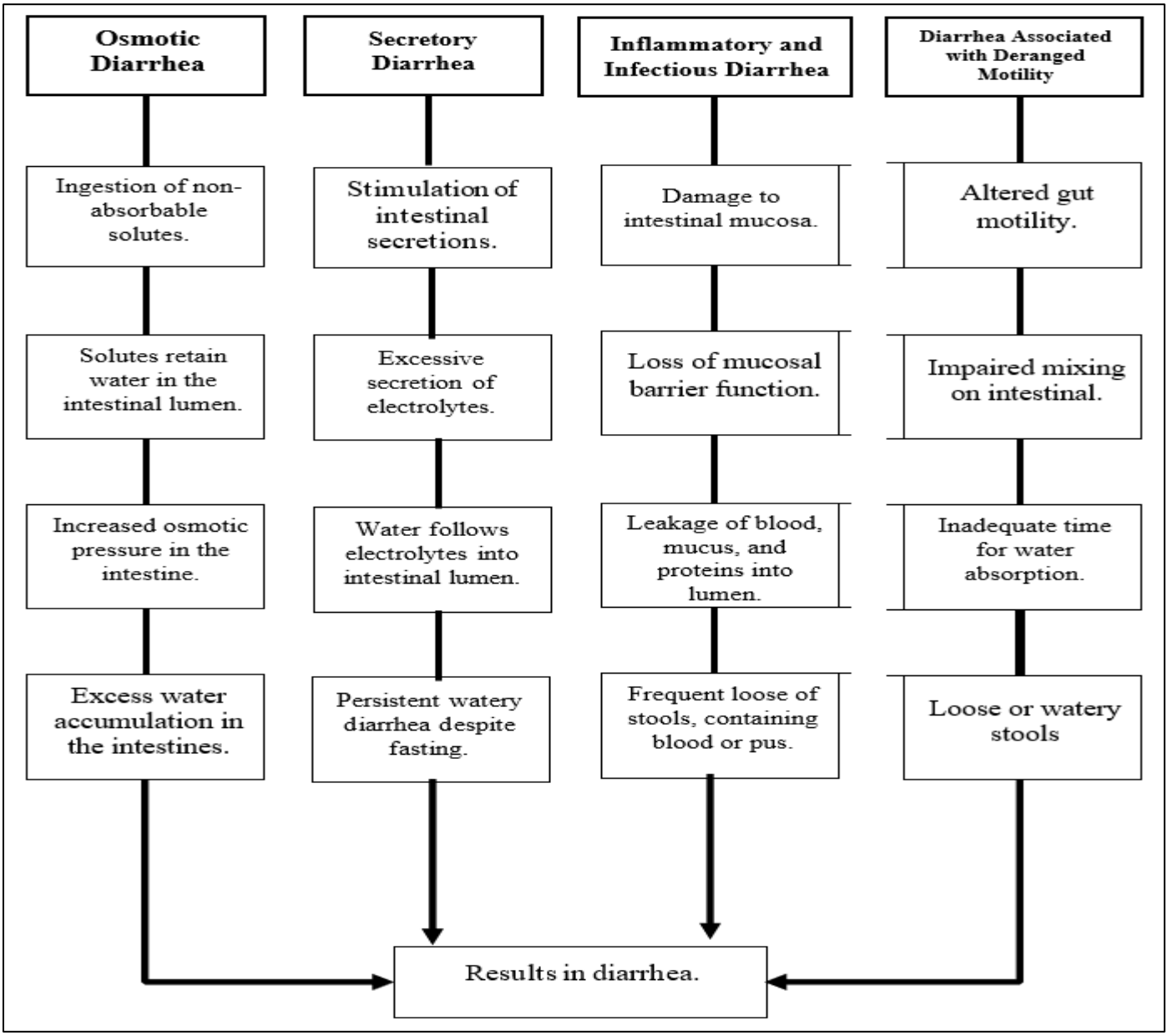

Fig 3 Pathophysiology of Diarrhea

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C. Antidiarrheal Drugs

According to the American Gastroenterological Association (AGA) in 2020, diarrhea is defined as the passage of loose, watery stools more than three times a day. It can be caused by various factors, including infections, certain medications, food intolerances, and underlying health conditions. Diarrhea can be acute (lasting a few days) or chronic (lasting more than four weeks), and the severity and treatmentdepend on the underlying cause. In managing diarrhea, antidiarrheal agents are used, it works by either decreasing GI motility or adding bulk to the stool. According to Bardal et al. (2016), the agent is suitable for various types of diarrhea treatment and can be safely used, provided close monitoring is performed. The mechanism that takes place in this class is the slowing of bowel movement, which allows for an increased time for absorption (Schiller 2017).

Antidiarrheal medications work through three main mechanisms: adsorbents assist in removing toxins or bacteria from the GI tract, antimotility agents slow down peristalsis, and probiotics aid in restoring the natural bacteria in the lower intestine. Oral rehydration agents can be administered to patients with diarrhea to replenish fluid and electrolytes, but they do not address the diarrhea itself. In cases of diarrhea caused by specific infections like campylobacter or giardia, antibacterial agents may be used, although they are not typically required (Libretexts 2023).

Table 6 provides a comprehensive summary of the pharmacological agents utilized in the treatment of diarrhea. According to Libretexts (2023), it details each drug's mechanism of action, generic name, therapeutic use, and associated adverse effects.

Table 6 Comparison of Antidiarrheal Medications

Class	Mechanism of Action	Prototype/ Generic	Therapeutic Use	Adverse Effect
Adsorbent	Adsorbing medications work by covering the GI tract walls and attaching to harmful bacteria or toxins, removing them from the GI tract through the stool.	Bismuth subsalicylate (Pepto Bismol)	Decreased diarrhea symptoms	Black tongue
Anticholinergic	The smooth muscleof the GI tract is affected by it, leading to the inhibition of propulsive motility and a reduction in gastric acid secretion.	Hyoscyamine	Decreased diarrhea symptoms	CNS Effect
Opiate-like medication	The mechanism of action involves reducing the influx of fluids and electrolytes into the intestine and delaying intestinal motility to reduce the frequency of bowel movements.	Loperamide (Imodium)	Decreased diarrhea symptoms	Black Box Warning
Probiotics	Assist in restoring the healthy balance of bacteria in the gut.	Lactobacillus	Prevention of diarrhea or decreased symptoms	Flatulen ce and bloating

D. Acute Oral Toxicity Testing

Acute Oral Toxicity Testing, specifically the Acute Toxic Class Method (OECD 423) is a testing procedure designed to evaluate the toxicity of a substance when administered orally. It refers to adverse effects that occur after the oral administration of a single dose of a substance or multiple doses administered within a 24-hour period (Anjankar et al. 2023). This method is used to determine the dose level that causes toxicity or death in test animals, typically rats, to help classify chemicals and substances for regulatory purposes.

E. Assays for Antidiarrheal Activity

To scientifically uphold the traditional use of *Z. zerumbet* as a potential antidiarrheal drug. Experimental procedure using albino mice is necessary. The castor oil-induced method and the Gastrointestinal Motility Test are amongst several antidiarrheal assays that can be used to assess such.

➢ *Castor Oil-Induced Method*

Castor oil has an active metabolite, ricinoleic acid, which is derived from the action of lipases and irritates the intestinal mucosa, causing inflammation and promoting the release of prostaglandins. Prostaglandins stimulate the intestines, resulting in increased motility and water secretion, thus inducing diarrhea. Ricinoleic acid is responsible for castor oil's laxative effect and induces diarrhea by directly affecting the intestines (Murugan et al. 2020). The previous study (Meite et al. 2009), records that castor oil induces diarrhea significantly in mice. Castor oil-induced diarrhea test is employed to evaluate the potential antidiarrheal effect of the ethanolic extract of *Z. zerumbet* that is given to mice. This is done by observing the mice'sloose stools. Lack of such loose stools indicates that the plant extract effectively manages diarrhea.

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➢ *Gastrointestinal Motility Test*

The gastrointestinal motility test, sometimes referred to as the Charcoal meal test, is commonly used for the measurement of gastrointestinal transit in mice (Evangelista 2013). It is used to evaluate the bowel movement and function of the gastrointestinal tract. This test involves administering activated charcoal to swiss albino mice and thus measures the speed at which it passes through the digestive tract. It can disclose how the food immediately passes through the gastrointestinal tract, which has to do with diarrhea when the transit is too rapid (Manjunath et al. 2011). The test can identify either increasing or decreasing motility that might be useful for evaluating the potential antidiarrheal effect of *Z. zerumbet.*

F. Related Studies

Zingiberaceae, a well-known family in which is quite diverse and so are the bioactive principles present within their rhizomes, that have been extensively studied for their therapeutic values. In such studies, compounds like gingerols, shogaols, curcuminoids, and essential oils have come forth with remarkable pharmacological properties, especially anti-gastrointestinal disorders. Zingiberaceae contains an abundant source of different bioactive phytochemicals, also known as ginger, with interestingly 52 genera and over 1300 species of aromatic perennial herbs with creeping or tuberous rhizomes, and it is widely distributed all around the tropics (Alolga et al. 2022). These active constituents of rhizomes traditionally have been used by many cultures to alleviate symptoms such as nausea, abdominal pain, diarrhea, and inflammation due to their anti-inflammatory, antioxidant, and antimicrobial effects.

This review gives a critical overview of the existing literature on the constituents of Zingiberaceae rhizomes concerning their bioactive profile and therapeutic applications for gastrointestinal health, focusing on well-investigated species like *Z. officinale* (ginger), *C. longa* (turmeric), and *A. galanga* (Thai ginger). The literature underlines the potential of these phytochemicals for the development of both traditional and modern medicinal approaches against gastrointestinal health. Table 7, as presented below, offers a comprehensive collection of sources and related information pertaining to the Zingiberaceae family, highlighting the origins, key references, and relevant data that provide a deeper understanding of this botanical group.

Table 7 Sources and Related Information on Zingiberaceae.

Constituents of Zingiberaceae Rhizomes with Gastrointestinal Applications Studies and Related Literature	
Study/ Journal Article	**Relevant/ Related Information**
Evaluation of anti-diarrhoeal activity of *C. zedoaria* rhizome	*C. zedoaria* (family: Zingiberaceae) is traditionally used in folk medicine for dyspepsia, cough, dermatosis, inflammatory ailments, diarrhoea, etc. The present paper deals with phytochemical screening and antidiarrhoeal activity of ethanol extracts of rhizomes of *C. zedoaria*. The results indicated that the extract of *C. zedoaria* dose dependently reduced the severity & frequency of diarrhea in mice as compared to the standard antimotility drug loperamide. (Azam et al. 2017).
Medicinal Plants Profile and Some Screening Method Used in the Treatment of Diarrhea: A Review	Examples of members of this family are ginger *Z. officinale* Roscoe, turmeric *C. longa* L., Javanese ginger *C. zanthorrhiza* Roxb., and Thai ginger *Alpinia galanga* L. In addition, many of the members in this family mentioned herbal plants that are used as antidiarrheal, more specifically in the rhizome part, such as *C. amada* roxb. (Herb) commonly known as Amahaldi, *Hedychium spicatum*, also known as *Kapur kachori*, and *Z. officinale*, which is called Ginger (Patil et al. 2020).
Anti-diarrhea effects of polysaccharides from *Z. officinale* rhizome on a rat diarrhea model	Under the Zingiberaceae family, one of its members contains a rich constituent of gingerol of *Z. officinale* in part of the plant, which is the rhizome. It commonly treats various gastrointestinal diseases, for example, abdominal cold pain, cough, and especially diarrhea (Su et al. 2019).
Pharmacological importance of	*Kaempferia galanga* is a source of valuable bioactive Compounds, it is renowned for its medicinal as well as edible
Kaempferia galanga (Zingiberaceae): A mini review	properties. It is used as folk medicine, the rhizome of *K. galanga* L. includes antibacterial, treatment of hypertension, asthma, Rheumatism, indigestion, cold and headache, abdominal relief. (Shetu et al. 2018).

Table 8 shown below is a collection of studies and related literature about the use of PNSS and 2% Tween 80 as the vehicle for negative control used in this study.

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Table 8 Sources and Related Information on PNSS and Tween 80.

Studies and Related Literature that Employed PNSS and 2% Tween 80 as Negative Control	
Evaluation of the Antidiarrheal Activity of 80% Methanol Extract and Solvent Fractions of the Leaf of *Bersama abyssinica* frese (Melianthaceae) in Mice	Negative controls received either distilled water or 2% Tween 80 (10 ml/kg), positive controls received 3 mg/kg loperamide or 1 mg/kg atropine, and the test groups received 100, 200, and 400 mg/kg doses of the extract.
In vivo Antidiarrheal Activity of Crude Extract and Solvent Fractions of *Rhamnus prinoides* (Rhamnaceae) Leaves	The *R. prinoides leaves* were macerated using absolute methanol and then fractionated using solvents of different polarity indexes. For *in-vivo* antidiarrheal activity evaluation of the crude extract and solvent fraction, castor oil-induced diarrhea, castor oil-induced anti-enteropooling, and intestinal transit models were used. One-way analysis of variance was used to analyze the data, followed by a Tukey post-test. The standard and negative control groups were treated with loperamide and 2% tween 80 respectively.
Evaluation of Antidiarrheal Activity of 80% Methanol Extract and Solvent Fractions of the Leaves of *Withania somnifera* (L.) Dunal in Swiss Albino Mice	The groups were allocated as group I (negative control) receiving 10 ml/kg 2% Tween 80 in distilled water; group II (positive control) receiving 3 mg/kg loperamide; and group III to group V (test groups) receiving 100, 200, and 400 mg/kg doses of the crude extract and solvent fractions, respectively.
Experimental Assessment of Antidiarrheal and Antisecretory Activity of 80% Methanolic Leaf Extract of *Zehneria scabra* in Mice	The animals were randomly assigned to different groups- as a negative control group, treatment groups for different doses of extracts of *Z.scabra*, and a positive control group. Group I was in negative control and was given a vehicle, (2% (v/v) Tween-80 in water). Group II (positive control group) was given standard drug, loperamide (3 mg/kg, orally) in both castor oil induced antidiarrheal test and misoprostol induced antisecretory test and atropine sulfate (0.1 mg/kg, i.p.) for gastrointestinal motility test by charcoal meal.

This leaves a high chance that the *Z. zerumbet* may carry those constituents, more specifically in the *Z. zerumbet*'s rhizome, that possibly helps to alleviate or bring the desired effect to the diarrheal symptoms.

G. Theoretical Framework

Diarrhea can be characterized by stool consistency, frequency, stool weight, and stool volume. Patients typically describe diarrhea as the passing of loose stools (Arasaradnam et al. 2018). The occurrence of diarrhea is very common, and it is important for healthcare providers to be knowledgeable about this condition, regardless of their specialty or scope of practice (Akhondi and Simonsen 2023). The antidiarrheal properties of plant-derived components are examined in this review, along with their potential mechanisms of action and chemical properties. Traditional uses of medicinal plants form one of the most essential bases for sharing this knowledge and a backdrop on which scientific research is conducted to establish their pharmacological activities (Petrovska 2012).

Z. zerumbet's rhizome is the most commonly utilized part for medicinal purposes (Norulaini et al. 2009). The rhizome of *Z. zerumbet* has been extensively employed with impressive therapeutic effects for addressing inflammation, diarrhea, stomach cramps, bacterial infections, fever, flatulence, allergies, and poisoning (Tewtrakul and Subhadhirasakul 2007; Okamoto et al. 2011; Acharya et al. 2011; Sidahmed et al. 2015). The dried rhizomes of *Z. zerumbet* were subjected to phytochemical screening, revealing the presence of steroids, terpenes, quinones, flavonoids, phenols, alkaloids, tannins, coumarins, and glycosides (Preshahdin et al. 2020).

The present study is to be done to investigate the antidiarrheal potential of *Z. zerumbet* by utilizing its major bioactive phytoconstituent. The null and alternative hypotheses would be as follows: H_1: The frequency of diarrhea and gastrointestinal transits of the ethanolic rhizome extract of *Z. zerumbet* are significantly less than that induced by the standard drug loperamide, while H_{01}: The extract will have no significant effect on the frequency of diarrhea and gastrointestinal transits when compared with the standard drug. The secondary hypothesis will be that the extract enhances the consistency of stool and diminishes intestinal motility, while the null hypothesis is that there is no significant improvement.

Formulation of these hypotheses is important for any future research since they give some background on how to assess the efficiency of *Z. zerumbet* in gastrointestinal disorders. Furthermore, the theoretical framework provides a systematic method of investigation and as such, allows the resultant analysis of the pharmacological effects of the extract to be aptly captured. The framework also serves to assist in the validation of the findings and in underlining the potentiality of further exploratory possibilities of *Z. zerumbet* into being a viable therapeutic option under both traditional and modern medical contexts. Figure 4 below illustrates the relationship between the input, process, and output of the research study, which focuses on evaluating the antidiarrheal activity of *Z. zerumbet*.

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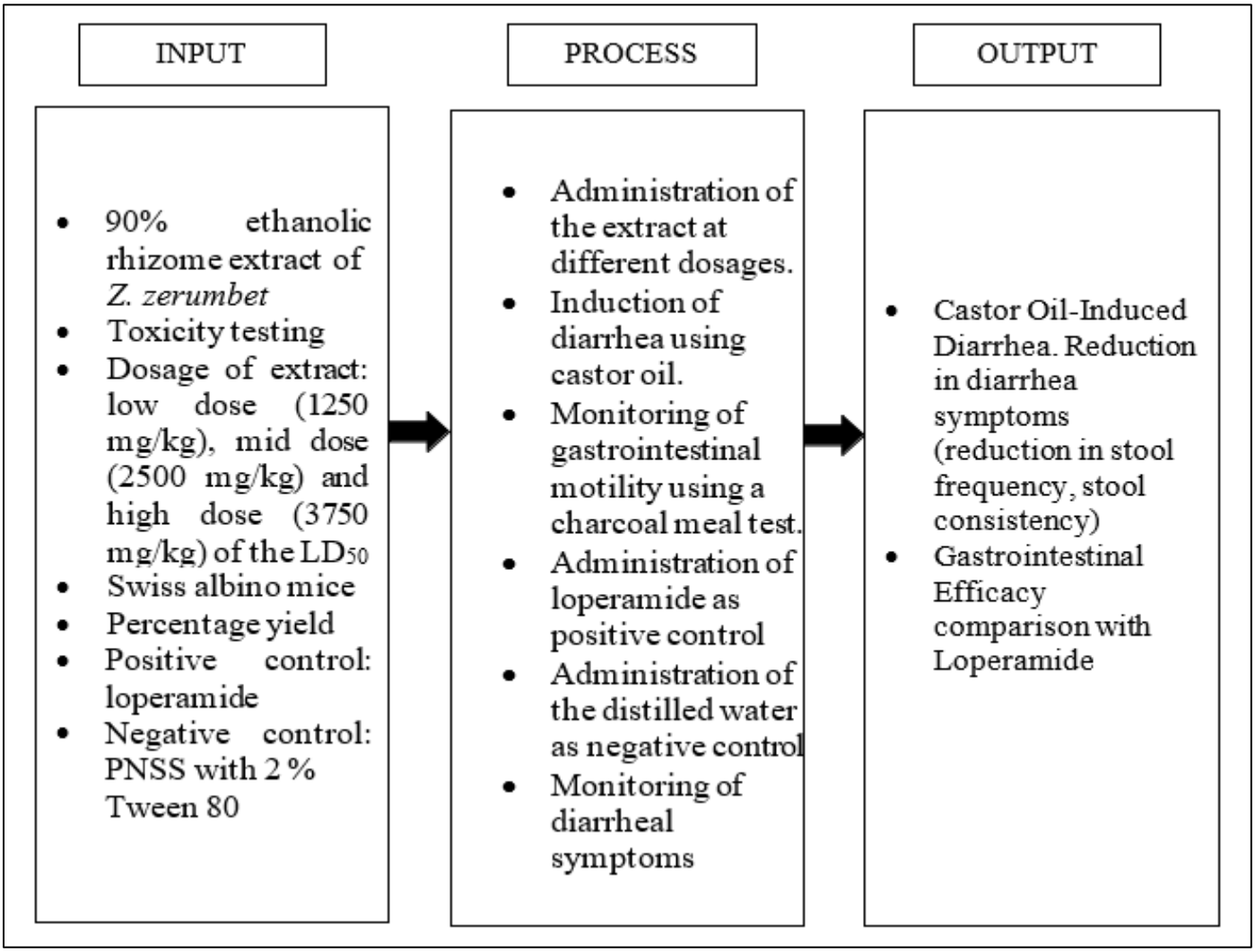

Fig 4 Theoretical Framework.

The relationship between the input, process, and output of the research study. This study aims to determine the antidiarrheal activity of *Z. zerumbet.*

The conceptual framework illustrates the primary agent to be investigated for the potential antidiarrheal activity of the 90% ethanolic rhizome extract of *Z. zerumbet*. The extract to be administered will be considered in three different dosages, specifically low dose (1250 mg/kg), mid dose (2500 mg/kg) and high dose (3750 mg/kg) of the LD50. Swiss albino mice will also be used as test subjects in the study. Additionally, in order to compare the effectiveness of the extract, the experiment will also include the administration of loperamide, a standard medicine to treat diarrhea, and PNSS with 2% of Tween 80 as a negative control for measuring its baseline effects without the treatment.

In this regard, the extract will be given to the Swiss albino mice in varied dosages, followed by induction of diarrhea using castor oil. It would hence serve as a standard against which the efficiency of the extract in treating diarrhea is measured. The researchers propose a charcoal diet test to precisely determine the rate of gastrointestinal tract motility and intestinal content movement. The intervention will, therefore, monitor symptoms of diarrhea in terms of the frequency and consistency of bowel movements as an indication of the extract's effectiveness in alleviating the symptoms. By comparing the results with the positive control, the researchers will draw a scientific relationship between the observed effects and the extract.

The expected outcomes will focus primarily on the possible decreased symptoms of diarrhea such as the reduction in stool frequency and an improvement in the consistency of the stool. The other key outcome would involve gastrointestinal motility, measured as the intestinal transit time of charcoal. The outcomes may conclude the evaluation of the extract's effectiveness against the standard for antidiarrheal treatment, loperamide.

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CHAPTER THREE
METHODOLOGY

This chapter, the methodology, presented the research methods, procedures, and sources of data employed in evaluating the antidiarrheal properties of *Zingiber zerumbet*. It outlined the research design, the research instruments utilized, and the procedures for data collection. Furthermore, it detailed the phytochemical analysis of the extract, the protocol for its administration, and the methods applied for data analysis. According to Bahishti (2022), it was a key component of the research process. The methodology offered a structured method for carrying out the research, ensured the validity and reliability of outcomes, encouraged effective communication and collaboration, and aided in the progression of knowledge within a specific domain.

A. Plant Material

The rhizome of *Z. zerumbet* was collected in Sawir, Masiu, Lanao del Sur, Mindanao, Philippines (7.7908° N, 124.3449° E), where the species was abundant. Thereafter, the obtained plant rhizome was identified and authenticated by the botanist at Mindanao State University - Iligan Institute of Technology (MSU-IIT) at the Chemistry Department of the College of Science and Mathematics. Confirmatory phytochemical tests were also conducted in the department's laboratory to accurately characterize the plant's bioactive compounds.

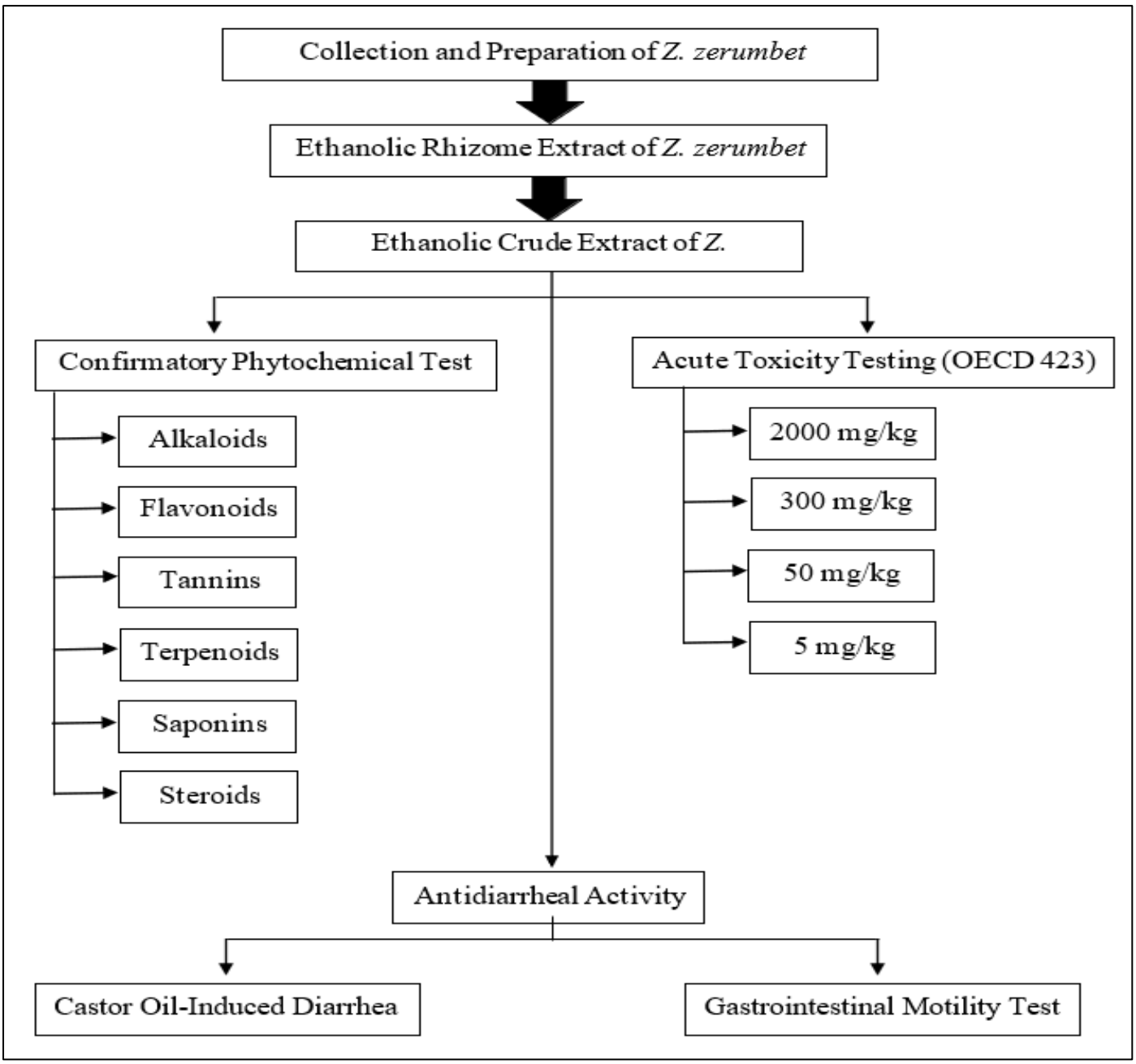

Fig 5 Flow Chart of Tests Conducted to the Crude Extracts of *Z. zerumbet's* Rhizome.

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B. Apparatus, Standards, Reagents, and Chemicals

The equipment, standards, reagents, and chemicals used for the evaluation of the ethanolic extract of *Z. zerumbet* (Zingiberaceae) had been acquired from and initially supplied by JBL Scientific (Manila, Philippines) and Nanjing Forever Pharmacy Co. Ltd (Nanjing Xin'gang Development Zone, Qixia District, Nanjing City) and selected apparatus were also sourced from the Pharmacy Department Laboratory of Adventist Medical Center College

Starting from extraction up to phytochemical analysis, the apparatus was a necessary tool in the extraction, processing, and examination of the rhizomes, these include: the Buchner funnel, desiccator, commercial pulverizer (Bobi), rotary evaporator (Biobase, RE-301), orbital shaker (Digisystem Laboratory Instruments Inc.), Soxhlet extractor, Whatman No. 1 filter paper, and a water bath. These items were sourced from the AMCC-Pharmacy Laboratory.

Loperamide, the standard or positive control, ensured the reliability of results, which was administered via oral gavage. *Z. zerumbet* was extracted using 90% ethanol, selected for its ability to isolate key bioactive compounds. A charcoal meal test was employed to analyze gastrointestinal motility, where the transit of charcoal through the gastrointestinal tract was observed (Margolis et al. 2016). Additionally, a castor oil- induced diarrhea test was conducted, using castor oil to induce gastrointestinal hypermotility and secretion (Rahman 2015). The necessary reagents and materials, including castor oil, were procured through JBL Scientific (Manila, Philippines), which served as a key supplier for the study.

C. Plant Extract Preparation

The rhizomes of *Z. zerumbet* were gathered in large quantities for further processing. The raw rhizomes had undergone several stages of handling, starting with a thorough wash under running water to eliminate any attached soil or dirt. As stated by Kamadyaapa (2018), the plant material was sliced thinly and air dried at room temperature of 22 ±3°C for a duration of three to four weeks or until the material was fully dried and then had been ground into a fine powder (0.25 mm) using a commercial grinder (Bobi). The powder was capable of passing through a 60-mesh sieve. Thereafter, one kilogram of the powdered material was macerated with five liters of 90% ethanol in a Erlenmeyer flask for a period of 168 hours (7 days) while being shaken continuously using an orbital shaker, at the speed of 100 rpm. The resulting mixture was filtered using Whatman (No 1) filter paper in the Buchner funnel, and the residue was then macerated twice using similar solvents to exhaustively extract the plant material. Then, the filtrate was concentrated in a rotary evaporator (Biobase, RE-301), with a constant temperature of 40 to 55℃ until it yielded a semi-solid mass. Thesemi- solid paste was then stored in the refrigerator under proper storage conditions.

D. Percentage Yield Analysis

The weight of the extracted *Z. zerumbet* rhizome and the initial powdered rhizome sample had been weighed and documented. The percentage yield of the extraction was then calculated using the formula below. As described by Afrin et al. (2021), a 10-gram sample of powdered rhizome had been used in quintuplicate analysis to determine the extract yield. The formula used for calculating the percentage yield was as follows:

$$\%\ \text{yield of extract} = \frac{Weight\ of\ extracted\ rhizome\ (g)}{Weight\ of\ powdered\ sample\ (g)} \times 100$$

E. Phytochemical Confirmatory Test

A confirmatory phytochemical test had been conducted using the standard protocols, to test for the existence of alkaloids, flavonoids, tannins, terpenoids, saponins, and steroids qualitatively. These phytochemical constituents may contribute to the antidiarrheal effects of *Z. zerumbet*. Table 9 presents the specific test, procedure and expected results for the phytochemical test.

Table 9 Phytochemical Confirmatory Tests Procedures for 90% Ethanolic Rhizome Extract of *Z. zerumbet.*

Phytochemicals	Test	Procedure	Expected Positive Result
Test for Alkaloids	Wagner's test	Wagner's reagent was produced by adding 1.27g of iodine and 2g of potassium iodide followed by distilled water to make the final volume of 100 mL. To test for alkaloids, a few mL of the extract was added with 1-2 drops of Wagner's reagent along the sides of the test tube (Shaikh and Patil 2020).	The brown or reddish precipitate
Test for Flavonoids	Alkaline reagent test	A 2 mL of 2% sodium hydroxide solution was added to a 1 mL of extract solution (Sharma et al. 2020).	Bright yellow was turned colorless upon dilution with a few drops of hydrochloric acid.
Test for Tannins	Ferric chloride test	A 5 mL of distilled water was added to an aliquot portion of the crude extract in the water bath and the mixture was heated. The mixture was then filtered, after which ferric chloride was added to the filtrate (Ramadanil et al. 2019).	Dark green solution

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Test for Terpenoids	Salkowski's test	The presence of terpenoids was assessed by dissolving 5 mg of the crude extracts in 2 mL of pure	Reddish-brown color
		chloroform and 5M sulfuric acid was added to it. A reddish-brown color formation suggested the presence of terpenoids (Ramzan et al. 2023).	
Test for Saponins	Froth test	A small amount of the crude extract was dissolved in 1 mL of ethanol followed by the addition of 5 mL of distilled water and it was then shaken vigorously (Preshahdin et al. 2023).	Formation of frothing that persisted within 5 minutes.
Test for Steroids	Liebermann – Burchard test	100 mg of the extract was mixed with chloroform in a test tube prior to adding a few drops of acetic anhydride. After that, it was boiled in a water bath and cooled rapidly in icy water followed by the addition of 2 mL concentrated sulfuric acid (Iqbal et al. 2015).	Formation of brown rings at the junction of two layers and turning the upper layers into green.

F. Acute Oral Toxicity: OECD 423 Acute Oral Toxic Class Method

➢ *Selection of Experimental Animals*

Healthy young adult female Swiss Albino mice within seven to twelve weeks old were used. Female mice were normally used according to the OECD 423 guidelines (Acute Toxic Class Method) as they were sensitive to acute toxic effects and assured that the mice were nulliparous and never had been pregnant.

➢ *Housing and Feeding Conditions*

Standardized conditions of housing and feeding were provided to ensure the well-being of experimental animals. Room temperature was maintained around 22ºC (± 3ºC), in addition, the qualified humidity which is 30% - 70% was maintained, and for the particular cleaning time, approximately it was at 50-60% room humidity. Since the animal tests were diurnal, artificial lighting was provided to them for 12 hours, and artificial darkness for 12 hours, subsequently. Distilled drinking water was available to the animals *ad libitum*, and an appropriate laboratory diet was given, consisting of standard rodent pellets. The study implemented a randomized controlled trial method, in which the test animals were randomly selected and marked to permit individual identification, afterward the test animals were housed and kept for 3-7 days, before dosing, to acclimatize to the laboratory environment.

➢ *Acute Oral Toxicity Testing: Acute Toxic Class Method (OECD 423)*

The acute oral toxicity was carried out following the OECD 423 guidelines (Acute Toxic Class Method). Before the procedure, the animals were fasted for three to four hours with free access to water. After fasting, the animals were weighed, and the ethanolic rhizome extract of *Z. zerumbet* was administered via oral gavage in a single dose of randomly selected Swiss albino mice. The starting dose was selected from one of four fixed levels with five mice in each level: 2000, 300, 50, and 5 mg/kg body weight. The initial dose level was selected with the possibility that it may result in mortality in some of the dosed animals. The time interval between dosing groups was based on the onset, duration, and severity of toxic signs. The limit test at one dose level of 2000 mg/kg body weight with 6 animals (three animals per step). The limit test was at one dose level of 5000 mg/kg body weight (with three animals). If the ethanolic rhizome extract of *Z. zerumbet* induced mortality, it may be necessary to conduct further testing at a reduced dose level to evaluate its safety and establish a more accurate toxicity threshold. After the administration of the ethanolic rhizome extract of *Z. zerumbet* dose, the animals were observed individually at least once or twice for the first thirty minutes with special attention for the first 4 hours and periodically checked for the first twenty-four hours, also daily for a total of fourteen days. The determination of the reaction is observed by the toxic signs, time of onset of action, and length of recovery period. The times in which the toxic reactions appear were essential for the observation and were must recorded. Observation included changes in skin and fur, eyes, and behavior patterns.

➢ *Administration of Test Substance*

The ethanolic extract of *Z. zerumbet* was administered at 2000 mg/kg body weight. Food was suspended for four hours before experimentation in the test animals. Experimental group, n = 3, a single dose of the test substance was given orally by oral gavage. After giving the test substance, the test animals were starved for two hours. For the administration of the test substances, the OECD 423 guidelines (2023) were followed. For the stepwise procedure on the determination of an approximate lethal dose, check Appendix C.

G. Test for Antidiarrheal Activity of the Ethanolic Rhizome Extract of Z. zerumbet (Zingiberaceae)

➢ *Selection of Experimental Animal*

Healthy Swiss albino mice of either sex, weighing 20–30 g and 6 to 8 weeks old were used and obtained at AMCC Animal House. The mice were handled based on the guidelines for the care and use of laboratory animals (Guide for the Care and Use of Laboratory Animals 2011).

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➢ *Housing and Feeding Conditions*

Standardized conditions of housing and feeding were provided to ensure the well- being of experimental animals. Room temperature was maintained around 25.0±2.0ºC. In addition, the qualified humidity was 30% - 70%, and for the particular cleaning time, it was approximately 50-60% room humidity. Since the animal tests were diurnal, the artificial lighting was provided to them for 12 hours and 12 hours of darkness, all was sequenced. Distilled drinking water was available to the animals *ad libitum*, and an appropriate laboratory diet was given. The animals were acclimatized to laboratory conditions for a week before experimentation (Guide for the Care and use of Laboratory Animals 2011).

➢ *Castor Oil-Induced Diarrhea*

Castor oil-induced diarrhea was carried out using the method described by Fokam Tagne et al. (2019) with slight modifications. Five cages contained five Swiss albino mice, with each cage containing individual compartments to separate the mice. Each of them was fast for 18 hours with free access to water before experimentation. To induced diarrhea, castor oil (10 mL/kg) was orally administered to all Swiss albino mice via oral gavage. 30 minutes after castor oil administration, the first group also known as diarrheal control (DC) received PNSS with 2% of Tween 80 (10 mL/kg bw) which had been a negative control, the second group received the standard drug, Loperamide (5 mg/kg) as a positive control, while the other (3) groups were received one of the *Z. zerumbet* ethanolic extract: low dose (1250 mg/kg), mid dose (2500 mg/kg) and high dose (3750 mg/kg) of the Acute Oral Toxicity test. After these treatments, each of the animal tests was individually placed in a partitioned cage with clean white filter paper beneath it, which had been used to collect the feces. These filter papers should be changed every hour for 5 hours. During each hour, it was used to inspect for diarrheal droppings (both wet and dry feces) and weighed; to calculate the total stool mass, diarrhea stool mass, total stool count, and the number of diarrheal stool wet feces had been recorded. The percentage inhibition of diarrhea and the stool emission frequency (SEF) was calculated as follows (Fokam Tagne et al. 2019). As outlined in Appendix Q.

$$Stool\ Emission\ Frequency = \frac{total\ number\ of\ stool}{time(5h)}$$

$$\%\ inhibition = \frac{SMDC - SMDT}{SMDC} \times 100$$

Where:

SMDC was the Stool mass of diarrheal control SMDT was the Stool mass of a diarrheal test

Table 10 Study Design for Castor Oil-Induced Diarrhea Test in Swiss Albino Mice.

Group Label	Treatment Group	Number of Animals
Group I	**Diarrheal Control (Negative Control):** Castor oil-induced + PNSS with 2% Tween 80 (10 mL/kg bw)	5 Animals
Group II	**Positive Control:** Castor oil-induced + Loperamide (5 mg/kg)	5 Animals
Group III	Castor oil-induced + *Z. zerumbet* rhizome low dose (1250 mg/kg) of the LD50	5 Animals
Group IV	Castor oil-induced + *Z. zerumbet* rhizome middle dose (2500 mg/kg) of the LD50	5 Animals
Group V	Castor oil- induced + *Z. zerumbet* rhizome high dose (3750 mg/kg) of the LD50	5 Animals

➢ *Gastrointestinal Motility Test by Charcoal Meal*

The gastrointestinal motility test was carried out using the method described by Mascolo et al. and Rahman et al (2012), as cited by Rahman et al. (2015) with slight modification. The animals were subdivided into five groups with five Swiss albino mice in each group and made to fast for 18 hours with free access to water prior to experimentation. Castor oil was administered to these animals to induce diarrhea. After one hour, group I (the control group) received PNSS with 2% Tween 80 (10 mL/kg). Group II, received the standard drug (loperamide 5 mg/kg) as a positive control. Groups III, IV, and V received the ethanolic rhizome extract of the *Z. zerumbet* at a dose of low dose (1250 mg/kg), mid dose (2500 mg/kg) and high dose (3750 mg/kg) of the Acute Oral Toxicity test. The administration of these substances were all given via oral gavage. The mice were then administered with 1 mL charcoal meal (10g charcoal, 5g gum acacia in 100 ml distilled water) after an hour with the same route of administration. One hour afterward, the animals were sacrificed in a humane way through cervical dislocation and celiotomy. The animals' intestines were removed and the movement of the charcoal meal from pylorus to caecum was measured. The distance was calculated and expressed as a percentage of the total length of the intestines. The percentage of the inhibition of the movement was calculated as follows (Tafesse and Mekonnen 2012). Refer to Appendix F. Table 11 shows the study design for gastrointestinal motility test.

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$$\%\ inhibition = \frac{MTLI - MDCC}{MTLI} \times 100$$

Where:

MTLI = mean total length of the intestine MDCC = mean distance covered by the charcoal

Table 11 Study Design for Gastrointestinal Motility Test in Swiss Albino Mice.

Group Label	Treatment Group	Number of Animals
Group I	**The Control Group (Negative Control):** Castor oil-induced + PNSS with 2% Tween 80 (10mL/kg)	5 Animals
Group II	**Positive Control:** Castor oil-induced + Loperamide (5 mg/kg)	5 Animals
Group III	Castor oil-induced + *Z. zerumbet* rhizome extract low dose (1250 mg/kg) of LD50	5 Animals
Group IV	Castor oil-induced + *Z. zerumbet* rhizome extract middle dose (2500 mg/kg) of LD50	5 Animals
Group V	Castor oil-induced + *Z. zerumbet* rhizome extract high dose (3750 mg/kg) of LD50	5 Animals

H. Data Analysis

The data obtained from the experiment from stool consistency observation, diarrhea frequency, and fecal weight were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26, and the results were expressed as mean ± standard error of the mean (SEM). The significant difference between the groups was analyzed via one-way ANOVA. Then, was followed by Tukey's post hoc test, and P-values below 0.05 were considered statistically significant (Mengesha 2022). Further evaluation of treatment effects was conducted using Analysis of Covariance (ANCOVA), pairwise comparisons adjusted by the Bonferroni method, and Poisson regression models, applied as appropriate to the data.

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CHAPTER FOUR
RESULTS AND DISCUSSION

This chapter presents the data of the study on the antidiarrheal effects of ethanolic rhizome extract of *Zingiber zerumbet* along with its analysis and interpretation. The data on this section are thoroughly analyzed and systematically interpreted to emphasize implicit contribution to addressing diarrheal conditions, corresponding with the objectives of the research mentioned in chapter one.

A. Preparation of the Plant Extract

The preparation and evaporation processes of the ethanolic rhizome extract of *Z. zerumbet* are illustrated in Figures 6 and 7. Figure 6 depicts five beakers containing the golden-yellow ethanolic extract, arranged with corresponding evaporating dishes for the drying procedure. The process released a characteristic herbal and resinous odor akin to turmeric. Following the evaporation of ethanol, as shown in Figure 7, the extract formed an orange-brown, semi-solid residue distributed across the surfaces of the evaporating dishes.

Fig 6 Ethanolic *Z. zerumbet* Rhizome Extract Prior to Evaporation.

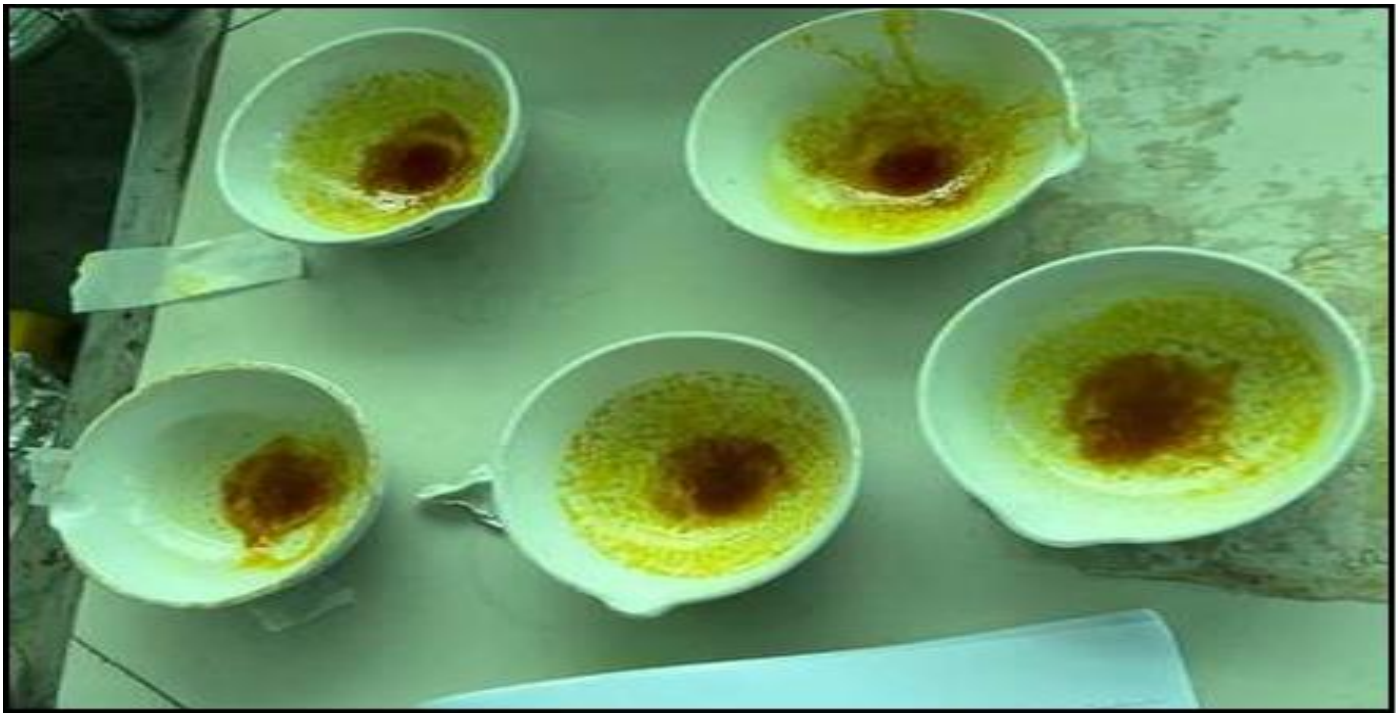

Fig 7 Dried ethanolic *Z. zerumbet* Extract after Solvent Removal, Yielding a Concentrated Residue.

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B. Percentage Yield

To determine the percentage yield of the extract, quintuplicate analysis was utilized by using five samples of 10-grams of powdered *Z. zerumbet* rhizome which had been macerated for 7 days. The evaporating dish (ED) was weighed prior to water- bath for extraction. The initial and final weight of the evaporating dish was inscribed. The results are then calculated. Table 12 below presents the individual yields from each trial, along with the calculated average yield of the ethanolic rhizome extract of *Z. zerumbet*. The analysis of a 10-gram sample conducted based on quintuplicate analysis resulted in an average percentage yield of 5±1.22%.

Table 12 Percentage Yield of the Ethanolic Rhizome Extract of *Z. zerumbet.*

Trial	Weight of Dried *Z. zerumbet (g)*	Weight of crude extract (g)	% yield
1	10	0.5	5%
2	10	0.5	5%
3	10	0.6	6%
4	10	0.6	6%
5	10	0.3	3%
		Mean ±SD	5±1.22%

C. Phytochemical Analysis Results

The phytochemical test for the 90% ethanolic rhizome extract of *Z. zerumbet* was conducted at Mindanao State University - Iligan Institute of Technology (MSU- IIT) by the Department of Chemistry shows a positive results for the phytochemicals such as alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids which are all known to contribute to antidiarrheal activity. Table 13 below provides a summary of the qualitative phytochemical analysis performed on the ethanolic rhizome extract of *Z. zerumbet*. The table highlights the identified bioactive compounds, offering insights into the extract's chemical profile and potential therapeutic applications.

Table 13 summarizes the confirmatory phytochemical screening of the crude extract of *Z. zerumbet*. Specific chemical tests were conducted to support preliminary screening and identify alkaloids, flavonoids, phenols, saponins, tannins, steroids, and terpenoids. Essential oil contents were also significant. Tests included Wagner's Test for alkaloids, Shinoda Test for flavonoids, Ferric Chloride Test for phenols and tannins, Foam Test for saponins, and Salkowski's Test for terpenoids, Liebermann–Burchard test for steroids, confirming the extract's richness in bioactive secondary metabolites.

Table 13 Confirmatory Test Analysis for the Presence of Phytochemical Constituents of *Z. zerumbet* (Bitter Ginger) Crude Extract.

Confirmatory Test	Visible Results		Result	Interpretation
Wagner's Test: Test for Presence of Alkaloids		Formation of dark brown precipitate	++	Positive for the Presence of Alkaloids.
Shinoda Test: Test for Presence of Flavonoids.		Appearance of dark reddish-brown color	+++	Positive for the Presence of Flavonoids.
Ferric Chloride Test: Test Presence of Phenols and Tannins.	Results for Tannins:	Appearance of light yellowish-brown color.	+	Positive for the Presence of Tannins.

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	Result for Phenols: 	Appearance of deep blue color.	+++	Positive for the Presence of Phenols.
Froth Test: Test Presence of Saponins.		Formation of frothing that persisted for 5 minutes	+	Positive for The Presence of Saponins.
Salkowski's Test: Test for the Presence of Terpenoids	Refer to Appendix G.	Appearance reddish-Brown color	+	Positive for The Presence of Terpenoids.
Liebermann–Burchard test: Test Presence of Steroids.	Refer to Appendix G.	Formation of brown rings at the junction of two layers and turning the upper layers green.	+	Positive for The Presence of Steroids.

+++: High Content, ++: Moderate, +: Positive, -: Negative

➢ *Remark:*

The ethanolic rhizome extract of *Z. zerumbet* showed significant presence of flavonoid content. Flavonoids compound has the ability to produce an inhibitory action on gastrointestinal motility (Kharal et al. 2023), which can possibly contribute to antidiarrheal activity. In addition, alkaloids had been suggested to lead to an inflammatory response which could contribute to diarrhea, including inhibiting intestinal motility. These results indicate that the extract could contain bioactive components that support its traditional treatment of gastrointestinal disorders.

D. Acute Oral Toxicity (OECD 423)

The acute toxicity of the plant was done in accordance with the OECD 423 guidelines using female Swiss Albino mice of average weight of 20g were selected in a stepwise procedure. The experimental animals were grouped into three mice each. The starting group mice received 2000 mg/kg where no mortality was recorded within 24 hours, hence, confirmation was conducted by repeating the procedure of 2000 mg/kg and still no mortality was observed which prompts the researchers to continue to the limit dose of 5000 mg/kg, no mortality. Since, there is no mortality recorded, as per the Globally Harmonized Classification System (GHS), the toxicity of the plant was considered to be Category 5 or Unclassified. Thus, the researchers concluded to use 5000 mg/kg as the

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LD50. Prior to that, signs of toxicity such as changes in skin and fur, respiratory activity, lethargy and salivation among others are closely monitored individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours. Observation was done over a period of 14 days. Necropsy findings of the experimental mice are detailed in Figure 8.

Doses used		
Low dose	**Mid Dose**	**High Dose**
1250 mg/kg	2500 mg/kg	3750 mg/kg

Fig 8 Swiss Albino Mice During Gross Necropsy

E. Castor-Oil Induced Diarrhea

➢ *Aggregated Stool Weight Over Time*

To address whether the ethanolic rhizome extract of *Z. zerumbet* exhibits antidiarrheal activity in a castor oil–induced diarrhea (COID) model, an analysis of covariance (ANCOVA) was conducted. This approach examined how aggregated stool weight (AgWeight) changed over time across treatment groups, using time as a continuous predictor and treatment as a between-subjects factor. Table 14 provides the regression estimates for both the main effects and the interaction terms, while the accompanying summary of estimated slopes (Table 14a) clarifies how each treatment's rate of change in stool weight compared to the negative control.

Table 14 Regression Estimates from the ANCOVA Examining the Effects of Time, Treatment, and their Interaction on Aggregated Stool Weight (AgWeight)

Characteristic	**Beta**	**95% CI**	**p-value**
Main Effects			
Time (slope for Negative Control)	14	[7.3, 20]	<0.001
Treatment (compared to Negative Control)			
Positive Control	18	[–13, 49]	0.30
Extract Low	5.0	[–26, 36]	0.70
Extract Mid	17	[–14, 47]	0.30
Extract High	4.5	[–26, 35]	0.80
Interaction (Difference in Slopes vs. Negative Control)			
Time × Positive Control	–22	[–31, –13]	<0 .001

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Time × Extract Low	–8.3	[–18, 1.0]	0.08
Time × Extract Mid	–16	[–25, –6.9]	< 0.001
Time × Extract High	–15	[–25, –6.1]	<0.001

Legend: Low: 1250 mg/kg Mid: 2500 mg/kg High: 3750 mg/kg

The ANCOVA results in Table 14 indicate that stool weight increased steadily over time in the negative control group, at approximately 14 mg per unit of time (p< 0.001), confirming the diarrheal effect of castor oil. In contrast, the positive control group treated with loperamide showed a significantly lower rate of stool weight accumulation, with a reduction of 22 mg per unit of time compared to the negative control (p<0.001), reflecting its strong antidiarrheal effect. Among the extract-treated groups, the low dose showed a slight reduction of 8.3 mg per unit time, but this effect was not statistically significant (p = 0.08), suggesting only a mild influence on stool output. However, both the mid and high extract doses demonstrated statistically significant reductions in stool weight gain over time, with slopes 16 mg (p < 0.001) and 15 mg (p < 0.001) lower than the negative control, respectively. These results suggest that the ethanolic rhizome extract of *Z. zerumbet* exhibits dose-dependentantidiarrheal activity, with the mid and high doses showing comparable effects to loperamide, while the low dose yielded minimal impact.

Table 14a Estimated Slopes of Aggregated Stool Weight (AgWeight) Over Time by Treatment.

Treatment	Time Trend (Slope)	SE	df	t-value	p-value
Negative Control	13.91	3.32	115	4.19	<0.001
Positive Control	–8.24	3.32	115	–2.48	0.01
Extract Low	5.63	3.32	115	1.70	0.09
Extract Mid	–2.28	3.32	115	–0.69	0.49
Extract High	–1.48	3.32	115	–0.44	0.66

Legend: Low: 1250 mg/kg Mid: 2500 mg/kg High: 3750 mg/kg

Table 14a translates the significant interaction terms into estimated slopes of stool weight over time for each group. The negative control (13.91 mg/time, $p < 0.001$) demonstrates a statistically significant positive slope, consistent with ongoing diarrhea severity, which indicated that there is a consistent increase in stool weight and confirming the strong diarrheal effect of castor oil. Conversely, the positive control group exhibits a negative slope (–8.24 mg/time, $p = 0.01$), indicating a reduction in stool weight accumulation, an established hallmark of loperamide's antidiarrheal effect.

The low dose extract group showed a positive slope of 5.63 mg per time unit, suggesting that stool weight continued to increase, yet compared to the negative control, it is at a slower rate. However, this result was not statistically significant (p = 0.09), indicating that the effect may be minimal or due to chance. In contrast, a potential reduction in stool accumulation was found in the mid and high extract groups, as both showed slightly negative slopes (–2.28 mg and –1.48 mg per time unit, respectively).

The graph below in Figure 6 displays AgWeight trends (with 95% confidence interval) with time treated as a continuous predictor. All groups are distinguished by color and error bars represent the 95% confidence intervals. Slight horizontal "dodge" adjustments were applied to prevent overlap and clarify differences between treatments at each time point.

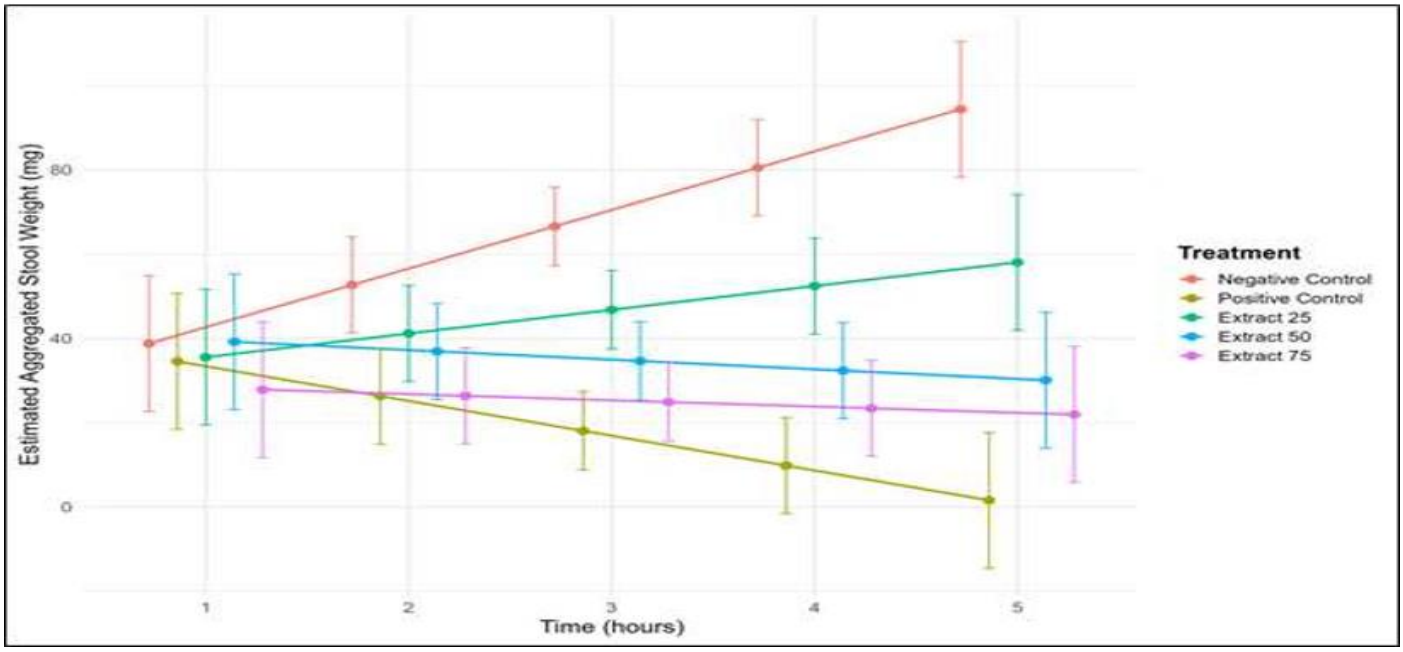

Fig 9 Estimated Marginal Means of AgWeight Over Time by Treatment.

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Table 15 Pairwise Comparisons of the Slopes of AgWeight Over Time Between Treatment Groups (Bonferroni Adjusted)

Comparison	Mean Difference	SE	df	t-value	Adj. p- value	Remark
Negative Control– Positive Ctrl	22.15	4.69	115	4.72	0.00	Significant
Negative Control – Extract Low	8.28	4.69	115	1.76	0.40	—
Negative Control – Extract Mid	16.19	4.69	115	3.45	0.01	Significant
Negative Control – Extract High	15.38	4.69	115	3.28	0.01	Significant
Positive Control – Extract Low	–13.87	4.69	115	–2.96	0.03	Significant
Positive Control – Extract Mid	–5.96	4.69	115	–1.27	0.71	—
Positive Control – Extract High	–6.77	4.69	115	–1.44	0.60	—
Extract Low – Extract Mid	7.91	4.69	115	1.69	0.45	—
Extract Low – Extract High	7.10	4.69	115	1.51	0.56	—
Extract Mid – Extract High	–0.81	4.69	115	–0.17	1.00	—

Table 15 presents the Bonferroni-adjusted pairwise comparisons of the slopes for aggregated stool weight (AgWeight) over time across the five treatment groups. These comparisons illuminate how each treatment's time trend differs from the others, highlighting where Castor oil–induced stool accumulation is most effectively mitigated. Consistent with the ANCOVA results (Tables 14 and 14a), the negative control's slope is significantly different from those of the positive control (mean difference = 22.15, $p < 0.001$), middle extract dose 16.19, $p = 0.01$), and high extract dose (15.38, $p = 0.01$). This pattern underscores that the positive control (loperamide), middle and high extract doses each yield marked decreases in stool accumulation compared to the negative control. By contrast, the difference between the negative control and low extract dose is not statistically significant ($p = 0.40$), reinforcing that the low dose does not diverge substantially from the baseline progression of diarrhea over time.

Comparisons involving the positive control reveal that it differs significantly only from low extract dose (–13.87, $p = 0.03$). This outcome implies that the slope for the lower extract dose is closer to the negative control than to the standard antidiarrheal drug, whereas mid and high extract doses show nonsignificant differences with loperamide, suggesting a more comparable degree of stool-weight reduction. Although differences among the three extract groups: low dose, mid dose and high dose are uniformly nonsignificant, the numerical trend indicates that mid and high doses are closer to loperamide in terms of slowing diarrhea progression.

- *Stool Frequency Over Time*

To further investigate whether the ethanolic rhizome extract of *Z. zerumbet* exhibits antidiarrheal activity in a Castor Oil–Induced Diarrhea (COID) model, stool frequency (i.e., the number of defecations per observation period) was analyzed using a Poisson Regression approach. By treating time as a continuous predictor, the model estimates how the incidence rate (frequency of stools) changes over time for each treatment group, relative to the negative control. Table 16 presents findings into estimated time trends for each group.

Table 16 Estimated Trends (Slopes) of Log Incidence Rate (Stool Rate) Over Time by Treatment

Treatment	Time Trend (Slope)	SE	t-value	p-value
Negative Control	0.18	0.09	1.90	0.06
Positive Control	–0.52	0.20	–2.60	0.01
Extract Low	0.10	0.10	0.93	0.35
Extract Mid	–0.06	0.13	–0.51	0.61
Extract High	–0.08	0.17	–0.50	0.62

For the time-dependent trend of each treatment, Table 16 translates the log(IRR) interactions into simple slope estimates. Although it falls short of statistical significance, the negative control's slope (0.18, p =0.06) is consistent with an increasing stool frequency over time. The positive control, on the other hand, shows a negative slope (–0.52, p =0.01), indicating a significant decrease in stool frequency over time. Among the groups treated with *Z. zerumbet* extracts, the low dose concentration resulted in a small positive slope (0.10, p = 0.35), indicating continued but non- significant increases in stool frequency. In contrast, both the mid (–0.06, p = 0.61) and high (–0.08, p = 0.62) extract groups showed negative trends, albeit non-significant, pointing to a potential dose-related reduction in stool frequency. Figure 10 graphically illustrates estimated marginal means of stool rate over time by treatment.

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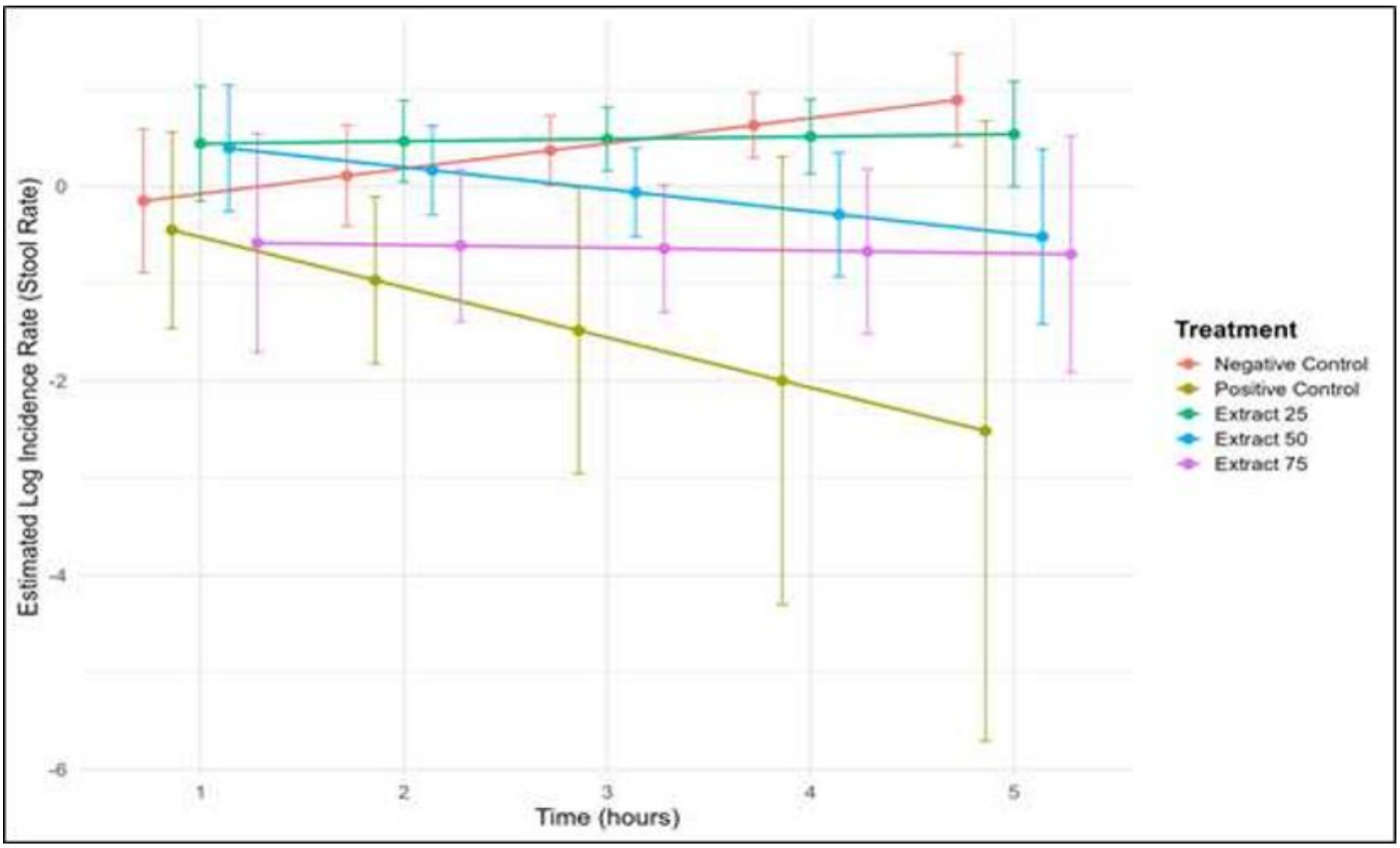

Fig 10 Estimated Marginal Means of Log Incidence Rate (Stool Rate) Over Time by Treatment.

Table 17 Pairwise Comparisons of the Slopes of Log Incidence Rate (Stool Rate) Over Time Between Treatment Groups (Bonferroni Adjusted).

Comparison	Mean log(IRR) Difference	SE	t-value	Adj. p- value	Remark
Negative Control – Positive Ctrl	0.70	0.22	3.15	0.02	Significant
Negative Control – Extract Low	0.08	0.14	0.58	1.00	—
Negative Control – Extract Mid	0.24	0.16	1.53	1.00	—
Negative Control – Extract High	0.26	0.19	1.36	1.00	—
Positive Control – Extract Low	–0.62	0.23	–2.73	0.06	—
Positive Control – Extract Mid	–0.46	0.24	–1.93	0.54	—
Positive Control – Extract High	–0.44	0.26	–1.68	0.93	—
Extract Low – Extract Mid	0.16	0.16	0.98	1.00	—
Extract Low – Extract High	0.18	0.20	0.91	1.00	—
Extract Mid – Extract High	0.02	0.21	0.09	1.00	—

Table 17 displays the Bonferroni-adjusted pairwise comparisons of the slopes for log incidence rate (stool rate) over time. These comparisons expand on the Poisson Regression findings (Tables 14 and 15) by assessing how each treatment group's rate of change differs from the others. As evidenced, the negative control differs significantly only from the positive control (mean difference = 0.70, $p = 0.02$). This outcome reinforces that loperamide (positive control) is the only treatment definitively reducing stool frequency over time relative to the untreated group.

➢ *Wet Stool Consistency Over Time*

In a further effort to address whether *Z. zerumbet* extract exhibits antidiarrheal effects in a castor oil–induced diarrhea (COID) model, stool consistency was evaluated by counting wet stool events over time. Using a Poisson Regression with time as a continuous predictor, the analysis estimated how each treatment condition diverged from the negative control in terms of wet stool occurrence. Table 18 presents the primary regression coefficients, while Table 19 summarizes the estimated slopes (log incidence rates) for each group.

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Table 18 Regression Estimates from the Poisson Regression Examining the Effects of Time, Treatment, and their Interaction on the Number of Wet-Detected Stool Consistency Events.

Characteristic	Log (IRR)	95% CI	p-value
Main Effects			
Time (slope for Negative Control)	0.26	[0.01, 0.52]	0.045
Treatment (compared to Negative Control)			
– Positive Control	0.48	[–1.60, 2.50]	0.60
– Extract Low	0.83	[–0.42, 2.10]	0.20
– Extract Mid	1.00	[–0.30, 2.40]	0.13
– Extract High	–0.14	[–2.10, 1.60]	0.90
Interaction (Difference in Slopes vs. Negative Control)			
Time × Positive Control	–0.78	[–1.90, 0.05]	0.11
Time × Extract Low	–0.24	[–0.58, 0.11]	0.20
Time × Extract Mid	–0.49	[–0.91, –0.09]	0.019
Time × Extract High	–0.29	[–0.85, 0.26]	0.30

The negative control group shows a statistically significant positive slope of 0.26 (p = 0.045), meaning that the rate of wet stool detection increases over time in untreated, castor oil–challenged mice. Conversely, the positive control (loperamide) has a negative time interaction (Time × Positive Control = –0.78), though this effect does not reach statistical significance (p = 0.11). Thus, while loperamide typically reduces diarrhea severity, the current data do not demonstrate a robust decline in wet stool detection over time compared to the negative control.

Among the *Z. zerumbet* extract doses, the Time × Extract Mid term emerges as noteworthy (β = –0.49, p = 0.019). The analysis displays that the middle extract dose significantly deviates from the negative control in how wet stool events accumulate over time. The middle extract shows a notable decline in trajectory, reducing the incremental rate by nearly half a log-IRR unit, while the negative control follows an increasing trend (slope = 0.26). In contrast, the Time × Extract Low and Time × Extract High interactions do not qualify for statistical significance, which suggests that compared to negative control these doses do not alter wet stool consistency. Interestingly, the Low extract shows a slight upward trend, hinting a mild yet nonsignificant in wet stool events.

- *Estimated Time Trends by Treatment*

Table 19 Estimated Trends (Slopes) of Log Incidence Rate (Wet Consistency Detection Rate) Over Time by Treatment

Treatment	Time Trend (Slope)	SE	t-value	p-value
Negative Control	0.26	0.13	2.01	0.04
Positive Control	–0.52	0.47	–1.09	0.27
Extract Low	0.02	0.12	0.20	0.84
Extract Mid	–0.23	0.16	–1.39	0.16
Extract High	–0.03	0.25	–0.12	0.91

Table 19 presents the estimated slopes for the log incidence rate of wet stool consistency detection over time across treatment groups. The negative control group exhibited a statistically significant positive slope (0.26, p = 0.04), indicating a progressive increase in wet stool detection, reflective of persistent diarrhea. In contrast, the positive control (–0.52, p = 0.27) and extract-treated groups (Extract Low: 0.02, p = 0.84; Extract Mid: –0.23, p = 0.16; Extract High: –0.03, p = 0.91) showed negative or near-zero slopes, none of which reached statistical significance. These results suggest that, while untreated mice experienced a worsening in stool consistency over time, neither loperamide nor the *Z. zerumbet* extracts produced a significant attenuation in wet stool frequency within the study period. Figure 11 graphically illustrates the Stool Wet Consistency Rate over time by treatment.

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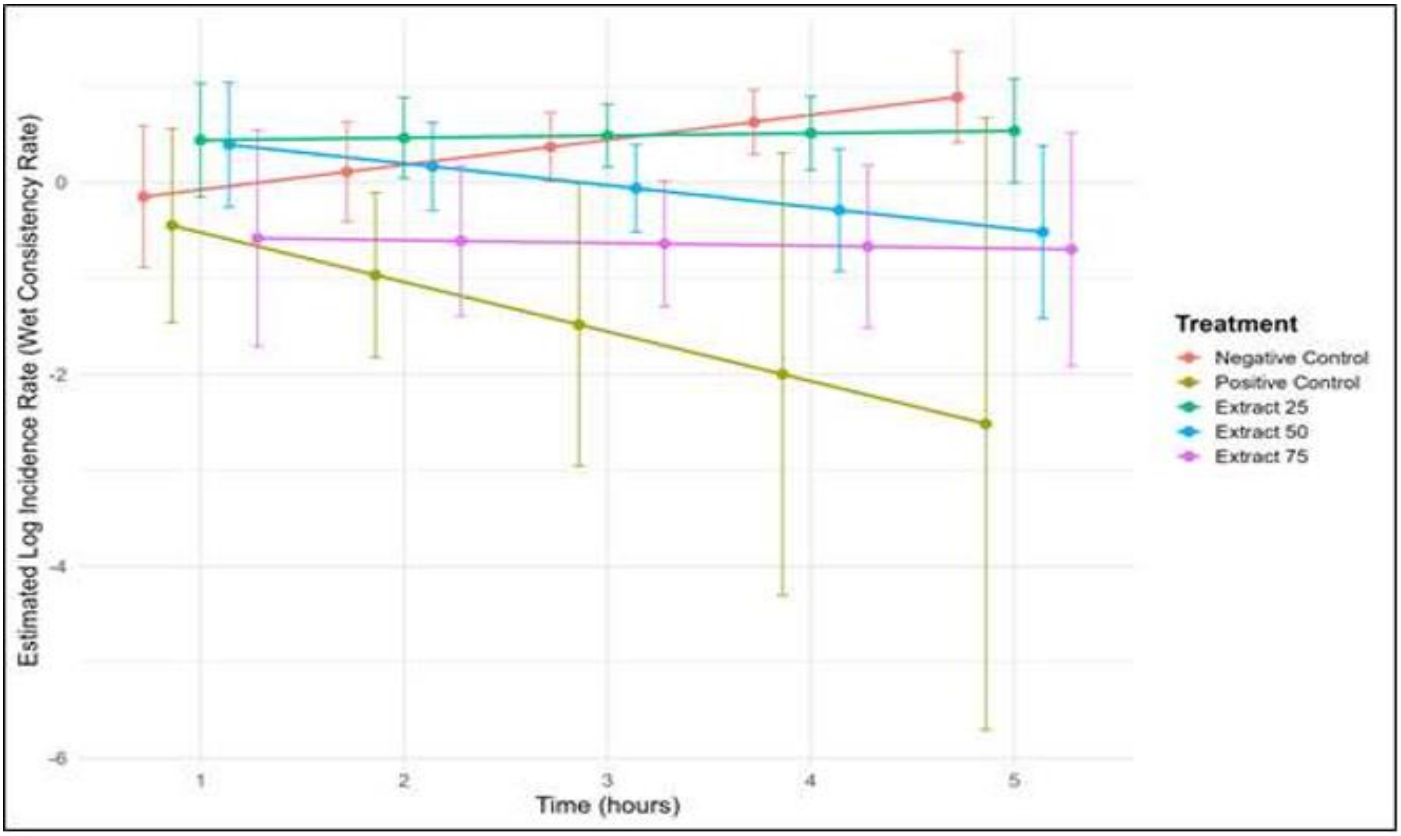

Fig 11 Estimated Marginal Means of Log Incidence Rate (Stool Wet Consistency Rate) Over Time by Treatment.

- *Pairwise Comparisons of Wet Consistency Slopes*

Table 20 Pairwise Comparisons of the Slopes of Log Incidence Rate (Wet Consistency Detection Rate) Over Time Between Treatment Groups (Bonferroni Adjusted).

Comparison	Mean log(IRR) Difference	SE	t-value	Adj. p- value	Remark
Negative Control – Positive Ctrl	0.78	0.49	1.59	1.00	—
Negative Control – Extract Low	0.24	0.18	1.34	1.00	—
Negative Control – Extract Mid	0.49	0.21	2.34	0.19	—
Negative Control – Extract High	0.29	0.28	1.03	1.00	—
Positive Control – Extract Low	–0.54	0.49	–1.11	1.00	—
Positive Control – Extract Mid	–0.29	0.50	–0.58	1.00	—
Positive Control – Extract High	–0.49	0.53	–0.92	1.00	—
Extract Low – Extract Mid	0.25	0.20	1.25	1.00	—
Extract Low – Extract High	0.05	0.27	0.19	1.00	—
Extract Mid – Extract High	–0.20	0.30	–0.67	1.00	—

Table 20 presents Bonferroni-adjusted pairwise comparisons of the slopes representing changes in the log incidence rate of wet stool consistency over time across treatment groups. Despite preliminary indications from Poisson regression that the middle dose of *Z. zerumbet* extract may reduce wet stool detection relative to the negative control, none of the pairwise contrasts reached statistical significance following correction for multiple comparisons. The observed difference between the negative control and Extract Mid (mean log(IRR) difference = 0.49, p = 0.19) fell short of significance under the stringent Bonferroni adjustment. These findings underscore the statistical conservatism of post hoc testing and indicate that, while mid-dose extract treatment may exhibit trends toward improved stool consistency, the effects are not yet robust enough to confirm a differential impact across groups with high confidence.

➢ *Stool Emission Frequency*

Another key measure for evaluating antidiarrheal efficacy in the castor oil– induced diarrhea (COID) model is stool emission frequency, reflecting how many times each mouse defecates over a specified period. Table 21 details the overall one-way ANOVA results for stool emission frequency across the negative control, positive control, and three *Z. zerumbet* extract groups while Table 22 presents the post-hoc Tukey HSD comparisons.

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- *One-Way ANOVA Results*

Table 21 Summary of One-Way ANOVA for Stool Emission Frequency Across Treatment Groups.

Source	df	Sum of Squares	Mean Square	F	p-value	Partial η²	Remark
Treatment	4	11.18	2.79	21.04	0.00	0.81	Significant
Residuals	20	2.66	0.13				

A one-way ANOVA yielded a highly significant effect of treatment on stool emission frequency, $F(4, 20) = 21.04$, $p < 0.001$, partial $\eta^2 = 0.81$ (Table 21). This indicates that 81% of the variance in how frequently the mice defecate can be attributed to the assigned treatments. Such a large effect size is consistent with earlier observations in which both the standard antidiarrheal (loperamide) and the higher doses of *Z. zerumbet* extract demonstrated meaningful reductions in diarrhea-related outcomes.

Table 22 Tukey HSD Pairwise Comparisons for Stool Emission Frequency Across Treatment Groups

Comparison	Mean Difference	95% CI	Adj. p- value	Remark
Positive Ctrl – Negative Ctrl	–1.72	[–2.41, –1.03]	0.00	Significant
Extract Low – Negative Ctrl	–0.52	[–1.21, 0.17]	0.20	—
Extract Mid – Negative Ctrl	–1.16	[–1.85, –0.47]	0.00	Significant
Extract High – Negative Ctrl	–1.68	[–2.37, –0.99]	0.00	Significant
Extract Low – Positive Ctrl	1.20	[0.51, 1.89]	0.00	Significant
Extract Mid – Positive Ctrl	0.56	[–0.13, 1.25]	0.15	—
Extract High – Positive Ctrl	0.04	[–0.65, 0.73]	1.00	—
Extract Mid – Extract Low	–0.64	[–1.33, 0.05]	0.08	—
Extract High – Extract Low	–1.16	[–1.85, –0.47]	0.00	Significant
Extract High – Extract Mid	–0.52	[–1.21, 0.17]	0.20	—

Table 22 provides the Tukey HSD pairwise comparisons. Several key differences stand out:

- ✓ **Negative Control vs. Positive Control.** The mice receiving loperamide defecated significantly less often, with a mean difference of –1.72 (95% CI [–2.41, –1.03], $p < 0.001$).
- ✓ **Negative Control vs. Extracts.** The middle dose (–1.16, $p < 0.001$) and high dose (–1.68, $p < 0.001$) extracts both show significant reductions in stool emission frequency compared to the negative control. However, the low dose extract (–0.52, $p = 0.20$) does not significantly differ from the negative control, implying that lower doses may be insufficient to elicit a robust antidiarrheal effect.
- ✓ **Extract Low vs. Positive Control.** The low extract dose group emitted stool significantly more often than the positive control ($p < 0.001$), confirming that this lower dose fails to match the standard drug's effectiveness.
- ✓ **Extract High vs. Extract Low.** The high extract dose displayed a meaningfully lower stool emission frequency (–1.16, $p < 0.001$), reinforcing that high concentrations of *Z. zerumbet* are more efficacious than minimal doses.
- ✓ **Comparisons Among Positive Control, Extract Mid, and Extract High.** No significant difference emerged in these pairwise tests, suggesting that the middle and high doses extracts approach the antidiarrheal performance of loperamide in terms of stool emission frequency.

➢ *Percentage Inhibition*

In percentage inhibition, it offers an additional metric for gauging the antidiarrheal efficacy of each treatment in the castor oil–induced diarrhea (COID) model. This measure typically reflects how much each intervention (e.g., loperamide or the *Z. zerumbet* extract) mitigates diarrhea-related parameters relative to a defined baseline—often the negative control. Table 23 summarizes the results of a one-way ANOVA for percentage inhibition, while Tables 24 and 25 present descriptive statistics and post-hoc comparisons, respectively.

- *One-Way ANOVA Results of Percentage Inhibition*

Table 23 Summary of One-Way ANOVA for Percentage Inhibition Across Treatment Groups

Source	df	Sum of Squares	Mean Square	F	p-value	Partial η²	Remark
Treatment	3	210.87	70.29	15.09	0.00	0.74	Significant
Residuals	16	74.51	4.66				

The one-way ANOVA results in Table 23 show a significant effect of treatment on percentage inhibition, with the positive control (loperamide) and *Z. zerumbet* extracts demonstrating varying levels of efficacy. Loperamide, as the positive control, significantly reduced stool emission frequency, leading to a strong inhibition. The higher concentrations of *Z. zerumbet* extracts (mid and high doses) achieved substantial inhibition, with the high dose extract showing the highest effect (mean percentage inhibition significantly greater than the low dose extract), and the mid dose extract also showing strong efficacy. Specifically, the high dose

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extract exhibited a mean inhibition of 1.68 compared to the negative control, and the mid dose extract displayed a mean inhibition of 1.16. These results suggest that the *Z. zerumbet* extracts, particularly at higher concentrations, exhibit antidiarrheal activity comparable to loperamide. Conversely, the low dose extract showed a mean inhibition of 0.52, which did not significantly differ from the negative control, indicating a less potent effect at lower doses. This highlights the dose-dependent efficacy of *Z. zerumbet* extracts in mitigating diarrhea.

Table 24 Descriptive Statistics for Percentage Inhibition by Treatment Group in Castor Oil-Induced Diarrhea Model

Treatment	n	Mean	SD	SE
Negative Control	5	—	—	—
Positive Control	5	72.88	1.70	0.76
Extract Low	5	29.72	3.51	1.57
Extract Mid	5	48	1.52	0.68
Extract High	5	62.61	1.05	0.47

Table 24 reports means and standard deviations for the positive control and the three extract concentrations. Loperamide (positive control) exhibits the highest mean percentage inhibition (72.88%), underscoring its well-known efficacy in halting diarrhea progression. Among the extracts, the high dose ranks next (62.61%), followed by the mid dose (48%) and the low dose (29.72%). Although no direct data appear for the negative control, the observed hierarchy is consistent with the premise that higher extract concentrations produce stronger antidiarrheal effects. Figure 12 displays the graph of percentage inhibition by treatment group in a Castor Oil-Induced Diarrhea model.

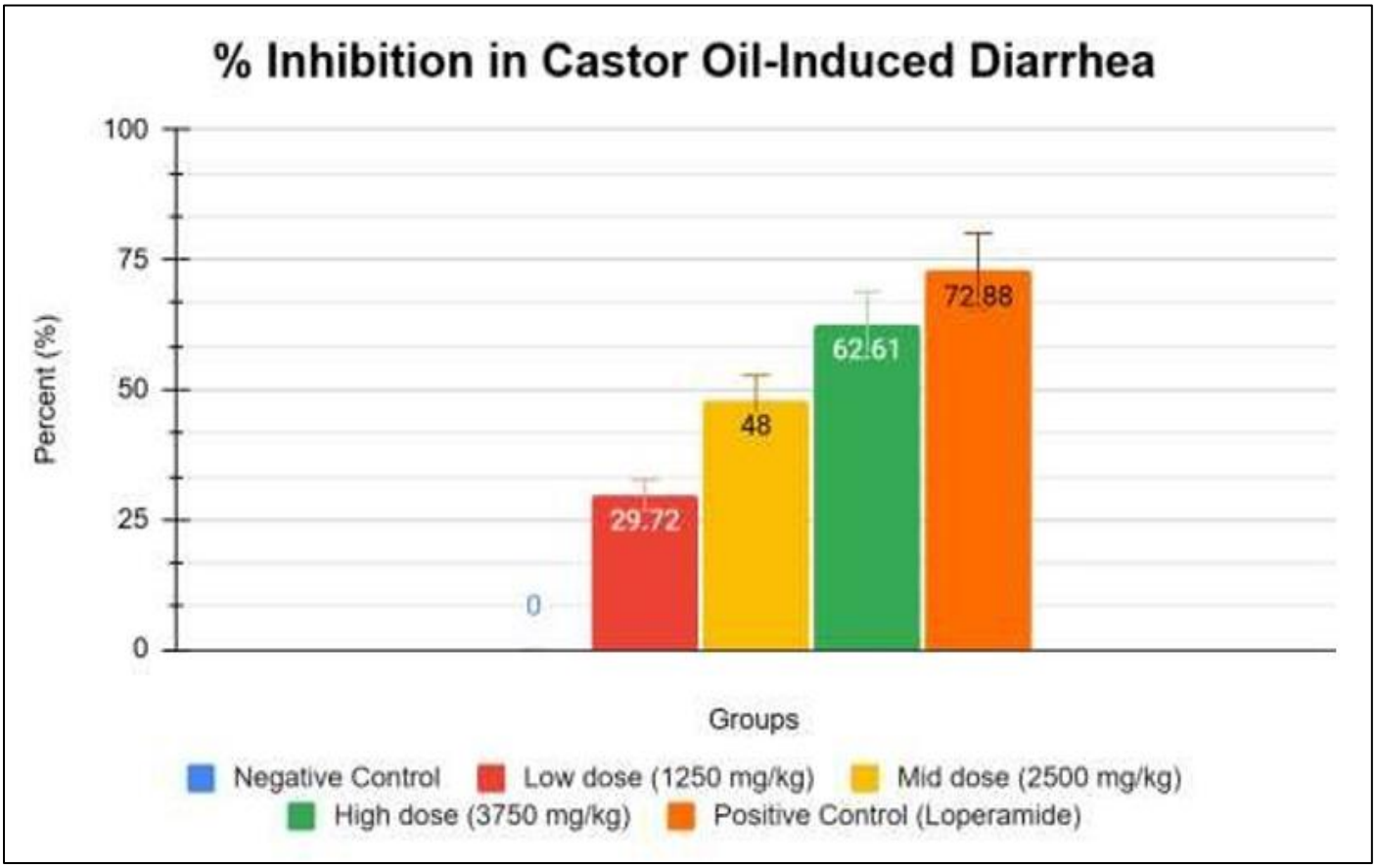

Fig 12 Percentage Inhibition of Diarrhea Across Treatment Groups in Castor Oil- Induced Diarrhea Model.

Table 25 Tukey HSD Pairwise Comparisons for Percentage Inhibition Across Treatment Groups

Comparison	Mean Difference	95% CI	Adj. p- value	Remark
Extract Low–Positive Ctrl	–8.63	[–12.54, –4.73]	0.00	Significant
Extract Mid–Positive Ctrl	–4.98	[–8.88, –1.07]	0.01	Significant
Extract High–Positive Ctrl	–2.05	[–5.96, 1.85]	0.46	—
Extract Mid–Extract Low	3.65	[–0.25, 7.56]	0.07	—
Extract High–Extract Low	6.58	[2.67, 10.48]	0.00	Significant
Extract High–Extract Mid	2.93	[–0.98, 6.83]	0.18	—

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The Tukey HSD post-hoc results (Table 25) help clarify how each extract concentration stacks up against the positive control and against each other.

- ✓ **Extracts vs. Positive Control.** Although all three extract doses produce high inhibition scores, Extract Low and Extract Mid differ significantly from loperamide (adjusted $p < 0.05$), indicating that these doses are statistically lower in inhibition than the standard drug. However, Extract High (mean difference = –2.05, $p = 0.46$) does not differ from the positive control, suggesting that this higher dose reaches an efficacy level akin to loperamide in suppressing diarrheal symptoms.
- ✓ **Dose–Dose Comparisons Among Extracts.** Extract High is significantly higher in inhibition than Extract Low (mean difference = 6.58, $p < 0.001$). The difference between Extract Mid and Extract Low (3.65, $p = 0.07$) trends toward significance but does not meet the adjusted threshold. Meanwhile, Extract High does not significantly differ from Extract Mid, implying that both concentrations deliver comparable, near-loperamide efficacy in percentage inhibition.

F. Gastrointestinal Motility Assessment Results

An essential component of evaluating gastrointestinal motility in this study is the measurement of total intestinal length distance after treatment administration, along with the distance traveled by the charcoal meal within the intestines as another important indicator. Together, these assessments provide valuable insights into the physiological response of the gastrointestinal tract under different interventions, addressing the study's fifth objective on how the ethanolic rhizome extract of *Z. zerumbet* (Zingiberaceae) affects gut function. The charcoal meal test, a widely accepted method for evaluating bowel transit speed and motility, was used to determine how treatments influenced intestinal movement (Evangelista 2013; Manjunath et al. 2011).

➢ *Distance Covered by Charcoal*

- *One-Way Analysis of Variance Results*

Table 26 One-Way Analysis of Variance for Distance Covered by Charcoal (cm) Among Treatment Groups

Source	df	Sum of Squares	Mean Square	F	p-value	Partial η²	Remark
Treatment	4	1652.49	413.12	9.81	0.00	0.53	Significant
Residuals	20	842.26	42.11				

An additional measure of gastrointestinal motility involved recording the distance traveled by the charcoal meal within the intestines. As summarized in Table 26, the one-way ANOVA yielded a statistically significant effect of treatment on the distance covered by charcoal, $F(4, 20) = 9.81$, $p < 0.001$, partial $\eta^2 = 0.53$. This partial η^2 value indicates a large effect size, implying that more than half of the variance in charcoal transit distance can be explained by the differences among the various treatment groups (i.e., negative control, positive control, and the three dosages of *Z. zerumbet* extract).

- *Descriptive Statistics for Distance Covered by Charcoal*

Table 27 Descriptive Statistics for Distance Covered by Charcoal (cm) by Treatment Group

Treatment	n	Mean	SD	SE
Negative Control	5	35.40	3.97	1.77
Positive Control	5	14.70	9.17	4.10
Extract Low	5	34.82	4.72	2.11
Extract Mid	5	28.32	4.74	2.12
Extract High	5	20.20	8.12	3.63F

Legend: Low: 1250 mg/kg Mid: 2500 mg/kg High: 3750 mg/kg

Table 27 provides the descriptive statistics for the distance (cm) that the charcoal meal traveled under each treatment condition. The negative control exhibited the greatest mean distance (35.40 cm), followed closely by the low extract group (34.82 cm). By contrast, the positive control (loperamide) evidenced the smallest mean distance (14.70 cm), implying markedly reduced gastrointestinal motility relative to all other groups. The mid extract group showed a moderate reduction in charcoal transit (28.32 cm), while the high extract group demonstrated a further reduction (20.20 cm).

- *Post Hoc Comparisons for Distance Covered by Charcoal*

Table 28 Tukey HSD Pairwise Comparisons for Distance Covered by Charcoal (cm) Across Treatment Groups

Comparison	Mean Difference	95% CI	Adj. p-value	Remark
Positive Control – Negative Ctrl	-20.70	[-32.98, -8.42]	0.00	Significant
Extract Low – Negative Ctrl	-0.58	[-12.86, 11.70]	1.00	—
Extract Mid – Negative Ctrl	-7.08	[-19.36, 5.20]	0.44	—
Extract High – Negative Ctrl	-15.20	[-27.48, -2.92]	0.01	Significant

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Extract Low – Positive Ctrl	20.12	[7.84, 32.40]	0.00	Significant
Extract Mid – Positive Ctrl	13.62	[1.34, 25.90]	0.03	Significant
Extract High – Positive Ctrl	5.50	[-6.78, 17.78]	0.67	—
Extract Mid – Extract Low	-6.50	[-18.78, 5.78]	0.52	—
Extract High – Extract Low	-14.62	[-26.90, -2.34]	0.01	Significant
Extract High – Extract Mid	-8.12	[-20.40, 4.16]	0.31	—

Legend: Low: 1250 mg/kg Mid: 2500 mg/kg High: 3750 mg/kg

The Tukey HSD test results (Table 28) confirm that both the low extract and the negative control are statistically similar (p = 1.00). In contrast, the high extract condition shows a significantly shorter transit distance than the negative control (mean difference = -15.20, p = 0.01), indicating reduced motility at higher dosages. Moreover, the high extract does not differ significantly from the positive control (p = 0.67), suggesting that at this higher dose, *Z. zerumbet* approximates the motility-slowing effects of loperamide.

➢ *Percent Inhibition*

- *One-Way Analysis of Variance Results*

Table 29 One-Way Analysis of Variance for Percent Inhibition Across Treatment Groups

Source	df	Sum of Squares	Mean Square	F	p-value	Partial η^2	Remark
Treatment	4	7727.79	1931.95	10.10	0.00	0.53	Significant
Residuals	20	3825.83	191.29				

The one-way ANOVA results, as detailed in Table 29, indicate a significant effect of treatment on percentage inhibition, $F(4, 20) = 10.10$, $p < 0.001$, with a partial $\eta^2 = 0.53$. This suggests that 53% of the variation in inhibition rates is attributable to the administered treatments. The findings highlight the strong influence of both loperamide (positive control) and *Z. zerumbet* extract at different concentrations on intestinal motility. The pronounced differences among treatment groups reinforce the extract's potential antidiarrheal properties and suggest a dose-dependent impact on gastrointestinal transit.

- *Descriptive Statistics for Percentage Inhibition*

Table 30 Descriptive Statistics for Percent Inhibition by Treatment Groups

Treatment	n	Mean	SD	SE
Negative Control	5	14.92	8.47	3.79
Positive Control	5	64.71	20.13	9.00
Extract Low	5	32.99	5.27	2.36
Extract Mid	5	38.99	12.10	5.41
Extract High	5	56.39	17.48	7.82

Table 29 displays the mean percentage inhibition across all treatment groups, providing insight into how effectively each condition can reduce or slow intestinal transit. The positive control (loperamide) reveals the highest mean inhibition (64.71%), whereas the negative control (distilled water) exhibits the lowest (14.92%). Notably, the high extract group yields a relatively high mean (56.39%), placing it close to the positive control's effect, while the low and mid groups register more moderate inhibitions (32.99% and 38.99%, respectively). Figure 13 below shows the graph of percentage inhibition of diarrhea across all treatment groups in the gastrointestinal motility model.

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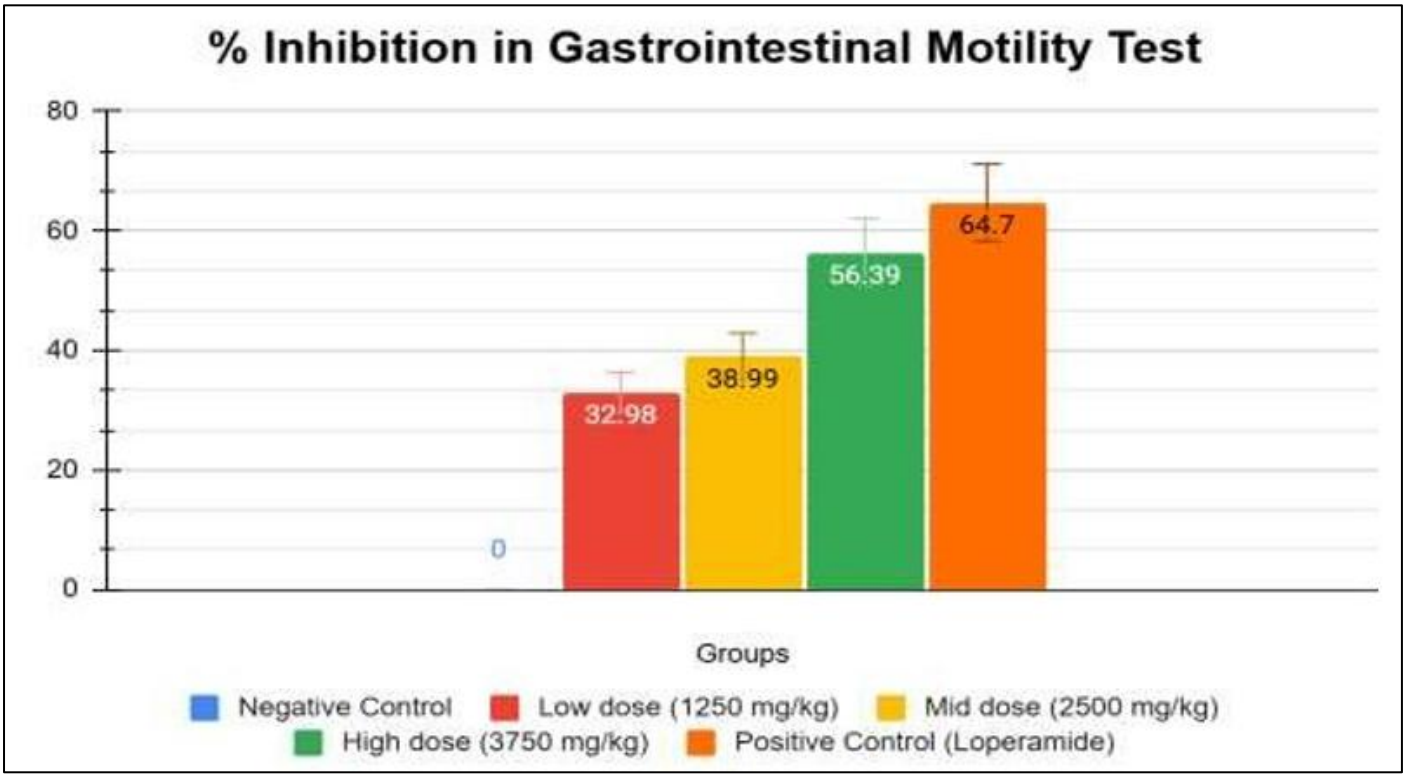

Fig 13 Percentage Inhibition of Diarrhea Across All Treatment Groups in Gastrointestinal Motility Test.

- *Post Hoc Comparisons for Percentage Inhibition*

Table 31 Tukey HSD Pairwise Comparisons for Percentage Inhibition Across Treatment Groups

Comparison	Mean Difference	95% CI	Adj. p-value	Remark
Positive Control – Negative Ctrl	49.79	[23.61, 75.97]	0.00	Significant
Extract Low – Negative Ctrl	18.07	[-8.10, 44.25]	0.27	—
Extract Mid – Negative Ctrl	24.08	[-2.10, 50.25]	0.08	—
Extract High – Negative Ctrl	41.47	[15.30, 67.65]	0.00	Significant
Extract Low – Positive Ctrl	-31.72	[-57.89, -5.54]	0.01	Significant
Extract Mid – Positive Ctrl	-25.71	[-51.89, 0.46]	0.06	—
Extract High – Positive Ctrl	-8.32	[-34.49, 17.86]	0.87	—
Extract Mid – Extract Low	6.00	[-20.17, 32.18]	0.96	—
Extract High – Extract Low	23.40	[-2.78, 49.57]	0.09	—
Extract High – Extract Mid	17.40	[-8.78, 43.57]	0.31	—

The Tukey HSD post hoc analysis (Table 31) indicates that the high extract group and positive control both demonstrate significantly greater inhibition relative to the negative control (p = 0.00). These findings parallel the pattern seen in descriptive statistics: the high dose closely approximates the inhibitory effect of loperamide (56.39% vs. 64.71% means, respectively), and their difference does not reach statistical significance (p = 0.87). The low extract group shows a significant difference from the positive control (p = 0.01) but remains statistically similar to the negative control, indicating that lower doses provide moderate inhibition without fully replicating the effect of standard treatments. Additionally, the comparison between the high and low extract groups results in a non-significant finding (p = 0.09), though it trends toward significance. This pattern suggests that higher doses may approach the efficacy of established antidiarrheal agents, while lower concentrations yield only partial inhibitory effects.

➢ *Discussion*

This section presents the summary and findings of the research study on the antidiarrheal activity of the ethanolic rhizome extract. The extract was prepared through maceration in 90% ethanol, followed by solvent evaporation. The color was initially golden-yellow, yet after drying it transformed into an orange-brown, semi-solid residue. The extraction yielded 5±1.22% from five 10-gram powdered rhizome samples based on triplicate analysis, which indicated consistent recovery of phytoconstituents.

The presence of bioactive compounds known for their pharmacological actions was discovered during the phytochemical screening. A dark brown precipitate was produced during Wagner's test for alkaloids (Shaikh and Patil 2020) confirming the presence of alkaloids; flavonoids were confirmed by the Shinoda test, which yielded a reddish-brown coloration (Sharma et al.

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2020); phenols and tannins were identified using the ferric chloride test — resulting in deep blue and light yellow-brown colors respectively (Ramadanil et al. 2019); using froth test, saponins were confirmed due to persistent froth formation for 5 minutes (Preshahdin et al. 2023). Salkowski's test was used to confirm the presence of terpenoids which resulted in a reddish-brown color (Ramzan et al. 2023), and Liebermann-Burchard test for steroids with a formation of brown rings at the junction of two layers and turning the upper layers green (Iqbal et al. 2015).

In accordance with OECD 423 Guidelines, an Acute Toxicity Test was conducted which resulted in no mortality or adverse behavioral effects in mice administered up to 5000 mg/kg of the extract. Hence, the extract falls within a favorable safety profile under Category 5 (Unclassified) in the Globally Harmonized Classification System.

The castor-oil induced diarrhea test was employed to evaluate *in vivo* antidiarrheal activity (Fokam Tagne et al. 2019). While the interpretation already demonstrated statistically significant reductions in stool weight and emission frequency for the mid and high doses of *Zingiber zerumbet* extract, a broader look at pharmacodynamic trends reveals a consistent dose-response relationship across all major endpoints—aggregated stool weight, stool emission, wet consistency, and percent inhibition. For the aggregated stool weight over time, 50% and 75% of the extract demonstrated a statistically significant difference, with slopes 16 mg ($p < .001$) and 15 mg ($p < .001$) lower than the set determinant which is <0.05. Both extracts also showed slightly negative slopes (-2.28 mg and -1.48 mg per time unit), indicating reduction in stool accumulation. Similar trends were observed in stool frequency, wet stool consistency over time, and in stool emission frequency, where 50% and 75% extracts exhibited inhibition of diarrhea. These findings highlight the dose-dependent efficacy of *Z. zerumbet* extracts in mitigating diarrhea, with the numerical trend indicating that 50% and 75% extract concentrations approach the effectiveness of loperamide in slowing diarrhea progression. The antidiarrheal activities observed in this method may be attributed to the presence of bioactive compounds such as flavonoids and tannins, which have been shown to inhibit intestinal motility and secretion (Kharal et al. 2023); Additionally, a similar study mentioned that a reduced water and electrolyte secretion into small intestine was observed which may due to the secondary metabolite such as alkaloids (Gudeta et al. 2020) and terpenoids which prevent the release of autocoids and prostaglandins hence preventing secretion and peristalsis caused by Castor oil (Kifle et al. 2021).

In the gastrointestinal motility test, the first parameter considered was the distance covered by the charcoal meal within the intestines (Rahman et al. 2015). As summarized in Table 25, the one-way ANOVA yielded a statistically significant effect of treatment on the distance covered by charcoal, $F(4, 20) = 9.81$, $p < .001$, partial $\eta^2 = 0.53$. Table 26 provides the descriptive statistics for the distance (in centimeters) that the charcoal meal traveled under each treatment condition. The negative control exhibited the greatest mean distance (35.40 cm), followed closely by the low dose extract (34.82 cm). By contrast, the positive control (loperamide) evidenced the smallest mean distance (14.70 cm), implying markedly reduced gastrointestinal motility relative to all other groups. The mid dose extract showed a moderate reduction in charcoal transit (28.32 cm), while the high dose extract demonstrated a further reduction (20.20 cm). This pattern aligns with past findings indicating that members of the Zingiberaceae family can modulate bowel motility in a dose-dependent manner (Kifle et al., 2021; Tiwari et al., 2011).

Additionally, the percentage inhibition of diarrhea was calculated specifically in the gastrointestinal motility test to compare which concentrations have the robust influence in inhibiting intestinal transit (Tafesse and Mekonnen 2012). The mean percentage inhibition across all treatment groups, provides insight into how effectively each condition can reduce or slow intestinal transit. Additionally, the percentage inhibition of diarrhea by treatment groups shows that positive control has the strongest inhibition of 64.71%, followed by high dose of extract 56.39%, mid dose having 38.99%, while the low dose 32.99% showing the least effect in inhibiting diarrhea. This variation indicates a dose-dependent relationship, in which higher concentrations of *Z. zerumbet* extract produce greater inhibition. This pattern also aligns with prior literature cited in Chapter 2, suggesting that key phytochemical constituents—especially terpenoids, flavonoids, and saponins—can influence gastrointestinal smooth muscle and peristalsis (Tiwari et al., 2011; Assiry et al., 2023). Specifically, moderate to higher doses of bioactive compounds in the Zingiberaceae family frequently elicit stronger antidiarrheal or antimotility effects, consistent with the observed elevation from low dose to high dose of the extract.

These findings suggest that *Z. zerumbet* extract—especially at higher concentrations—possesses significant antidiarrheal potential by modulating intestinal motility, reducing stool frequency, decreasing aggregated stool weight over time, and increasing percent inhibition. These results support its potential use as a natural therapeutic agent for managing diarrhea.

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CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

- *Conclusions*

This study aimed to evaluate the antidiarrheal effects of the ethanolic rhizome extract of *Zingiber zerumbet* through *in vivo* models. The extract was consistently obtained through ethanol maceration followed by solvent evaporation, with confirmation of multiple bioactive phytoconstituents relevant to gastrointestinal pharmacology. Acute toxicity assessment demonstrated the extract's safety at high doses, with no adverse effects observed in test subjects. Pharmacological evaluation using castor oil-induced diarrhea and gastrointestinal motility tests revealed that the extract significantly decreased diarrhea frequency, improved stool consistency, and inhibited intestinal transit compared to controls. These results provide strong evidence to reject the null hypotheses, which posited that the extract would not significantly affect diarrhea parameters or gastrointestinal motility relative to loperamide. Conversely, the findings support acceptance of the alternative hypotheses that the ethanolic rhizome extract of *Z. zerumbet* exerts a dose-dependent antidiarrheal effect comparable to the standard drug. Overall, the results demonstrate that the ethanolic rhizome extract exhibits significant efficacy and a favorable safety profile as a natural antidiarrheal agent, justifying further research to clarify its pharmacological mechanisms and support its development for clinical application.

- *Recommendation*

The findings of this study reveal that *Z. zerumbet* rhizome extract has the potential to inhibit diarrhea. The researchers thereby recommend the use of other plant parts of *Z. zerumbet* for antidiarrheal activity, for example, the leaves where it is traditionally used to produce essential oils and reported to have alkaloids, flavonoids and terpenoids which can all contribute to antidiarrheal effects (Chan et al. 2017).

Additionally, they can evaluate antidiarrheal properties of *Z. zerumbet* by considering additional models such as anti-enterpooling tests to enhance support for the study. While this study shows the ZZRE has antidiarrheal activity, a further study should be conducted to isolate, purify, and identify bioactive principles responsible for the antidiarrheal activities to better understand which specific compound contributes the most to the therapeutic effect which is critical for potential safety and drug development (Susanti et al. 2024).

The researchers also recommend to further study the essential oils on the rhizome of *Z. zerumbet* due to the result of phytochemical analysis conducted on this study showing that it contains a strong amount of essential oil which can inhibit the release of autocoids and prostaglandins (Tiwari et al. 2017).

Due to the limitations, the researchers recommend performing antidiarrheal activity tests with various solvent like aqueous, methanol and others to reveal if one extract has a more potent antidiarrheal effect than the current solvent used in this study, ethanol.

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APPENDIX A

Schedule of Activities SCHEDULE OF ACTIVITIES

Thesis Title: The Evaluation of the Antidiarrheal Properties of Ethanolic Rhizome Extract of *Zingiber zerumbet* (Zingiberaceae)
***Project Dates*:** December 2024 to May 2025

RESEARCH OBJECTIVES		2024					2025				
		AUG	SEP	OCT	NO V	DEC	JAN	FEB	MAR	APR	MAY
1	Choosing a Research Topic										
2	Submission of Abstract Proposal										
3	Proposal for Candidate Adviser										
4	Finalization of Research Topic										
5	Writing of Chapter 1: The Problem and Its Background										
6	Writing of Chapter II: Reviews of Related Literature and Studies										
7	Writing of Chapter III: Methodology										
8	Writing of Other Parts of the Proposal Manuscript and Making PowerPoint Presentation for Defense										
9	Proposal Defense Application										
10	Thesis Proposal Hearing										
11	Revision of Proposal Manuscripts According to Thesis Panel Suggestions, Recommendations, and Annotations										
12	Submission of Approved and Signed Proposal Manuscript										
13	Plant Authentication										
14	Plant Collection										
15	Requesting and Purchasing of Chemicals and Reagents to be Used										

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	RESEARCH OBJECTIVES	2024					2025				
		AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
16	Collection and Preparation of *Z. zerumbet* Rhizome										
17	Air drying and Powderization of *Z. zerumbet* Rhizome										
18	Processing of 90% Ethanolic Rhizome Extract of *Z. zerumbet*										
19	Determining Percentage Yield and Conduction of Confirmatory Phytochemical Testings										
20	Testing of Acute Oral Toxicity to Swiss Albino Mice										
21	Testing for Antidiarrheal Activity of Extract using Castor Oil-Induced Diarrhea										
22	Testing for Antidiarrheal Activity of Extract using Gastrointestinal Motility Test										
23	Collecting and Analysis of All Data										
24	Writing of Chapter 4 and Chapter 5 and the rest of the study.										

Legend:

	Done in Proposal Period		Proposed Schedule Upon Proposal Acceptance

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APPENDIX B

Budget Proposal

BUDGET PROPOSAL

Thesis Title: The Evaluation of the Antidiarrheal Properties of Ethanolic Rhizome Extract of *Zingiber zerumbet* (Zingiberaceae)
***Project Dates*:** December 2024 to May 2025

This Budget Proposal provides necessary possible costs that are associated with the above-named research project. The proposed possible cost for the research project is itemized below:

Expenditures	Budget
Oral Defense Presentation Fee	PHP 12,000
Identification and Authentication	PHP 300
Chemical and Reagents	PHP 26,968
Phytochemical Tests	PHP 1,000
Mice	PHP 5,525
Mice Materials	PHP 3,450
Transportation	PHP 2,000
Laboratory Materials	PHP 3,000
Pharmacy Laboratory Fee	PHP 2,000
Statistician's Fee	PHP 4,500
Print	PHP 1500
Plant Collection	PHP 1500
Feeds and distilled water	PHP 1,500
Miscellaneous	PHP 3,500
TOTAL	**PHP 68,243**

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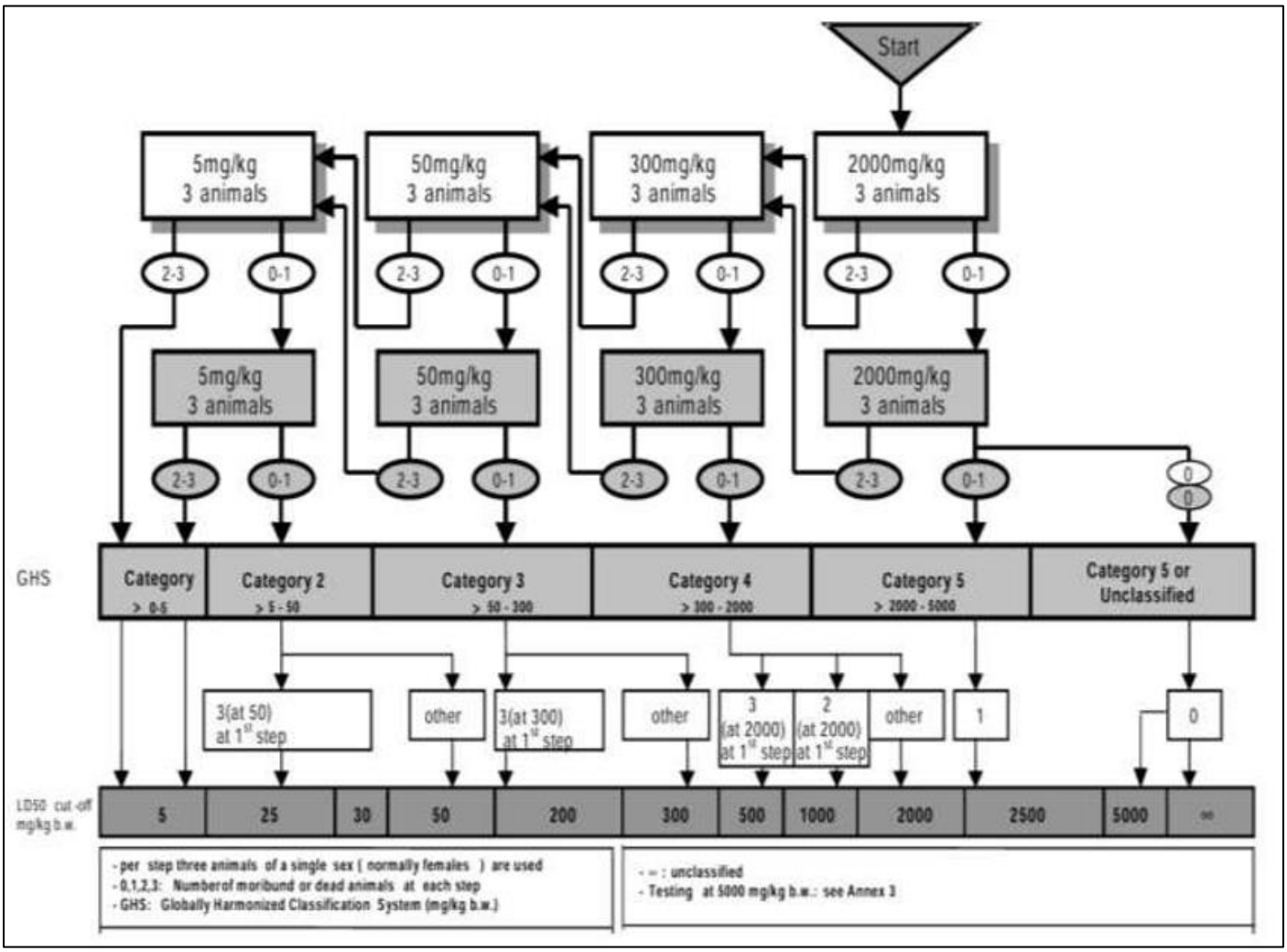
APPENDIX C

Acute oral toxicity testing (OECD 423: Acute Toxic Class Method)

TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



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APPENDIX D

Plant Authentication

DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF SCIENCE AND MATHEMATICS
www.msuit.edu.ph
csm.biosc@g.msuit.edu.ph

CERTIFICATE OF AUTHENTICATION OF BOTANICAL SPECIMENS

This is to certify that the BOTANICAL specimen/s herein listed and presented by the persons/s herein noted were identified and authenticated by this office.

Name: **Johayber M. Macud**
Sophia C. Magbanua
Raudha D. Abdulrauf
Princess Nhurliza S. Ramber
School/Institution: **Adventist Medical Center College – Iligan City**

Family	Scientific Name	Local/Common Name
Zingiberaceae	*Zingiber zerumbet* (L.) Roscoe ex Sm	Wild Ginger, shampoo ginger(Eng), Luiang-usiu(Tag.)

Collected from: Sawir, Masiu, Lanao del Sur
Date of Collection: 11 December 2024

Muhmin Michael E. Manting, MSc.
Assistant Professor
Department of Biological Sciences
MSU-Iligan Institute of Technology

(loc)-4137

Influencing the Future

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APPENDIX E

Ethanol Certificate of Conformance

ChemSupply Australia

Certificate of Conformance

PRODUCT: ETHANOL Undenatured 100% AR

PRODUCT CODE: EA043-2.5L-P

BATCH NO. : 427427 **DATE MANUFACTURED:** 31/05/2024

Product General Information

Properties	Result
Assay	99.5% v/v min.
Colour (APHA)	≤ 10
Solubility in water	Passes test
Residue after evaporation	≤ 0.001%
Acetone, isopropyl alcohol	Passes test
Titrable acid	≤ 0.0005 meq/g
Titrable base	≤ 0.0002 meq/g
Methanol (CH3OH)	≤ 0.1%
Subs. darkened by H2SO4	Passes test
Subs. reducing KMnO4	Passes test
Water (H2O)	≤ 0.2%
Aluminium (Al)	≤ 0.00001%
Barium (Ba)	≤ 0.000002%
Boron (B)	≤ 0.000002%
Cadmium (Cd)	≤ 0.000002%
Calcium (Ca)	≤ 0.0002%
Chromium (Cr)	≤ 0.000002%
Cobalt (Co)	≤ 0.000002%
Copper (Cu)	≤ 0.00005%
Iron (Fe)	≤ 0.0001%

26/09/2024

J. Krieg
Quality and Regulatory Lead

Chem Supply Australia Pty Ltd - An ISO 9001 Accredited Company
38 - 50 Bedford Street, Gillman SA 5013, Australia ABN 19 008 264 211 PO Box 201, Port Adelaide SA 5015, Australia
Telephone +61 8 8440 2000 E-mail: sales@chemsupply.com.au Web: www.chemsupply.com.au

ChemSupply Australia Pty Ltd does not warrant that this product is suitable for any particular use or purpose. The user must ascertain the suitability of the product for any intended purpose. Preliminary testing of the product before use or application is recommended. Any reliance or purported reliance upon ChemSupply Australia Pty Ltd with respect to any skill or judgement or advice in relation to the suitability of this product for any particular purpose is, to the extent permitted by law, disclaimed. Except to the extent prohibited by law, any condition implied by any statute as to the merchantable quality of this product or fitness for any particular purpose is hereby excluded. This product is not sold by description. For clarity, nothing in this document shall be read or applied as to purport to exclude, restrict, or modify or have the effect of excluding, restricting, or modifying the application in relation to the supply of goods or services by ChemSupply Australia Pty Ltd of all or any of the provisions of the Competition and Consumer Act 2010 or any relevant state or federal legislation which by law cannot be excluded, restricted, or modified. Where the purchaser of this product is deemed to be a consumer for the purposes of Schedule 2 of the Competition and Consumer Act 2010 (The Australian Consumer Law), the liability of ChemSupply Australia Pty Ltd is limited to the replacement or supply of equivalent goods or payment of the cost of replacing the goods or acquiring equivalent goods.

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APPENDIX F

Certificate of Analysis

FOREVER

南京九佳科技有限公司

Nanjing Forever Pharmacy Co., Ltd.

地址： 南京市栖霞区新港开发区27号

Certificate of Analysis

检 验 报 告 书

产品名称 Product Name	Loperamide HCl	CAS No.	34552-83-5
批号 Batch No	N19763758	数量 Quantity	5G
生产日期 Manufacture Date	2025.02.08	有效日期 Exp. Date	2028.02.07
检测日期 Test Date	2025.02.09	储存 Storage	RT

检测项目 Inspection Items	检测标准 Standard	检测结果 Results
APPEARANCE	WHITE POWDER	WHITE POWDER
PURITY	≥98%	98.55%
LOSS ON DRYING	≤0.5%	0.06%
SOLUBILITY IN CHCL3	COLORLESS,CLEAR,25MG/ML	CONFORMS
LC-MS FOR IDENTIFICATION	CONFORMS	CONFORMS
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS TO STRUCTURE

结论：按企业标准检验，符合公司标准

CONCLUSION: COMPLIED WITH COMPANY STANDARD

分析员(ANALYST)： 审核(CHECKED)： 质量经理(QC MANAGER)：

南京九佳科技有限公司 质检专用章 Quality Inspection Nanjing Forever Pharmacy Co., Ltd.

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APPENDIX G

Certificate of Phytochemical Screening Result

MSU- Iligan Institute of Technology
College of Science and Mathematics
Department of Chemistry
Andres Bonifacio Avenue, Iligan City 9200

DEPARTMENT OF CHEMISTRY
MSU-Iligan Institute of Technology

CERTIFICATE OF RESULTS

Name of Student: **Johayber Macud**
Name of School: **Adventist Medical Center, Iligan City**
Name of Analysis **Phytochemical Screening**
Sample Tested: Cryude exctarct of Bitter Ginger
Date Analyzed: March 1, 2025

Sample code	Alka-loids	Flavo-noids	Phe-nols	Sapo-nins	Tannins	Ste-roids	Terpe-noids
Bitter Ginger	++	+++	+++	+	+	+	+
Remarks: The sample contains significant amount of essential oil							

Analyzed by:

ENJELYN C. GOMEZ, RCh.
PRC Reg. No. 0007319

Noted:

for:
Dr. ANELYN P. BENDOY.
Chairman, Chemistry Department

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APPENDIX H

Animal Research Clearance

Republic of the Philippines
Department of Agriculture
BUREAU OF ANIMAL INDUSTRY
Visayas Avenue, Brgy. Vasra, Quezon City

ANIMAL RESEARCH CLEARANCE

NAME OF INSTITUTION : **ADVENTIST MEDICAL CENTER COLLEGE**	**REFERENCE NO :** *AR - 2025 - 0081*
	DATE/VENUE : February 2025 - April 2025 AMCC Laboratory
BUSINESS ADDRESS : Brgy. San Miguel, Iligan City	**LEAD RESEARCHER/VETERINARIAN/IACUC CHAIR:** **Abdulrauf, Raudha D., et.al.** – Researcher **Maria Carmela Grace T. Flor, DVM** - Veterinarian **Darmi Wena A. Maquilan, RPh** – IACUC Chair

Pursuant to the provisions of Republic Act 8485 or the Animal Welfare Act of 1998 as amended by RA 10631 and DA-Administrative Order (AO) No. 40, series of 1999, on the Rules and Regulations on the Scientific Procedure Using Animals, this Permit is hereby issued to **ADVENTIST MEDICAL CENTER COLLEGE** with BAI Registration No. **LAF - 0011** after completing the requirements to conduct the research entitled **"The Evaluation of Antidiarrheal Properties of Ethanolic Rhizome Extract of *Zingiber zerumbet* (Zingiberaceae)"** on the date and venue stipulated above.

The Institution pis hereby reminded to observe the provisions of DA-AO no. 40 s. 1999.

Prepared on February 20, 2025

Approved By Authority of the Director

HYACINTH G. NAPILOY, DVM, MPS-PA
Chief
Animal Health and Welfare Division

RF AHWD-49 Animal Research Clearance
Rev. No. 03
April 19, 2023

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1866

APPENDIX I

Certificate of Statistical Analysis

StatRex Consulting
For more details, please follow and contact us:
statrexconsulting
statrexclass@gmail.com

CERTIFICATION FOR STATISTICAL ANALYSIS

This is to certify that the study titled "**The Evaluation of the Antidiarrheal Properties of Ethanolic Rhizome Extract of *Zingiber zerumbet* (Zingiberaceae)**" conducted by **Raudha D. Abdulrauf, Johayber M. Macud, Sophia C. Magbanua,** and **Princess Nhurliza S. Ramber**, Bachelor of Science in Pharmacy students of Adventist Medical Center College, has undergone statistical analysis performed by the undersigned, **Rey R. Cuenca, MSc**, as the Statistician.

This certification is issued at the request of the researchers as part of their defense requirements.

Issued this 15th day of May 2025.

Signed,

Rey R. Cuenca, MSc
Statistician

Note:
- *This certification is authenticated through the QR code provided above.*
- *The official e-copy is exclusively owned and maintained by the statistician at rey.cuenca@g.msuit.edu.ph.*
- *Certifications lacking this footer, QR code verification, or confirmed ownership via the drive link of rey.cuenca@g.msuit.edu.ph are invalid.*
- *Report suspected forgery immediately to rey.cuenca@g.msuit.edu.ph.*

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APPENDIX J

Request for Acknowledgement of Vehicle Modification

Adventist Medical Center College
Department of Pharmacy
San Miguel, Iligan City, Lanao del Norte, 9200,
Northern Mindanao

Adventist Medical Center College (AMCC)
Brgy. San Miguel, Iligan City
April 22, 2025

Mrs. Patricia Mae B. Jarabe, RPh
Panelist – AMCC
Ms. Darmi Wena A. Maquilan, RPh
Panelist – AMCC
Ms. Roselle L. Remulta-Lauron, RPh, MS Pharm
Thesis Chair – AMCC
Ms. Junnin Gay L. Garay, RPh, CPh, MS Pharm
Research Adviser – AMCC

Subject: Request for Acknowledgement of Vehicle Modification in Our Research Study

Dear Mrs. Jarabe, Ms. Maquilan, Mrs. Remulta-Lauron,

We, the undersigned researchers, respectfully submit this letter to formally inform you of a procedural modification in our research study entitled "The Evaluation of the Antidiarrheal Properties of Ethanolic Rhizome Extract of *Zingiber zerumbet (Zingiberaceae)."*

After initial preparations and trials, we found that the ethanolic extract of *Zingiber zerumbet* exhibits limited solubility and dispersibility in distilled water due to its relatively hydrophobic and resinous components. As such, to improve the suspension and ensure consistent dosing, we opted to change the vehicle used in Gastrointestinal Motility and Castor Oil-Induced Diarrhea tests from distilled water to 0.9% Plain Normal Saline Solution (PNSS) with Tween 80 as a suspending agent.

This modification promotes better homogeneity of the extract during administration. Furthermore, PNSS is uniformly used across all groups — negative control, positive control, and the extract treatment groups (25%, 50%, and 75% concentrations).

We kindly seek your understanding and approval regarding this necessary methodological adjustment.

Respectfully yours,
Raudha D. Abdulrauf
Johnyber M. Macud
Sophia L. Magbanua
Princess Nhuriza S. Ramber

Noted by:
Ms. Junnin Gay L. Garay, RPh, CPh, MS Pharm
Research Adviser

Mrs. Patricia Mae B. Jarabe, RPh
Panelist

Ms. Darmi Wena A. Maquilan, RPh
Panelist

Mrs. Roselle L. Remulta-Lauron, RPh, MS Pharm
Thesis Chair

Scanned with CamScanner

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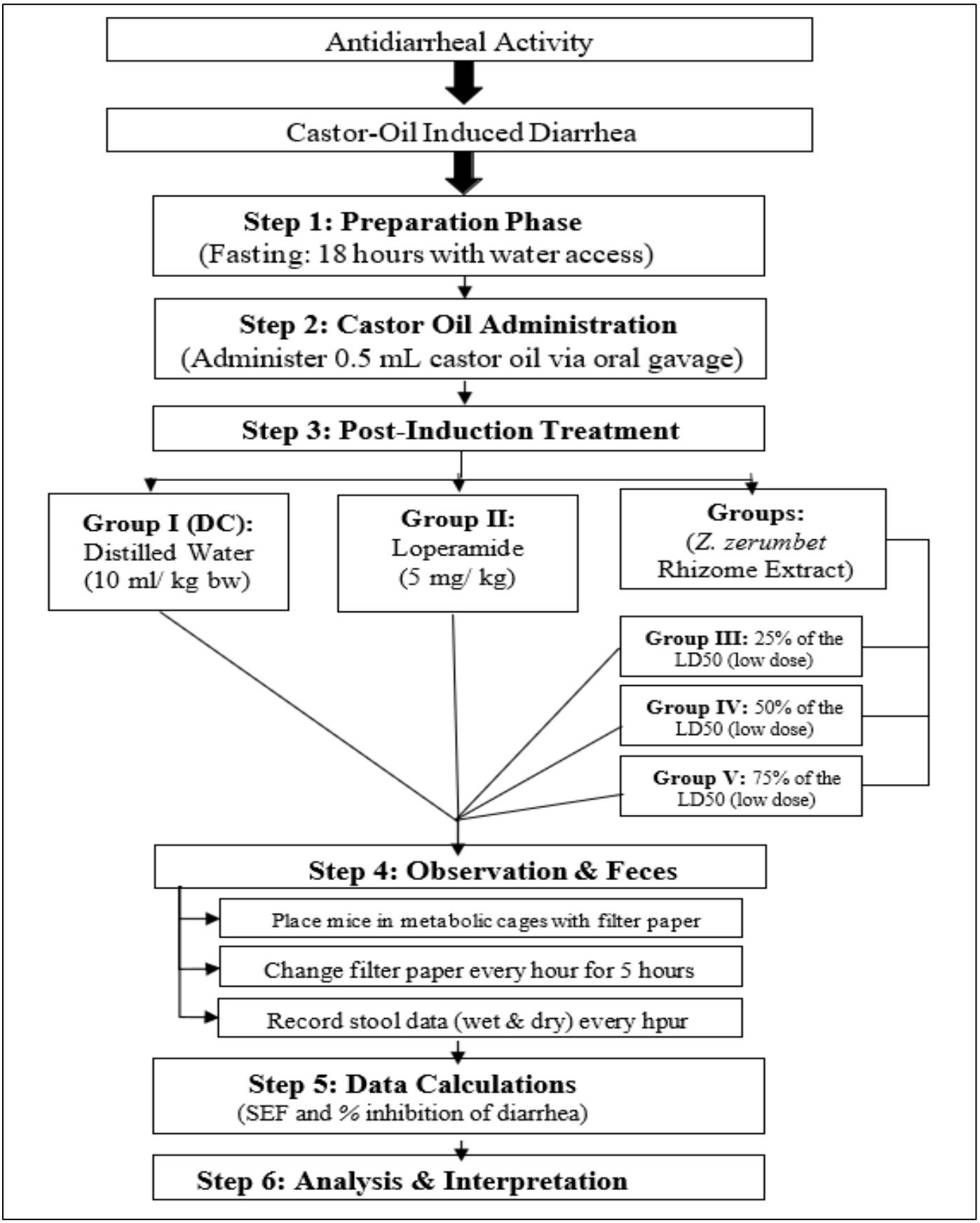
APPENDIX K

Castor Oil-Induced Diarrhea



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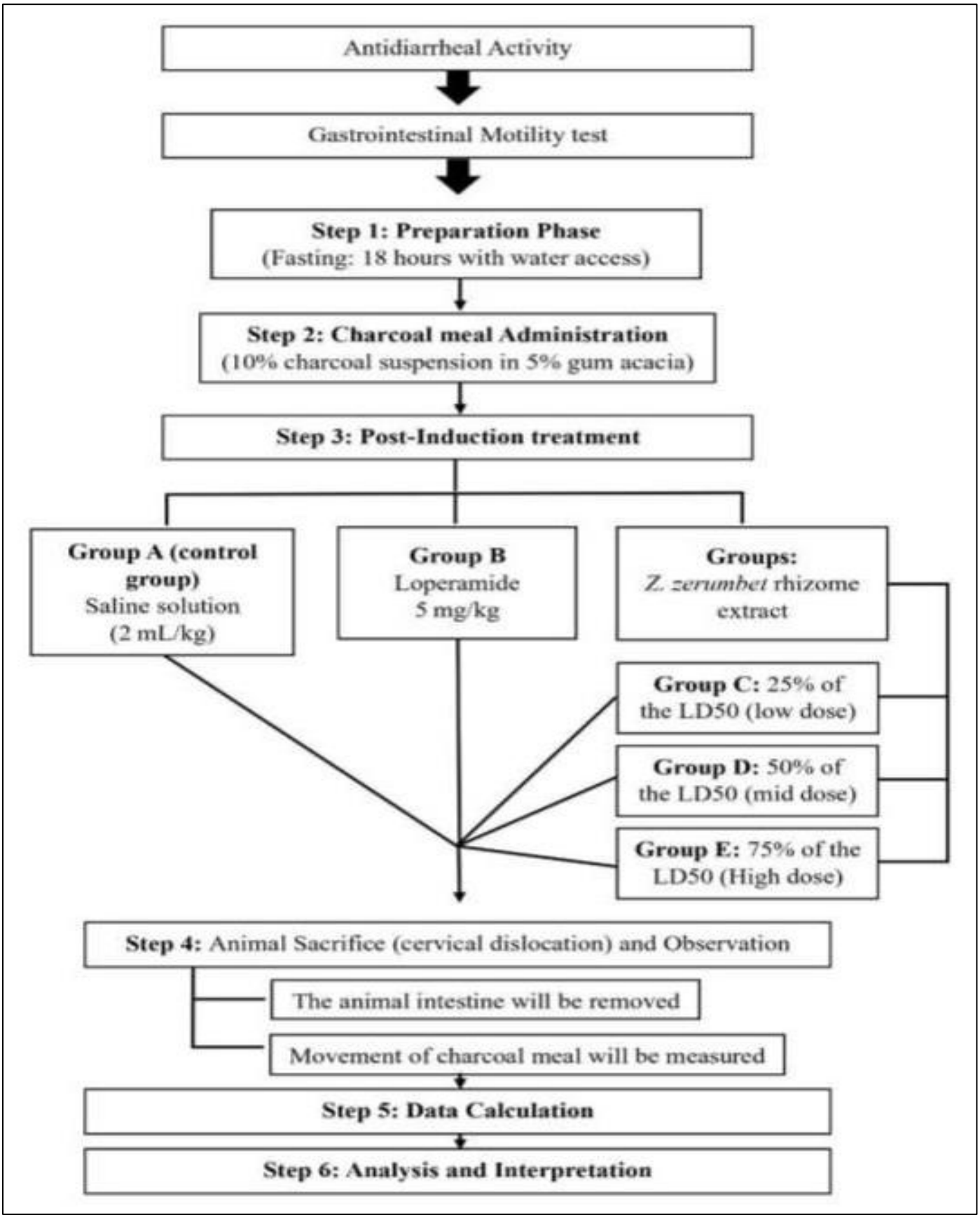
APPENDIX L

Gastrointestinal Motility Test



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APPENDIX M

Collection, Air Drying, and Preparation of *Z.zerumbet* Rhizomes

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1866

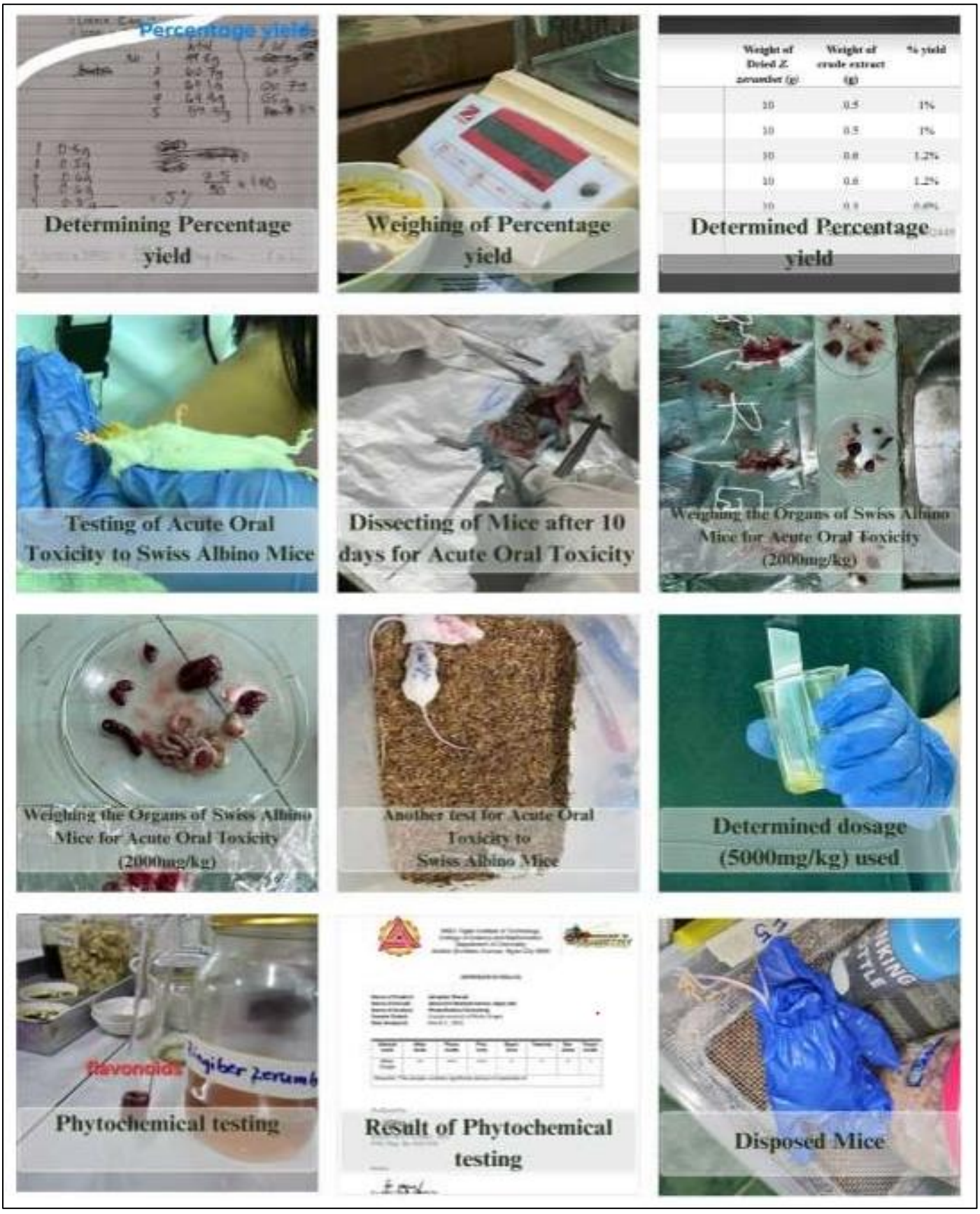
APPENDIX N

Z. zerumbet Rhizome Extraction

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1866

APPENDIX O

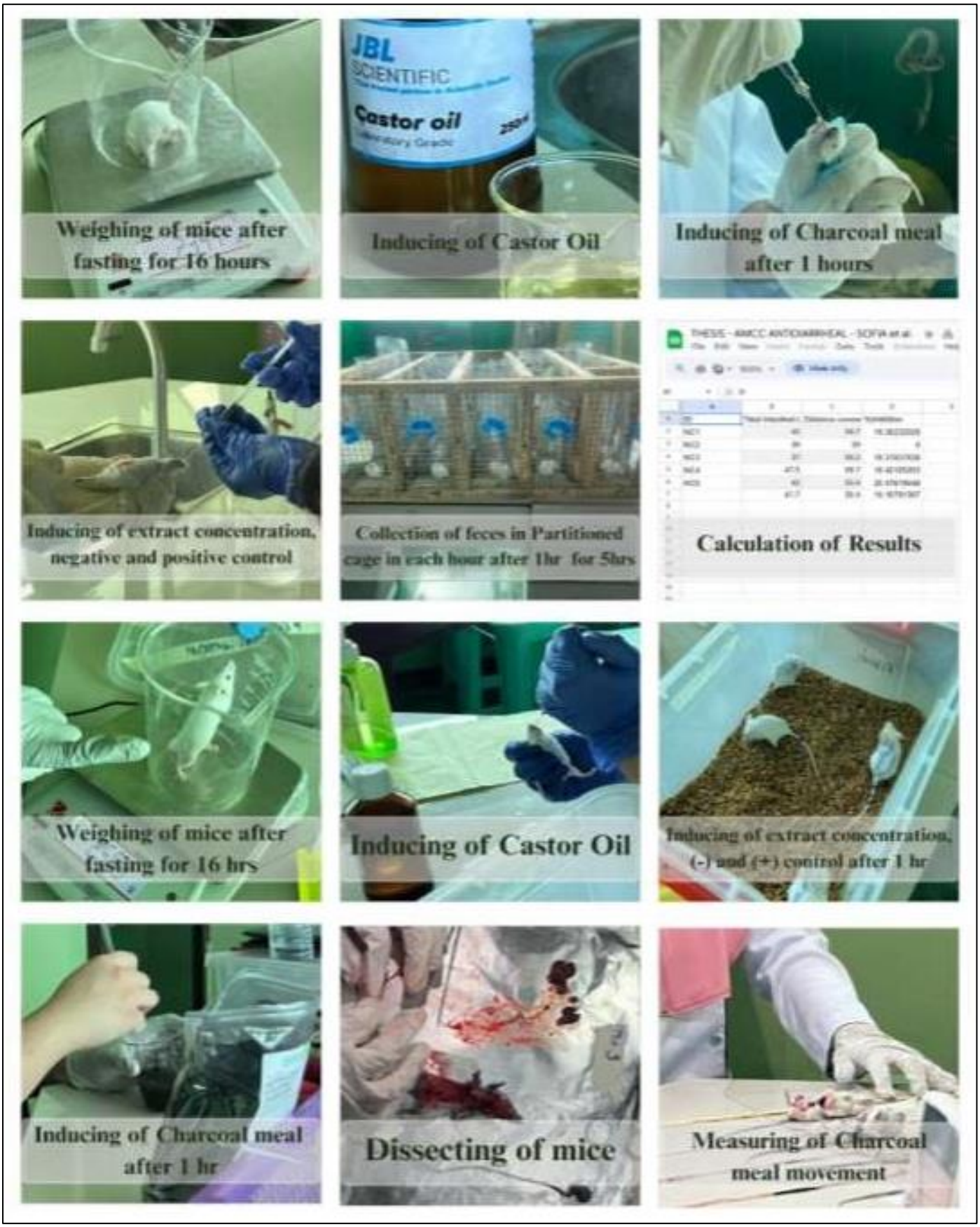
Determining Percentage Yield, Acute Oral Toxicity, Phytochemical Picture and Results



ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jun1866

APPENDIX P

Gastrointestinal Motility Test and COID Result and Pictures



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APPENDIX Q

Raw Data

Castor Oil-Induced Diarrhea – Negative Control						
ID	**Time**	**Freq**	**Consistency**	**Aggregated weight (mg)**	**Stool Emission Frequency**	**% Inhibition**
NC1	1	1	1	23.5		
	2	3	3	86.2		
	3	1	1	38.05		
	4	1	1	35.2		
	5	4	2	66.91		
		10	8	249.86	2	0
NC2	1	0	-1	0		
	2	1	0	35.21		
	3	2	2	62.71		
	4	3	3	87.96		
	5	2	2	63.4		
		8	6	249.28	1.6	0
NC3	1	0	-1	27.21		
	2	2	0	47.05		
	3	3	0	76.8		
	4	4	2	99.5		
	5	5	4	117.7		
		14	6	368.26	2.8	0
NC4	1	2	1	29.56		
	2	4	2	106.21		
	3	2	2	88.81		
	4	1	1	56.64		
	5	5	3	154.26		
		14	9	435.48	2.8	0
NC5	1	2	0	24.61		
	2	4	1	94.76		
	3	5	3	112.9		
	4	2	2	69.1		
	5	1	1	60.86		
		14	7	362.23	2.8	0
	Total Freq	60	**Total Wt.**	1665.11		
				Total SEM	12	
					G. Inhibition	0
St. Dev.		2.828427125	1.303840481	81.42651055	0.5656854249	0

Castor Oil-Induced Diarrhea – Positive Control						
ID	**Time**	**Freq**	**Consistency**	**Aggregated weight (mg)**	**Stool Emission Frequency**	**% Inhibition**
PC1	1	2	1	40.5		
	2	1	1	27.8		
	3	0	-1	0		
	4	0	-1	0		
	5	0	-1	0		
		3	-1	68.3	0.6	95.89816889
PC2	1	1	2	30.5		
	2	1	1	32.9		
	3	0	-1	0		
	4	0	-1	0		
	5	0	-1	0		
		2	1	63.4	0.4	96.1924437

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PC3	1	1	0	21.2		
	2	1	1	34.8		
	3	1	0	49		
	4	0	-1	0		
	5	1	0	20.55		
		4	1	125.55	0.8	92.4599576
PC4	1	2	0	38.6		
	2	1	1	35.1		
	3	1	0	21.5		
	4	1	0	20		
	5	0	-1	0		
		5	1	115.2	1	93.0815381
PC5	1	1	1	33		
	2	1	0	15		
	3	1	0	31.2		
	4	0	-1	0		
	5	0	-1	0		
		3	1	79.2	0.6	95.2435574
			Total Wt.	**451.65**		
				Total SEF	**3.4**	
					G.Inhibition	**72.8756658**
St. Dev.		1.140175425	0.894427191	28.25503495	0.228035085	1.69688698

Castor Oil-Induced Diarrhea – LOW						
ID	**Time**	**Freq**	**Consistency**	**Aggregated weight (mg)**	**Stool Emission Frequency**	**% Inhibition**
E21	1	3	3	65.51		
	2	4	4	80.6		
	3	1	0	21.5		
	4	2	2	80.6		
	5	3	2	65.87		
		13	11	314.08	2.6	81.1375825
E22	1	1	0	15.5		
	2	2	1	55.8		
	3	3	3	99		
	4	2	1	30.92		
	5	2	2	47.11		
		10	7	248.33	2	85.08627058
E23	1	2	2	40.15		
	2	0	-1	0		
	3	1	1	21.17		
	4	2	1	31.5		
	5	2	2	60.88		
		7	4	153.7	1.4	90.7693786
E24	1	1	1	40.5		
	2	3	2	70.57		
	3	2	2	50.9		
	4	0	-1	0		
	5	3	2	81.42		
		9	7	243.39	1.8	85.38294767
E25	1	0	-1	0		
	2	1	0	21.34		
	3	2	2	57.52		
	4	3	2	84.31		
	5	2	1	47.6		
		8	5	210.77	1.6	87.34197741
			Total Wt.	**1170.27**		

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				Total SEF	**9.4**	
					G. Inhibition	**29.71815676**
St. Dev.		2.302172887	2.683281573	58.5107591	0.4604345773	3.513927554

Castor Oil-Induced Diarrhea – MID						
ID	**Time**	**Freq**	**Consistency**	**Aggregated weight (mg)**	**Stool Emission Frequency**	**% Inhibition**
E51	1	2	2	53.9		
	2	1	1	20.5		
	3	0	-1	0		
	4	1	1	41.56		
	5	1	0	20.55		
		5	4	136.51	1	91.80174283
E52	1	0	-1	0		
	2	2	1	38.95		
	3	1	0	20.11		
	4	2	2	50.8		
	5	1	1	55		
		6	4	164.86	1.2	90.09915261
E53	1	2	2	55.71		
	2	1	1	45.13		
	3	1	1	55.16		
	4	0	-1	0		
	5	1	0	25.96		
		5	4	181.96	1	89.07219343
E54	1	2	2	66.1		
	2	1	0	20.4		
	3	2	1	50.3		
	4	2	1	40.55		
	5	0	-1	0		
		7	4	177.35	1.4	89.34905201
E55	1	1	1	30.55		
	2	2	2	60.52		
	3	1	0	15.2		
	4	2	1	50.5		
	5	2	1	48.79		
		8	5	205.56	1.6	87.65486965
			Total Wt.	**866.24**		
				Total SEF	**6.2**	
					G. Inhibition	**47.97701053**
St. Dev.		1.303840481	0.4472135955	25.28255268	0.2607680962	1.51837132

Castor Oil-Induced Diarrhea – HIGH						
ID	**Time**	**Freq**	**Consistency**	**Aggregated weight (mg)**	**Stool Emission Frequency**	**% Inhibition**
E71	1	1	1	46.5		
	2	0	-1	0		
	3	1	0	31.5		
	4	0	-1	0		
	5	1	0	30.2		
		3	1	108.2	0.6	93.5019308
E72	1	0	-1	0		
	2	1	0	30.5		
	3	1	1	44.6		
	4	0	-1	0		
	5	1	0	30.5		
		3	1	105.6	0.6	93.65807664

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E73	1	1	0	20.6		
	2	1	1	40.5		
	3	1	1	42.9		
	4	1	1	42.6		
	5	0	-1	0		
		4	3	146.6	0.8	91.19577686
E74	1	2	1	72.9		
	2	1	0	20.5		
	3	0	-1	0		
	4	1	1	40.55		
	5	0	-1	0		
		4	1	133.95	0.8	91.95548642
E75	1	0	-1	0		
	2	1	0	20.5		
	3	1	1	40.5		
	4	1	0	20.7		
	5	1	1	46.5		
		4	2	128.2	0.8	92.30080896
			Total Wt.	**622.55**		
				Total SEF	**3.6**	
					Group Inhibition	**62.61207968**
St. Dev.		0.5477225575	0.894427191	17.42341815	0.1095445115	1.04638241

Gastrointestinal Motility Test – Negative Control			
ID	**Total Intestinal Length Distance (cm)**	**Distance covered by charcoal (cm)**	**%inhibition**
NC1	43	34.7	19.30232558
NC2	39	39	0
NC3	37	30.2	18.37837838
NC4	47.5	39.7	16.42105263
NC5	42	33.4	20.47619048
Average wt.	41.7	35.4	15.10791367
St. Dev.	4.024922359	3.967996976	8.469000426

Gastrointestinal Motility Test – Positive Control			
ID	**Total Intestinal Length Distance (cm)**	**Distance covered by charcoal (cm)**	**%inhibition**
PC1	40	1	97.5
PC2	48	20	58.33333333
PC3	36	14.3	60.27777778
PC4	36	12.7	64.72222222
PC5	44.5	25.5	42.69662921
Average	40.9	14.7	64.70599251
St. Dev.	5.296225071	9.173058378	20.12510332

Gastrointestinal Motility Test – LOW			
ID	**Total Intestinal Length Distance (cm)**	**Distance covered by charcoal (cm)**	**%inhibition**
E21	58.5	34.6	40.85470085
E22	46.8	32.3	30.98290598
E23	56.5	41	27.43362832
E24	53.7	37.5	30.16759777
E25	44.5	28.7	35.50561798
Average	52	34.82	32.98889018
St. Dev.	6.09672043	4.723028689	5.269001191

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Gastrointestinal Motility Test – MID			
ID	**Total Intestinal Length Distance (cm)**	**Distance covered by charcoal (cm)**	**%inhibition**
E51	48.5	22.2	54.22680412
E52	42.7	32.5	23.88758782
E53	46.7	29.8	36.18843683
E54	48.5	32.6	32.78350515
E55	47	24.5	47.87234043
Average	46.68	28.32	38.99173487
St. Dev.	2.375289456	4.744154298	12.09863984

Gastrointestinal Motility Test – HIGH			
ID	**Total Intestinal Length Distance (cm)**	**Distance covered by charcoal (cm)**	**%inhibition**
E71	47.8	27	43.51464435
E72	45.2	22.4	50.44247788
E73	45.8	27.8	39.30131004
E74	46	14.8	67.82608696
E75	47	9	80.85106383
Average	46.36	20.2	56.38711661
St. Dev.	1.033440855	8.115417426	17.48069517

Doses used		
Low dose	**Mid Dose**	**High Dose**
1250 mg/kg	2500 mg/kg	3750 mg/kg