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Laxative Potential of the Ethanolic Leaf Extract of Ipomoea Triloba (Convolvulaceae) in a Loperamide-Induced Constipation Model in Swiss Albino Mice

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ABSTRACT

Constipation is a common gastrointestinal disorder characterized by infrequent bowel movements, hard stool consistency, and straining during defecation (Shi et al. 2024). *Ipomoea triloba* has been reported to contain flavonoids and saponins (Uka & Ukeke, 2020; StuartXchange, 2023), which are associated with potential laxative activity. This study evaluated the laxative effect of *I. triloba* leaf extract using Swiss albino mice. Acute oral toxicity was assessed following OECD Guideline 425, and no signs of toxicity or mortality were observed at the limit dose of 2000 mg/kg, confirming the safety of the extract. Based on this, *Ipomoea triloba* extract was administered at low (500 mg/kg), mid (1000 mg/kg), and high (2000 mg/kg) doses. Two experiments were conducted using twenty-five male mice each, randomly assigned into five groups: negative control (distilled water, 10 mL/kg), positive control (bisacodyl 0.25 mg/kg), and three treatment groups receiving the extract. Constipation was induced using loperamide for six days, followed by five days of treatment. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.01$). In the first experiment, fecal parameters showed significant dose-dependent improvement, with the most pronounced effects at mid and high doses ($p < 0.001$). In the second experiment, intestinal transit was significantly increased ($p < 0.001$), with effects comparable to the positive control and without a clear dose-dependent pattern. Overall, *I. triloba* exhibited significant laxative activity in loperamide-induced constipated mice.

Keywords: *Ipomoea triloba*, laxative activity, loperamide-induced constipation, Swiss albino mice, OECD Guideline 425, fecal parameters, intestinal transit, flavonoids, saponins

CHAPTER I

1.1 Introduction

Constipation is a common digestive disorder that affects people of all ages. It is usually characterized by infrequent bowel movements, hard stools, straining during defecation, and a feeling of incomplete evacuation, which can lead to discomfort and reduce quality of life (Ma et al. 2022; Chiarioni et al. 2023). Conventional laxatives, including bulk-forming, osmotic, and stimulant agents, provide relief but

may cause side effects such as abdominal cramps, electrolyte imbalance, and dependence when used long-term (Ma et al. 2022).

This has fueled increasing interest in plant-based therapeutics, which have been suggested as potentially safer and more sustainable alternatives (Kongdang et al. 2022). Members of the Convolvulaceae family, in particular, have drawn scientific attention for their pharmacological properties. For example, species such as *Convolvulus spinosus*, *Ipomoea pes-caprae*, *Ipomoea batatas*, Morning Glory seeds, and *Operculina turpethum* have been reported to exhibit laxative activity (Arif et al. 2023; Akinniyi et al. 2022; Liu et al. 2023; Ren et al. 2020; Nafees et al. 2020).

Ipomoea triloba, a member of the Convolvulaceae family, has been traditionally used for various medicinal purposes, and its leaves have been reported to contain flavonoids and saponins (Uka and Okeke 2020; StuartXchange 2023). Although other species of *Ipomoea* have been evaluated for laxative activity, no experimental study has yet evaluated the laxative potential of *I. triloba*.

Thus, this study sought to evaluate the *in vivo* laxative activity of ethanolic leaf extract of *I. triloba* in a loperamide-induced constipation model in Swiss albino mice.

1.2 Background of the study

The loperamide-induced constipation model is a well-established and widely used preclinical method for evaluating laxative activity. Loperamide is a drug that slows intestinal movement and reduces fluid secretion, effectively mimicking constipation in rodents with reproducible outcomes (Gao et al. 2022). Its safety, predictability, and consistency make it a preferred choice for natural product screening and pharmacological evaluation (Gao et al. 2022; Jia et al. 2024).

Ipomoea triloba L. (family Convolvulaceae), commonly known as littlebell morning-glory, is widely distributed in tropical regions and has been used traditionally in folk medicine. Phytochemical evaluations of the species and related members of the *Ipomoea* genus have shown the presence of flavonoids and saponins, which are phytochemical considerations known for their potential gastrointestinal and smooth-muscle modulatory properties (Uka and Okeke 2020). Preclinical *in vivo* studies of medicinal plant extracts have demonstrated various pharmacological effects, supporting the potential of natural agents in modulating gastrointestinal activity (Gao et al. 2022). Ethanol is also recognized as an effective solvent for extracting phytochemical constituents, capturing both polar and non-polar compounds essential for pharmacological evaluation (Abubakar and Haque 2020).

By assessing fecal parameters and gastrointestinal motility, the study aimed to provide scientific validation for the traditional use of *I. triloba*, to explore its potential as a natural laxative, and to contribute to the growing evaluation of plant-based alternatives for constipation management.

1.3 Conceptual Framework

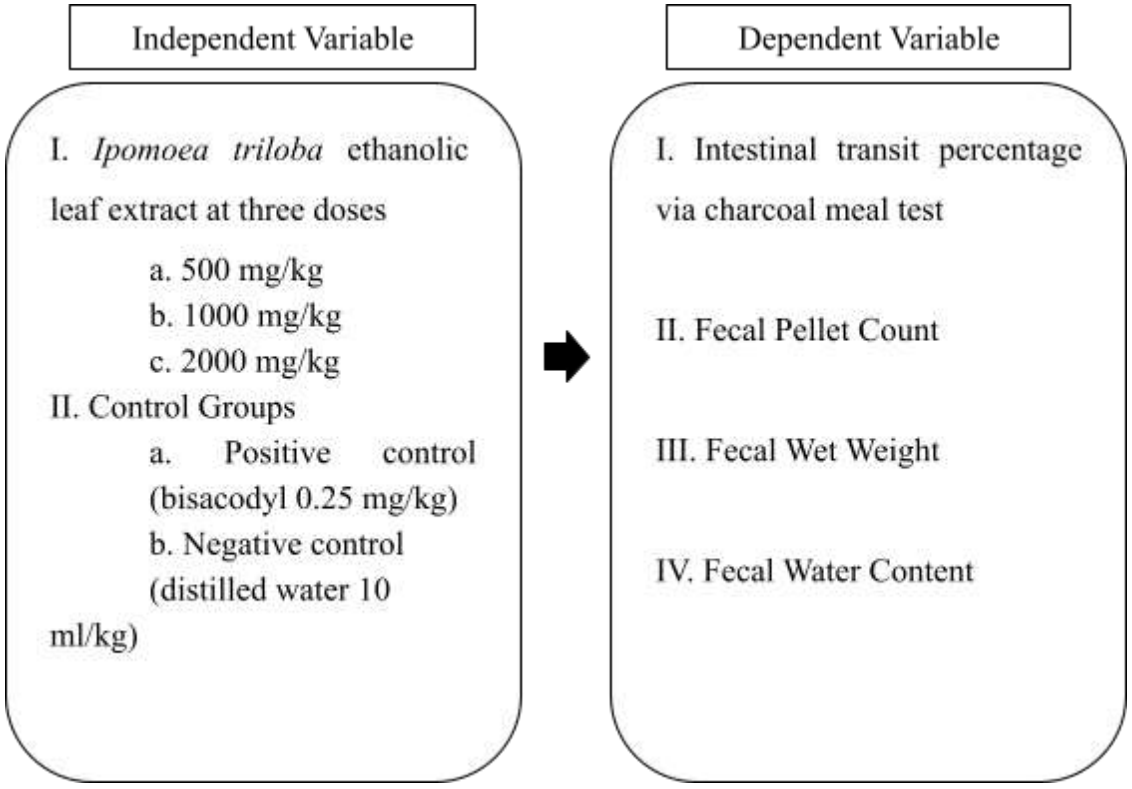


Figure 1. Conceptual Framework of the Study

The independent variables comprised the treatment groups administered to Swiss albino mice: *Ipomoea triloba* ethanolic leaf extract at low, medium, and high doses; a negative control (distilled water); and a positive control (bisacodyl). These conditions allowed comparative evaluation of the extract's potential laxative activity against standard models of constipation.

The dependent variables included: (1) intestinal transit percentage, measured via the activated charcoal meal test; (2) fecal pellet count; (3) fecal wet weight; and

(4) fecal water content. Together, these parameters assessed stool output and intestinal transit, enabling evaluation of the dose-dependent laxative effects of *I. triloba* extract.

1.4 Research Objectives General Objective

To evaluate the laxative potential of *Ipomoea triloba* leaves ethanolic extract in a loperamide-induced constipation model in Swiss albino mice.

Specific Objectives:

1. To determine the percentage yield of the *Ipomoea triloba* leaf extract.
2. To conduct confirmatory phytochemical tests of *I. triloba* leaf extract.
3. To determine the approximate toxic dose of *I. triloba* crude extract through acute toxicity test.
4. To determine the specific concentrations of the crude extract as low dose, mid dose and high dose.
5. To evaluate the potential laxative property of *I. triloba* leaf extract by assessing fecal parameters and intestinal transit percentage.
6. To compare the laxative effect of *I. triloba* leaf extract with the positive control Bisacodyl drug.

1.5 Hypothesis of the Study Alternative Hypothesis (H_1):

The ethanolic leaf extract of *Ipomoea triloba* significantly increases fecal pellet count, fecal wet weight, fecal water content, and intestinal transit percentage in Swiss albino mice

with loperamide-induced constipation compared to the standard drug (bisacodyl).

Null Hypothesis (H_0):

The ethanolic leaf extract of *Ipomoea triloba* produces no significant difference in fecal pellet count, fecal wet weight, fecal water content, and intestinal transit percentage in Swiss albino mice with loperamide-induced constipation compared to the standard drug (bisacodyl).

1.6 Significance of the Study

This study aimed to provide scientific evidence on the therapeutic potential of *Ipomoea triloba* leaves as a natural laxative, demonstrating how traditional plant-based remedies can be validated through research. The findings are expected to offer valuable contributions to multiple sectors:

To the Pharmaceutical Industry. The use of *Ipomoea triloba* leaves extract may provide a cost-effective and safer alternative to synthetic laxatives. This could help reduce production costs, support the use of plant-based treatments, and promote more sustainable pharmaceutical practices.

To Local Agriculture and Communities. Since *Ipomoea triloba* is widely distributed in tropical regions, its medicinal use could create new applications for the plant. This may support local farmers, add value to agricultural products, and strengthen the connection between farming and healthcare.

To Adventist Medical Center College. The research serves as a practical example of natural product evaluation, offering educational value in classrooms and laboratories and demonstrating the process of scientifically validating traditional remedies.

To the Department of Pharmacy. The study contributes to the department's knowledge of medicinal plants with gastrointestinal benefits. It encourages faculty and students to explore natural alternatives in drug development and helps enhance research and teaching in pharmacognosy and pharmaceutics.

To Future Researchers. This study provides useful data and a systematic method for testing natural laxatives. It can guide other researchers working on similar plant-based treatments and supports more studies on using local plants in medicine.

1.7 Scope and Limitations of the Study

This study evaluated the laxative potential of the ethanolic leaf extract of *Ipomoea triloba* using male Swiss albino mice in a loperamide-induced constipation model. The scope included the collection and authentication of plant material, preparation of ethanolic extracts, and phytochemical confirmatory test for constituents such as flavonoids and saponins. A preliminary toxicity test was conducted following OECD 425 guidelines to determine a safe dose range for administration.

The pharmacological assessment included fecal parameter analysis, specifically pellet count, wet weight, water content, and intestinal transit percentage, determined using the activated charcoal meal test. The extract's effects were compared with bisacodyl as a standard laxative to establish dose-dependent efficacy.

Only male Swiss albino mice were used to ensure consistency and minimize hormonal variability as the female populations, whose hormonal cycles, such as the estrous cycle, cause fluctuations in estrogen and progesterone may significantly affect gastrointestinal motility, fluid secretion, and smooth muscle activity (Yoon & Kim, 2021). The study focused solely on functional evaluation of laxative activity and did not include gastrointestinal histopathological analysis or mechanistic evaluation.

The study did not evaluate the specific mechanism of action of the *Ipomoea triloba* leaf extract due to the limited time frame and the unavailability of advanced laboratory equipment needed to identify and analyze the chemical components responsible for its effect. Instead, the research focused on assessing the potential laxative activity of the extract.

Additional limitations included the use of a single extraction solvent (ethanol), potential variability in plant composition due to environmental and seasonal factors, and laboratory constraints such as limited equipment and sample size. Although the OECD 425 test provided preliminary safety data, further subchronic and clinical studies were not conducted, as the study was designed solely to evaluate the acute safety profile of the extract.

1.8 Definition of Terms

Bisacodyl – Referred to a synthetic stimulant laxative that stimulates intestinal peristalsis to induce bowel movement. It is usually used as a reference standard in laxative studies (Corsetti et al. 2021)

Charcoal Meal Transit Test – Referred to an *in vivo* assay in rodents in which activated charcoal is orally administered, and intestinal motility is assessed by measuring the distance traveled by the charcoal (Bawazeer et al. 2020).

Constipation – Referred to a gastrointestinal disorder marked by infrequent or difficult bowel movements, often defined clinically as fewer than three defecations per week, with hard stool consistency, straining, or incomplete evacuation. In this study, constipation is experimentally induced by loperamide to simulate slowed intestinal transit in mice (Kongdang et al. 2022; Liu et al. 2020).

Ethanolic Extract – Referred to a concentrated preparation obtained by maceration or Soxhlet extraction of plant material using ethanol (70-95% v/v), a solvent capable of dissolving both polar and non-polar phytochemicals (Patil et al. 2020).

Fecal Pellet Count – Referred to the number of fecal pellets excreted within a defined period; reduced pellet count indicates impaired bowel movement frequency in constipation models (Arif et al. 2023)

Fecal Water Content – Referred to the percentage of water present in feces, calculated using the difference between fecal wet weight and dry weight relative to wet weight. It is an important parameter used in laxative studies to evaluate stool hydration, where decreased fecal water content is associated with constipation and increased levels indicate improved bowel function (Liu et al. 2020; Arif et al. 2023; Gao et al. 2022).

Fecal Wet Weight – Referred to the weight of freshly excreted fecal matter before drying; reflects both stool mass and hydration status, serving as a direct measure of laxative or pro-motility activity (Arif et al. 2023).

Gastrointestinal Motility – Referred to the spontaneous muscular contractions (peristalsis) of the digestive tract that move contents along the intestines; in experimental studies, motility is often measured by the distance a marker (e.g., activated charcoal) travels relative to total intestinal length (Bawazeer et al. 2020).

In Vivo – Referred to a term used to describe experiments conducted within a living organism, such as animal models. In this study, *in vivo* testing involves administering *Ipomea triloba* extract to Swiss albino mice and measuring physiological outcomes like fecal parameters and intestinal motility (Gao et al. 2022).

Loperamide – Referred to a synthetic μ-opioid receptor agonist commonly used as an anti- diarrheal drug in humans. In preclinical research, it is administered to rodents to induce constipation by inhibiting intestinal peristalsis and enhancing water absorption. This makes it a reliable pharmacological model for testing laxative agents (Gao et al. 2022).

OECD Test Guideline 425 - Referred to a standardized method for assessing acute oral toxicity in rodents, and in this study, it will be used to determine the safety of *Ipomoea triloba* leaf extract before evaluating its laxative effects (OECD 2022).

Phytochemical constituents – Referred to naturally occurring bioactive compounds in plants, including flavonoids, alkaloids, glycosides, tannins, and organic acids. These secondary metabolites often contribute to therapeutic effects, such as modulation of gastrointestinal activity (Patil et al. 2020).

Preclinical Experimental Model – Referred to a controlled laboratory model (animal or in vitro) used to evaluate the pharmacological activity of a compound before human trials. The loperamide-induced constipation model in mice is widely accepted for testing natural laxatives (Kongdang et al. 2022).

Ipomoea triloba – Referred to a species of the morning-glory family (Convolvulaceae) with twining stems and three-lobed leaves. Its phytochemical screening of its stems and leaves shows the presence of bioactive secondary metabolites such as saponins, tannins, and flavonoids, which may contribute to therapeutic effects (Uka and Okeke 2020).

CHAPTER II
REVIEW OF LITERATURE AND RELATED STUDIES

This chapter reviewed the literature on *Ipomoea triloba*, a member of the Convolvulaceae family, focusing on its taxonomy, morphology, ethnomedicinal uses, pharmacological properties, and phytochemical constituents. The chapter also discussed constipation as a health condition, experimental assays for evaluating laxative activity, conventional laxative drugs, and relevant local and international studies, forming the basis for the theoretical framework. Finally, to ensure both safety and ethical compliance, the acute toxicity of *I. triloba* leaf extracts was assessed following OECD guidelines.

2.0 Literature Review

2.1 *Ipomoea triloba (*Convolvulaceae)

Ipomoea triloba L., a member of the Convolvulaceae family, is an annual climbing herb widely distributed in tropical and subtropical regions, including Southeast Asia, Africa, and the Americas (StuartXchange 2023; Uka and Okeke 2020). It is characterized by its slender, twining stems, three-lobed leaves, and funnel-shaped pink to purple flowers. The species commonly thrives in disturbed habitats such as roadsides, fields, and open grasslands, where it grows abundantly and rapidly (StuartXchange 2023).

Traditionally, *I. triloba* has been used for various medicinal purposes. Leaf decoctions have been used to relieve stomach aches and other digestive discomforts, while poultices made from its leaves are applied for headaches. The plant has also been utilized in local communities to manage abdominal pain, indigestion, and general gastrointestinal ailments (Shaikh 2022; Kauniyah Journal 2021). These ethnobotanical applications highlight the therapeutic relevance of *I. triloba* and

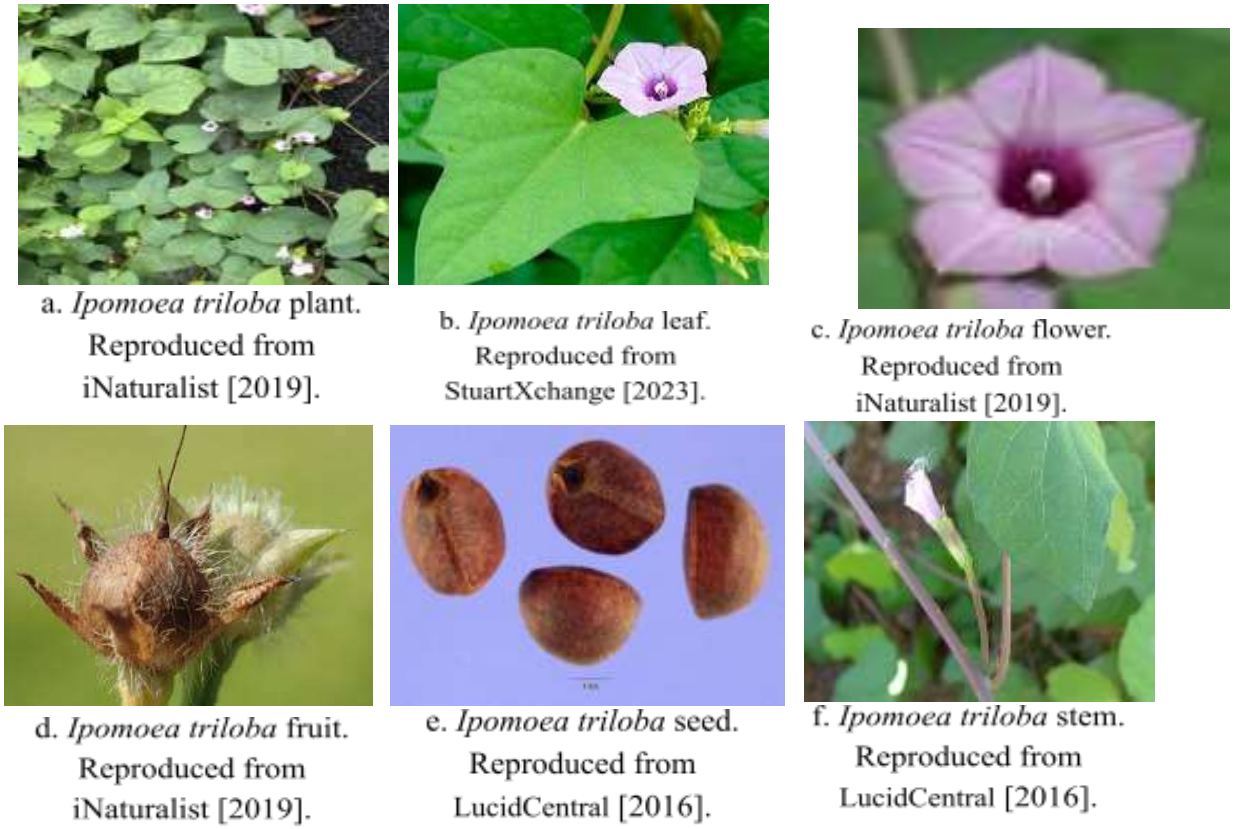
support further pharmacological investigation, particularly in evaluating its potential laxative activity.

Phytochemical confirmatory tests of *I. triloba* have revealed the presence of flavonoids and saponins, which are among the most abundant compounds in its leaves (Uka and Okeke 2021; StuartXchange 2023). These phytochemicals are known in other plant species to promote intestinal motility and fluid secretion, suggesting a potential mechanism for laxative effects (Kongdang et al. 2021). Despite these promising properties, no experimental study has directly evaluated the in vivo laxative potential of *I. triloba*, highlighting a significant research gap that this study aimed to address.

2.1.1 Taxonomy of *Ipomoea triloba* (Convolvulaceae) Table 1. Taxonomy of *Ipomoea triloba*.

Kingdom:	Plantae
Phylum:	Streptophyta
Class:	Equisetopsida
Subclass:	Magnoliidae
Order:	Solanales
Family:	Convolvulaceae
Genus:	*Ipomoea*
Species:	*Ipomoea triloba* L.

2.1.2 Morphological Description of *Ipomoea triloba* L..



a. *Ipomoea triloba* plant. Reproduced from iNaturalist [2019].

b. *Ipomoea triloba* leaf. Reproduced from StuartXchange [2023].

c. *Ipomoea triloba* flower. Reproduced from iNaturalist [2019].

d. *Ipomoea triloba* fruit. Reproduced from iNaturalist [2019].

e. *Ipomoea triloba* seed. Reproduced from LucidCentral [2016].

f. *Ipomoea triloba* stem. Reproduced from LucidCentral [2016].

Figure 2. *Ipomoea triloba* (Convolvulaceae) (a) plant (b) leaf (c) flower (d) fruit (e) seed (f) stem

Ipomoea triloba L., commonly known as "three-lobe morning-glory," is a climbing or trailing herbaceous vine widely distributed in tropical and subtropical regions. It typically grows as a twiner

or creeper with slender stems reaching 1–3 m in length, producing a milky sap when cut (World Flora Online 2024; StuartXchange 2023). The stems are smooth or sparsely hairy, frequently twining around other vegetation.

Leaves are alternate and variable in shape, ranging from broadly ovate to deeply three-lobed, measuring approximately 4–10 cm long and 3–8 cm wide; petioles may reach 1–10 cm in length (World Flora Online 2024). Leaf bases are typically cordate, with margins entire or slightly dentate. Inflorescences are axillary cymes or umbels with peduncles of variable length.Flowers are funnel-shaped, pink to pale-purple, occasionally white, about 1.2–2.5 cm across, with five fused petals and a calyx of five sepals (EDDMapS 2023).

The fruit is a dry, subglobose to slightly depressed capsule, about 5–8 mm in diameter, typically containing 2–4 dark brown to black, smooth seeds (World Flora Online 2024). These morphological traits—slender twining stems, variable leaf-lobing, and small capsules—allow the species to thrive in disturbed habitats such as roadsides and open fields, facilitating rapid spread (PlantwisePlus Knowledge Bank 2024).

2.1.3 Ethnobotanical and Pharmacologic Uses of *Ipomoea triloba* (Convolvulaceae)

Table 2. Ethnobotanical and pharmacologic uses of *Ipomoea triloba*

Study title	Result	Reference
Acute Toxicity and In vivo Antidiarrhoeal Activity of Ethanol Extract and Fractions of *Ipomoea triloba* by Model Infection and Protection Tests in Mice	The ethanol extract of *I. triloba* was non-toxic at tested doses and reduced diarrhoea in mice by 50–83%.	Alozie MF et al. 2024. Acute toxicity and *in vivo* antidiarrhoeal activity of ethanol extract and fractions of *Ipomoea triloba* by model infection and protection tests in mice. Biotechnology Journal International 28(6):35–45.
		https://imsear.searo.who.int/items/b18eed06-df42-4 c64-b33f-955f82853a1d
Evaluation of the antibacterial properties of the extracts and fractions of *Ipomoea triloba L.* (Convolvulaceae) on selected enteric diarrhoeagenic bacteria	Ethanol and aqueous extracts (and fractions) showed antibacterial activity against enteric diarrhoeagenic bacteria; MIC ranged 250–500 mg/mL, MBC 500–1000 mg/mL, supporting traditional antidiarrhoeal use.	Favour AM, Samuel EU, Ekpa ED, Jumbo UE, Joseph AO. 2022. Evaluation of the antibacterial properties of the extracts and fractions of *Ipomoea triloba* L. (Convolvulaceae) on selected enteric diarrhoeagenic bacteria. Journal of Biological Research and Biotechnology 20(1):1398-1408. https://www.bio-research.com.ng/index.php/home/a

		rticle/view/112
Allelopathic Effects of Ginger and Turmeric on the Germination of	Laboratory experiments using juice and water extracts from ginger and turmeric stems and leaves	Ece I, Kitiş YE. 2023. Allelopathic effects of ginger and turmeric on the germination of *Ipomoea*
Ipomoea triloba and *Avena sterilis*	were tested on the germination of *I. triloba* and Avena sterilis. Both extracts significantly reduced germination, with *I. triloba* showing greater sensitivity. This indicates that I. triloba can serve as a model weed species for allelopathic interaction studies and that it is responsive to bioactive plant compounds.	*triloba* and *Avena sterilis*. Proceedings of the 2nd International Conference on Frontiers in Academic Research, Kütahya, Turkey. pp 376-379. https://www.researchgate.net/publication/376470931_Allelopathic_Effects_of_Ginger_and_Turmeric_on_the_Germination_of_*Ipomoea_triloba*_and_Avena_sterilis
A Systematic Review on Ethnobotany and Bioactive Compounds of the Genus *Ipomoea* (Convolvulaceae)	Review of 152 selected studies from 4,297 records. Genus shows therapeutic potential for diabetes, cancer, skin, and gastrointestinal disorders. Bioactive compounds include antioxidants, antibacterial, anti-inflammatory, and anticancer agents.	Borlongan KM, Torres MAJ, Demayo C, Llantos OE. 2024. A systematic review on ethnobotany and bioactive compounds of the genus *Ipomoea* (Convolvulaceae). Himalayan Journal of Health Sciences 9(2):7-30.

		https://doi.org/10.22270/hjhs.v9i2.191

2.1.4 Mechanism of Action of Phytochemical Constituents Table 3. Mechanism of Action of Phytochemical Constituents

Phytochemical Constituents	Mechanism of Action
Flavonoids	Flavonoids have been reported to enhance intestinal motility and improve bowel function through multiple physiological pathways. Several flavonoids, such as quercetin, stimulate gut contractility by modulating neuromuscular and cholinergic signalling, thereby increasing intestinal transit and promoting fecal propulsion (Liu and Zhi 2021). In addition, flavonoid-rich extracts can regulate colonic water transport by influencing the expression and activity of aquaporin channels (AQP3 and AQP4), which enhances luminal water retention and reduces excessive re-absorption, resulting in softer stools and relief from
	constipation (Lv et al. 2024; Zhu et al. 2023).
Saponins	Surface-active (surfactant) interaction with epithelial membranes → increased secretion / permeability. Saponins can complex with membrane sterols and transiently increase epithelial permeability and mucosal secretion (more luminal fluid; stool softening). This membrane interaction can contribute to stool softening / laxation. (Sharma et al. 2023; Cao et al. 2024)

2.1.5 Phytochemical Composition and Pharmacological Relevance

Studies on the *Ipomoea* genus indicate that many species contain phytochemical constituents such as flavonoids, saponins, alkaloids, glycosides, and tannins, which are associated with antimicrobial, antioxidant, and anti-inflammatory effects (Borlongan et al. 2024). Although *I. triloba* itself was not specifically reviewed in that study, its inclusion in the genus suggests potential pharmacological relevance.

Analysis of the leaves of *I. triloba* indicates that they contain particularly high amounts of flavonoids (around 23%) and saponins (around 20%), whereas other phytochemicals such as alkaloids, tannins, phenols, glycosides, terpenoids, and steroids are found in smaller quantities (Uka & Okeke, 2020). Phytochemical screening also confirmed the presence of saponins, flavonoids, tannins, and alkaloids, while anthraquinones and terpenes were absent (Deena & Keerthana, 2021). These dominant compounds are especially relevant because flavonoids and saponins are known to support intestinal motility and gut health, providing a possible explanation for the plant's traditional use in gastrointestinal conditions.

2.1.6 Members of Convolvulaceae with Laxative Activity Table 4. Members of Convolvulaceae with Laxative Activity

Study Title	Members of Convolvulaceae	Results	Reference
Laxative effect of 70% ethanol extract of *Convolvulus spinosus* Burm. f. in mice.	*Convolvulus spinosus* Burm.f.	Thestudy confirmed that *Convolvulus spinosus* extract contains tannins, alkaloids, terpenoids, and flavonoids, providing scientific support for its traditional use as a laxative.	Arif J, Sultana N, Urooj R, Arif R. 2023. Laxative effect of 70% ethanol extract of *Convolvulus spinosus* Burm. f. in mice. J Popul Ther Clin Pharmacol. 30(18):158-169. doi:10.53555/jptcp .v30i18.3043
A Medicinal Halophyte *Ipomoea*	Ipomoea	Notably, its resin glycosides, common in the	Akinniyi G, Lee J, Kim H, Lee JG, Yang I. 2022. A
pes-caprae (Linn.) R. Br.: A Review of Its Botany, Traditional Uses, Phytochemistry, and Bioactivity	pes-caprae (Linn.)	Convolvulaceae family, exhibit strong purgative or laxative effects, supporting its traditional use in relieving constipation and promoting bowel movement, alongside its antispasmodic and antiulcer activities.	medicinal halophyte *Ipomoea pes-caprae*(Linn.) R. Br.: a review of its botany, traditional uses, phytochemistry, and bioactivity. Mar Drugs. 20(5):329. doi:10.3390/md20 050329.
Gastrointestinal Fermentable Polysaccharide Is Beneficial in Alleviating Loperamide-Induc ed Constipation in Mice	*Ipomoea batatas*	The results suggest thatthe neutral polysaccharide with superior GI fermentation properties exerted beneficial effects on constipation, while the less	Liu B, Zhang Z, Liu X, Hu W, Wu W. 2023. Gastrointestinal fermentable polysaccharide is beneficial in alleviating loperamide-induce

		fermentable pectic fraction might act	d constipation in mice. Nutrients.

		as a stool-bulking agent.	15(20):4364. doi:10.3390/nu15204364.
Morning glory seed keeps laxative effect while retains less subchronic toxicity after being fried	Morning Glory Seeds (MGS)	While the MGS group demonstrated a stronger laxative effect by downregulating the AQP3 protein, it also resulted in lower body weights compared to theMGSF group, which contained less phenolic acids and more fatty acids.	Ren SM, Zhang W, Xu XJ, Zhou Y, Guo JY, Zhang XL, Wang DM, Pan YN, Liu XQ. 2020. Morning glory seed keeps laxative effect while retains less subchronic toxicity after being fried. J Ethnopharmacol. 251:112522. doi:10.1016/j.jep.2020.112522.
A comprehensive review on Turbud (*Operculina* turpethum L): A potential unani drug	Operculina turpethum	The chloroform and methanol extract of O. turpethum produced a significant ($P < 0.05$) dose and time dependent increase in the percentage of wet faeces. There was a dose dependent increase in the intestinal motility in the treated mice	Nafees H, Nafees S, Nizamudeen SA.2020. A comprehensive review on Turbud (*Operculina turpethum* (L.)): a potential unani drug. Int J Herb Med. 8(5):88-92. Available from: https://www.researchgate.net/publication/349337435_A_comprehensive_review_on_Turbud_Operculina_turpethum_L_A_potential_unani_drug

2.2 Local Related Studies

In the Philippines, *Ipomoea triloba* (locally known as "marakamote") is commonly found along roadsides, open fields, and disturbed areas, and has been recognized in local communities for its medicinal applications (StuartXchange, 2023). Leaf decoctions have traditionally been used to relieve stomach aches, indigestion, and abdominal discomfort, while poultices prepared from the

leaves are applied for headaches. These ethnobotanical practices highlight the cultural familiarity of *I. triloba* in Filipino folk medicine and justify scientific evaluation of its pharmacological activities, including laxative effects (Kokila and Jeevan 2021).

In a recent ethnobotanical survey of medicinal plants used by Indigenous People of Cawilan, Tubod (Surigao del Norte, Philippines), *Ipomoea triloba* was among the 25-plant list documented. The study recorded that the leaves of *I. triloba* were most frequently used, prepared as decoctions, topical applications, or poultices, to treat a variety of ailments including gastrointestinal discomfort and wounds (Adjarani et al. 2024). These findings underscore the plant's cultural importance and suggest a foundation for further pharmacological investigation.

A community-based ethnobotanical survey conducted among indigenous groups in Sto. Niño, Barangay Ambassador, Tublay (Benguet Province, Philippines) documented a variety of herbs used by locals for digestive ailments and general health care. Although the study did not isolate each plant's use individually, it listed *Ipomoea triloba* among the frequently cited herbal plants in the area, underscoring its cultural presence and local therapeutic relevance (Andalan et al. 2024).

2.3 Constipation

Constipation is a common gastrointestinal disorder characterized by infrequent bowel movements, hard stools, and difficulty in stool passage. It affects quality of life and is associated with multiple factors such as diet, lifestyle, medications, and underlying health conditions (Black et al. 2021).

2.3.1 Types of Constipation

Primary (functional) constipation refers to cases without any identifiable secondary cause. It is typically classified into normal-transit, slow-transit, and defecatory dysfunction types. In normal-transit constipation, patients experience hard stools and abdominal bloating despite normal stool frequency. Slow-transit constipation is associated with reduced colonic motility or delayed transit time, whereas defecatory dysfunction results from impaired pelvic floor coordination or dyssynergia during defecation (Bharucha and Lacy 2020; Vlismas et al. 2024; Fulan 2025).

Secondary constipation occurs when difficulty in bowel movement is caused by another factor such as medication or a systemic condition. One of the most common examples is opioid-induced constipation, which happens when opioid pain relievers, such as morphine or codeine, slow down the movement of the intestines by activating μ-opioid receptors in the gut. This reduced intestinal activity causes stool to remain longer in the colon, leading to excessive water absorption and hard, dry stools. It affects about 40–80% of patients who receive long-term opioid therapy (Argoff 2020; Camilleri et al. 2020).

2.3.2 Laxative Drugs

Laxative drugs are considered one of the primary treatments for constipation because they help restore normal bowel function. They work by softening the stool, enhancing intestinal motility, and increasing fluid secretion in the colon, stimulating intestinal nerves and smooth muscles, and promoting peristalsis. Additionally, some laxatives increase water and electrolyte secretion into the colon, facilitating stool passage. They are particularly useful for short-term relief of constipation and for patients with slow-transit bowel disorders (Bashir 2024; Corsetti et al. 2021; Bharucha and Lacy 2020).

Osmotic Laxatives (e.g., lactulose, polyethylene glycol) work by drawing water into the intestines, which increases stool water content, softens stools, and enhances bowel volume, thereby triggering natural peristaltic movements (Bashir 2024).

Bulk-Forming Laxatives (e.g., psyllium, dietary fiber) absorb water to form a gel-like mass in the

stool, increasing fecal bulk and stimulating intestinal motility.

These agents are generally considered safe for long-term use and help maintain regular bowel habits (Bashir 2024).

Lubricants and Stool Softeners (e.g., mineral oil, docusate sodium) facilitate easier stool passage. Mineral oil coats the stool and intestinal lining, retaining moisture, while docusate sodium reduces stool surface tension, allowing water and fats to penetrate the stool and soften it (Bashir 2024).

2.3.3 Limitations of Synthetic Laxatives

Synthetic laxatives are effective in relieving constipation, but prolonged or excessive use can cause side effects such as abdominal cramps, diarrhea, electrolyte imbalances such as losses of potassium and sodium. (Rao and Brenner 2021). Stimulant laxatives, in particular, can cause dehydration and disturbances in electrolytes, including potassium and sodium, which may affect normal muscle and nerve function (Bashir and Sizar 2024). Over time, habitual use can lead to laxative dependency, where the colon requires external stimulation to function properly and loses some of its natural contractility (Bashir and Sizar 2024). These drawbacks highlight the potential value of exploring natural alternatives that may support bowel regularity with fewer side effects.

2.3.4 Bisacodyl

In experimental pharmacology, bisacodyl serves as a positive control in laxative activity studies because of its well-defined mechanism and consistent ability to stimulate bowel movement. Bisacodyl is a stimulant laxative that works directly on the colon to increase muscle movement, speed up stool passage, and draw water into the intestines to help bowel movements (Corsetti et al. 2021; Lawrensia et al. 2024). Because of its rapid and predictable laxative effect, bisacodyl is commonly used as a standard reference drug in studies evaluating the laxative activity of plant extracts and natural compounds. It is effective in managing various types of constipation, including slow-transit and functional constipation, as well as secondary constipation associated with medications or disease (Corsetti et al. 2021). In this study, bisacodyl was used solely as a reference standard for comparison in a loperamide-induced primary (acute) constipation model. Its well-established mechanism of action and consistent efficacy made it an appropriate standard for assessing the laxative potential of *Ipomoea triloba* leaf extract (Corsetti et al. 2021).

2.3.5 Loperamide-Induced Constipation Model

Loperamide is a synthetic opioid that primarily targets μ-opioid receptors in the gastrointestinal tract. Activation of these receptors reduces the release of excitatory neurotransmitters in the gut, which slows intestinal muscle contractions and peristalsis. As a result, intestinal transit time is prolonged, and water is absorbed for a longer period from the stool, leading to harder, drier, and fewer feces (Sahi 2024).

Because of these effects, loperamide is widely used to induce constipation in laboratory animals, particularly mice. This model is valuable for evaluating the laxative potential of natural products and pharmacological agents. Substances are considered effective if they increase fecal output, soften stool consistency, and reduce intestinal transit time, counteracting the effects of loperamide.

The loperamide-induced constipation model is preferred in research because it is reproducible, straightforward, and reproduces key features of constipation in rodents, making it a reliable tool for screening potential laxative agents and studying underlying mechanisms (Yao et al. 2022).

2.3.6 Review of Experimental Parameters for Evaluating Laxative Effects

Fecal analysis and intestinal transit percentage are widely accepted and reliable methods for evaluating the laxative activity of plant extracts in experimental models. The two primary parameters measured are fecal output and gastrointestinal motility. Fecal output was determined by fecal pellet count, fecal water content, and fecal wet weight. Pellet count reflects the frequency of bowel movements and stool output, indicating the extract's effect on overall bowel function. While gastrointestinal motility tests measure the movement of intestinal contents, together these assays provide complementary information, offering a more complete assessment of the extract's laxative activity. Water content indicates the level of hydration in the stool and reflects the extract's ability to enhance intestinal secretion and reduce water reabsorption, resulting in softer feces and easier defecation. Wet weight represents the total mass of feces excreted and demonstrates the extract's ability to promote stool bulk and passage. Gastrointestinal motility is assessed as the percentage of intestinal transit, providing a direct measure of how efficiently the bowel moves its contents. Together, these parameters provide standardized and quantifiable methods for comparing the efficacy of different plant extracts in promoting bowel function

Recent studies have confirmed the reliability of these parameters in assessing the laxative potential of Convolvulaceae members. The 70% ethanol leaf extract of *Convolvulus spinosus* significantly increased fecal pellet count and intestinal transit in constipated mice, demonstrating a strong laxative effect (Arif et al. 2023). Similarly, *Ipomoea batatas* polysaccharides improved fecal pellet count and fecal wet weight in loperamide-induced constipated mice, supporting its traditional use as a laxative (Liu et al. 2023). In another study, Morning Glory Seeds (MGS) promoted fecal pellet count and wet fecal weight while modulating AQP3 protein expression, indicating both efficacy and mechanistic insights into laxative action (Ren et al. 2020). *Operculina turpethum* extracts also enhanced fecal pellet count, wet fecal weight, fecal water content, and intestinal transit in a dose-dependent manner in mice (Nafees et al. 2020).

Some studies additionally measured intestinal transit percentage to complement fecal analysis, which provides insight into the speed of gastrointestinal motility. Collectively, these post-2020 studies highlight the consistent use of fecal pellet count, fecal wet weight, fecal water content, and intestinal transit in evaluating the laxative activity of Convolvulaceae plants.

2.3.7 Charcoal Meal Test

The charcoal meal test is a widely used method in animal pharmacology to evaluate intestinal motility by administering an inert marker (activated charcoal) and measuring its transit through the small intestine. For example, in a laxative study the charcoal meal's progression was used to assess how a root extract affected small intestinal transit in mice (Ayele et al. 2022). Similarly, another study measured transit using charcoal meal in a mouse model of reduced motility (Chen et al. 2022). In this procedure, activated charcoal acts as an inert and non-absorbable marker that moves through the small intestine, allowing researchers to assess the influence of test substances on intestinal transit. These methods confirm that charcoal-based transit tests are established models for studying how test substances influence gut movement (Tandur et al. 2022).

The suspension is typically prepared using 10% (w/v) activated charcoal in 5% acacia solution, in which acacia functions as a suspending agent to maintain even dispersion of the charcoal particles. Activated charcoal is widely used in this test because it is non-toxic, easily visible, and physiologically inert, making it suitable for accurate assessment of motility without interfering with normal digestion. An increased distance traveled by the charcoal marker along the intestine indicates

enhanced motility, suggesting a potential laxative effect, a preparation and method that have been validated in mice (Ayele et al. 2022).

2.3.8 Ethical Considerations in Animal Assays

Ethical considerations are essential in studies involving laboratory animals to ensure humane treatment and scientific validity. Experimental procedures are typically conducted in accordance with established guidelines such as Organisation for Economic Co-operation and Development Guideline 425 for acute toxicity testing. These guidelines emphasize minimizing animal suffering, using the least number of animals necessary, and ensuring proper housing, handling, and care (OECD, 2022).

2.4 Theoretical Framework

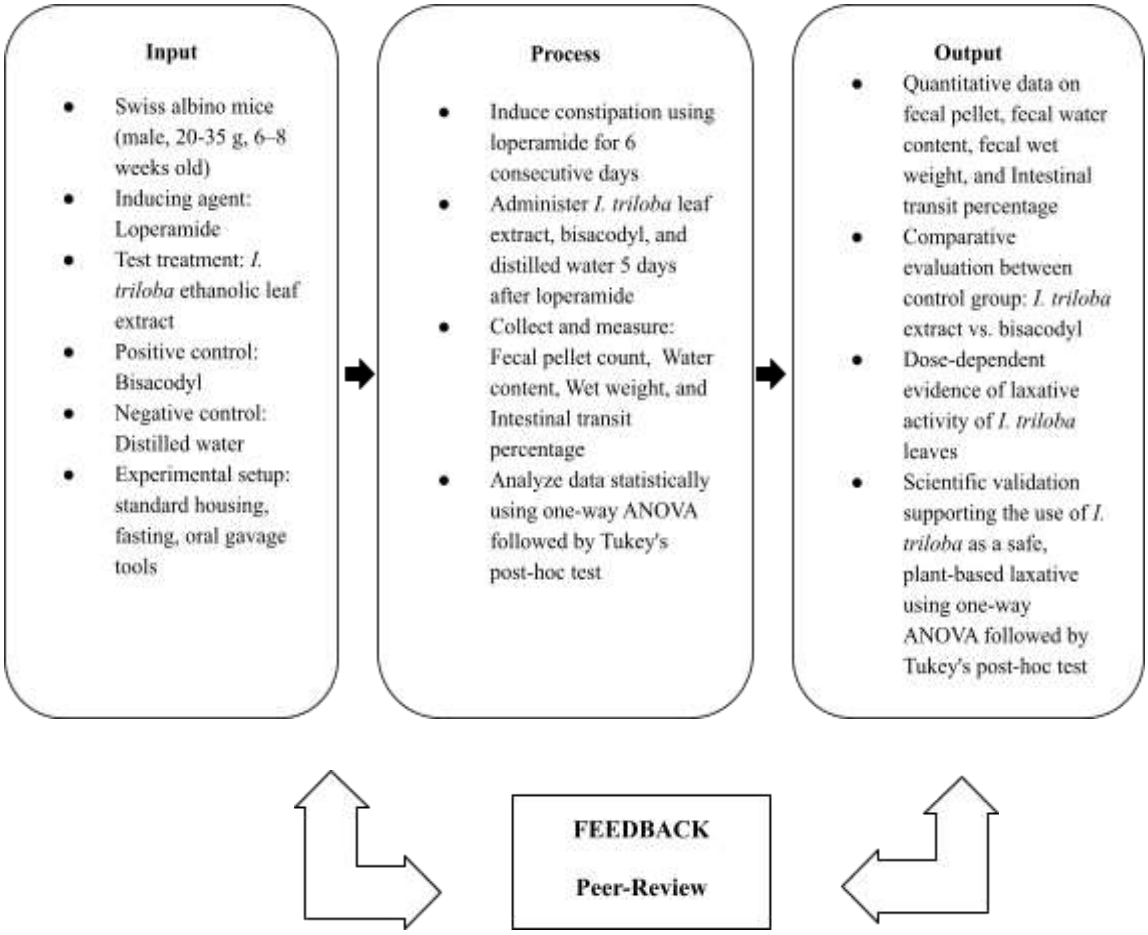


Figure 3. Theoretical framework of the study

In the theoretical framework of this study, a system approach was utilized to explain how the researchers investigated the potential laxative effects of *Ipomoea triloba* ethanolic leaf extract *in vivo*. The framework consisted of three major components: input, process, and output. The input included the ethanolic leaf extract of *Ipomoea triloba* administered at varying doses as determined from the OECD toxicity test, the experimental animals (male Swiss albino mice), and the test substances and comparators. These included loperamide as the constipation-inducing agent, bisacodyl as the positive control, and distilled water as the negative control.

The process involves the interaction of these substances within the gastrointestinal system of the experimental animals. Loperamide induces constipation by decreasing intestinal motility and prolonging transit time, resulting in reduced stool frequency and harder stool consistency. In contrast,

laxative agents such as the plant extract and bisacodyl are expected to counteract these effects by enhancing intestinal motility, improving water secretion, and facilitating stool passage.

The output refers to the observed physiological responses, which include changes in fecal output and gastrointestinal motility. These outcomes serve as indicators of laxative activity and provide evidence for the potential therapeutic effect of *Ipomoea triloba* as a plant-based laxative.

2.5 Synthesis and Summary of Related Literature

The Convolvulaceae family has been recognized in traditional medicine for its therapeutic potential, including uses related to digestive health. Several species within this family have demonstrated bioactive properties such as antioxidant, antimicrobial, and anti-inflammatory activities, which support their pharmacological relevance.

Ipomoea species, in particular, have been noted for their physiological effects on the digestive system. For example, *Ipomoea batatas*, *Ipomoea pes-caprae (Linn.)*, *Convolvulus spinosus* Burm.f., have demonstrated bowel-stimulating and purgative actions due to the presence of secondary metabolites. These findings highlight the therapeutic potential of this genus as a natural source of compounds that may influence intestinal motility and promote bowel evacuation.

Among these, *Ipomoea triloba* (Morning Glory) has attracted interest for its diverse biological effects. Although its traditional use includes applications for digestive wellness, its laxative activity has not been extensively documented through scientific validation. Evaluating this aspect is valuable for confirming traditional claims and providing evidence-based support for its potential role in promoting bowel regulation.

This study aims to evaluate the laxative activity of *I. triloba* leaves to validate their traditional use and contribute to the growing body of research on plant-based approaches for maintaining healthy digestive function.

CHAPTER III MATERIALS AND METHODOLOGY

This chapter describes the experimental procedures that were used to evaluate the potential laxative activity of the ethanolic leaf extract of *Ipomoea triloba*. It also presents the materials and instruments utilized, as well as the methods employed for data collection and statistical analysis. Furthermore, it outlines the experimental design, including the grouping of test subjects and the administration of treatments. The parameters measured, such as intestinal motility and fecal output, are described to provide a clear basis for data interpretation.

3.1.Plant Collection and Material Identification

Fresh leaves of *Ipomoea triloba* were collected from Brgy. Tubod, Iligan City, Lanao del Norte, Philippines (Latitude: ~ 8.2117° N and Longitude:
~ 124.2410° E). The site was selected based on the accessibility of mature *Ipomoea* plants. After harvesting, the leaves were cleaned with running tap water to remove soil, dust, and possible surface contaminants, followed by rinsing with distilled water to ensure purity of the sample. The plant material was then authenticated by a botanist from the Department of Biological Sciences, Mindanao State University–Iligan Institute of Technology (MSUIIT), and a voucher specimen was prepared and deposited in the herbarium for proper identification and future reference. Once the plant material was identified, the leaves were shade-dried at room temperature (25–35 °C) for two weeks to prevent degradation of heat-sensitive and light-sensitive phytochemicals, thereby preserving the plant's biological activity. After drying, the air-dried plant material was pulverized into a fine powder using a household electric blender (BOBI Electric Powder Machine, Philippines).

3.1.1 Reagents and Chemicals Used

The study employed analytical-grade reagents and solvents to ensure accuracy, reliability, and reproducibility of results. Ethanol (90%) was used as the primary extraction solvent during maceration. Ethanol was chosen due to its safety, polarity, and effectiveness in extracting a broad range of bioactive metabolites. This approach aligned with recent literature supporting cold maceration as a reliable and practical method for obtaining bioactive compounds from medicinal plants for pharmacological evaluation (Bhandare & Kardile, 2025; Bitwell et al. 2023). Distilled water was used throughout the study for dilutions, washing, and solvent adjustments, while Whatman No. 1 filter paper with a particle size of approximately 11 µm and a thickness of approximately 180 µm was employed for filtration to separate plant residues from liquid extracts.

In the qualitative confirmatory tests, saponins were detected using distilled water to create stable froth, while flavonoids were identified by the formation of a yellow precipitate upon the addition of a 10% lead acetate solution (Sharma et al. 2023).

For preparation of the oral administration, 1% Tween-80 (polyoxyethylene sorbitan monooleate) and distilled water were used to prepare the extract suspension. In the acute oral toxicity test following OECD 425, the same vehicle (distilled water + 1% Tween-80) was used as the dosing medium for the ethanolic extract, which was administered via oral gavage. Tween-80 acted as a suspending agent, improving the dispersion of the extract in the vehicle and ensuring that the extract remained evenly suspended, preventing sedimentation or aggregation of particles that could have led to inconsistent dosing (Ravichandran et al. 2021; Taher et al. 2024).

To maintain aseptic conditions during handling and dosing, 70% ethanol or an equivalent disinfectant solution was used for sterilizing gavage needles and equipment before and after administration (Ayele, 2024; OECD, 2022; Arif et al. 2023).

All reagents and solvents were of analytical grade and were procured from Nanjing Forever Pharmacy (27th, Nanjing Xin'gang Development Zone, Jiangsu Province, China), a certified supplier providing Certificates of Analysis (COA) and Safety Data Sheets (SDS) to ensure product quality and authenticity. All chemicals were stored under recommended conditions to preserve stability and safety prior to use (JBL Scientific, 2024).

3.1.2 Instruments to be Used

The study utilized a range of instruments and laboratory equipment essential for extraction, phytochemical confirmatory, toxicity testing, and in vivo evaluation of the laxative effects of *Ipomoea triloba*. A No. 120 mesh sieve (≈0.125 mm) was used to obtain a uniform and fine particle size. An Erlenmeyer flask and orbital shaker set at 100 rpm were used during the maceration process to ensure thorough solvent interaction with the powdered leaf material. The ethanolic extract was concentrated under reduced pressure (approximately 100–150 mbar) at a water bath temperature of 45 °C using a Digital Water Bath (Premiere HH-6-YCYDW2370054) to gently remove the solvent while minimizing thermal degradation of heat-sensitive constituents. An evaporating dish served as the container for evaporation. A clean airtight container was used to store the final extract at 4 °C to maintain stability.

For phytochemical confirmatory tests, test tubes served as reaction vessels for all qualitative tests. A glass rod was used for mixing solutions, and droppers were employed for the precise addition of reagents. Beakers and measuring cylinders were used to prepare solutions and ensure accurate volume measurements.

For the acute oral toxicity study and *in vivo* experiments, individual metabolic cages housed each

mouse to prevent cross-contamination and enable accurate fecal monitoring. An analytical balance with 0.001 g sensitivity was used to weigh fecal pellets. Sterile forceps were employed for handling fecal pellets during collection to maintain sample integrity. Gavage needles attached to 1 mL syringes were used to administer all treatments and test substances orally, ensuring controlled dosing. Metabolic cages were used during extended fecal collection periods to prevent urine and food contamination.

These instruments and materials were integral to ensuring methodological accuracy, animal welfare, and the reliability of the phytochemical and pharmacological assessments in this study.

3.2 Research Flow

Figure 4. Flowchart of the Study Process

3.3 Crude Drug Extraction by Maceration

The extraction of the plant sample was conducted using the cold maceration method, a widely recommended approach for extracting phytochemical constituents from medicinal plants while preserving thermolabile compounds (Bhandare & Kardile, 2025; Bitwell et al. 2023). The dried leaf powder of *I. triloba* weighing 20 g was used for extraction. The powdered sample was sieved through a No. 120 mesh sieve (≈0.125 mm) to obtain a uniform and fine particle size. The powder was then soaked in 90% ethanol at a ratio of 1:10 (w/v) for 72 hours, with continuous shaking using an orbital shaker set at 100 rpm to improve solvent contact. The prolonged soaking ensured efficient mass transfer between the solvent and plant material, maximizing extraction yield while

preventing compound degradation (Haido et al. 2024). After soaking, the mixture was filtered using Whatman No. 1 filter paper to separate the liquid extract from the solid residue. The filtrate was concentrated under reduced pressure using a digital water bath (Premiere HH-6-YCYDW2370054) at 40°C. The concentrated extract was then transferred to empty jar containers to facilitate easier use during subsequent procedures.

3.3.1 Percentage Yield

A representative 20 g portion of the dried powder was used to determine the percentage yield of the ethanolic extract, which was then applied to the entire sample. After extraction and drying, the weight of the obtained extract was measured using an analytical balance. The percentage yield was calculated using the formula:

$$\text{Yield (\%)} = \frac{\textit{Weight of dried extract}}{\textit{Weight of dried leaves}} \times 100\ \%$$

Where the weight of dried extract referred to the mass of the extract obtained after solvent removal, and the weight of dried powdered leaves referred to the initial mass of the plant material subjected to extraction. A higher percentage yield indicated a more efficient extraction process, suggesting that a greater proportion of ethanol-soluble phytoconstituents was obtained from the *I. triloba* leaves. Factors such as solvent polarity, extraction duration, particle size, and drying conditions influenced the final yield.

The determination of the percentage yield was performed in triplicate analysis to ensure accuracy and reliability of the results. The average yield was calculated and expressed as mean ± standard deviation (SD) to reflect the precision of the data.

3.4 Preparation of the Extract

The ethanolic extract of *Ipomoea triloba* leaves was formulated as a uniform mixture in distilled water, following established methods for plant extracts intended for *in vivo* administration (Alozie et al. 2024). The crude extract was directly dispersed in distilled water prior to each administration, and the dose was calculated based on the body weight of each mouse. Since the extract showed poor solubility, 1% Tween-80 (v/v) was incorporated as a suspending agent to facilitate uniform dispersion.

Dose levels were selected based on the results of the acute oral toxicity limit test, conducted according to OECD 425 guidelines (Up-and-Down Procedure, 2022) (OECD, 2022). To evaluate potential dose-dependent effects while maintaining animal safety, lower doses were chosen as fractional proportions of the safe limit—specifically ½ (1000 mg/kg) and ¼ (500 mg/kg) of the limit dose of 2000mg/kg following the approach recommended for *in vivo* studies when the maximum safe dose has been established. Each mixture was gently homogenized before administration to ensure a smooth and uniform consistency.

3.5 Confirmatory Test of Plant Extract

To confirm the presence of phytochemical constituents in the crude extract, a qualitative phytochemical confirmatory test was conducted. The table below illustrates the selected phytochemical constituents, the corresponding confirmatory tests, and the outcomes that confirmed the presence of flavonoids and saponins in the ethanolic leaf extract of *Ipomoea triloba*.

Table 5. Phytochemical Confirmatory Tests Conducted on *I. triloba* Ethanolic Leaf Extract

Phytochemical Constituents Test	Procedure	Positive Indication
Flavonoids (Lead Acetate Test)	10 mg of the extract is treated with a few drops of 10% lead acetate solution. (Sharma et al. 2023)	The formation of a yellow precipitate indicates the presence of flavonoids. (Sharma et al. 2023)
Saponins (Froth Test)	5 mg of the extract were combined with 20 ml of distilled water. The mixture is then agitated for 15 minutes, then allowed to stand for 10–15 minutes (Sharma et al. 2023; Aliyu et al. 2024).	Formation of a stable froth layer (≥ 1 cm height) persisting for at least 10 minutes indicates the presence of saponins (Sharma et al. 2023; Aliyu et al. 2024).

3.6 Toxicity Testing and Lethal Dose (OECD 425 Acute Oral Toxicity - Limit Test Procedure)

A limit test was performed using a single high dose of the extract (2000 mg/kg) to determine whether it caused any signs of toxicity or mortality. No adverse effects were observed, and therefore, the main test was not conducted, as the extract was considered safe at the limit dose.

At the end of the acute toxicity study, all animals were sacrificed, and a gross necropsy was performed to examine major organs for any visible signs of toxicity or pathological changes, in accordance with OECD 425 guidelines (OECD, 2022).

3.6.1 Animal Selection

Mice are chosen because they have a relatively short gastrointestinal transit time, which makes them suitable for evaluating drugs that affect bowel motility. Their previous use in similar pharmacological evaluation, such as constipation and diarrhea models, further supports their suitability. Male Swiss albino mice weighing 20–35 g and aged 6–8 weeks were used as the experimental model, as this range represents young adult mice with relatively stable metabolism and sensitivity to pharmacological agents (Malik et al. 2022; Gao et al. 2022; Ayele and Kawet, 2024).

All animals were sourced from a reputable breeding facility to ensure they are healthy and disease-free at the start of the study. An application for animal use permit was submitted to the Adventist Medical Center College (AMCC) Institutional Animal Care and Use Committee (IACUC) prior to the conduct of the experiment.

3.6.2 Housing and Feeding Condition

In accordance with OECD 425, mice were acclimatized for seven days before testing to reduce stress and permit baseline stabilization.

A total of five mice were used for the acute oral toxicity study following OECD 425 limit test guidelines, with animals dosed sequentially to allow careful observation of any signs of acute toxicity before proceeding to the next animal. The animals were observed closely for clinical signs, mortality, and changes in body weight over a 14-day period, and all procedures were conducted in accordance with standard ethical guidelines for animal care. The mice were housed on softwood shavings (Chipsi® brand) under standard laboratory conditions maintained at 21–24 °C (70–75 °F), with 70% relative humidity, adequate ventilation, and a 12-hour light/12-hour dark cycle. Environmental parameters followed internationally accepted housing standards for laboratory mice to

ensure welfare, comfort, and reproducibility of experimental results (Jackson Laboratory, 2024; NC3Rs, 2023; UCLA Research Safety & Animal Care, 2024). They were fed Integra 3000® rodent pellets and provided with water ad libitum, meaning with free and unlimited access. The Integra 3000® rodent pellet served as a nutritionally complete maintenance diet. Cages were cleaned two to three times per week using water and liquid soap, and bedding was replaced two to three times per week to keep the animals clean, dry, and relatively odor-free. The described housing, feeding, and handling procedures were consistent with standard laboratory practices and were applied in both the actual experimental study and the acute oral toxicity testing.

3.6.3 Limit Test and Dose Administration

The ethanolic extract of *Ipomoea triloba* leaves was administered orally by gastric gavage as a single limited dose of 2000 mg/kg body weight to five (5) mice, which were dosed sequentially in accordance with OECD 425. Before dosing, the animals were fasted for 4 hours but had free access to water, and food was reintroduced 2 hours after dosing to standardize gastrointestinal conditions.

After administration, the animals were closely monitored at specific time intervals to detect any signs of acute toxicity (OECD, 2022). On Day 1, observations were made immediately after dosing (0 minutes), then at 15, 30, 60, 120, and 240 minutes. The first three observations (0–30 minutes) were taken at 15-minute intervals to capture rapid-onset reactions such as tremors, salivation, or hypoactivity. The next observations at 60 and 120 minutes represented hourly checks designed to detect slightly delayed responses that could develop as the extract was absorbed and distributed. The final observation at 240 minutes (4 hours post-dose) ensured detection of any later-onset acute effects within the early post-treatment period.

During the first 24 hours, animals were then checked every 6 hours—specifically at 6, 12, 18, and 24 hours post-dosing—to identify early-delayed signs that might not appear immediately but could develop within the first day (e.g., changes in posture, grooming, or food intake). From Day 2 through Day 14, monitoring continued once daily to detect delayed or progressive toxic manifestations such as weight loss, reduced motor activity, or organ-related effects.

The remaining mice were administered the limit dose of 2000 mg/kg sequentially. This stepwise approach was employed to minimize the risk of mortality by allowing close monitoring of each animal's response before treating additional mice. Parameters such as body weight (recorded on Days 1 [pre-dose], 2, 7, and 14), food and water intake (recorded daily), grooming behavior, and general activity were monitored. Daily cage-side observations and body-weight tracking throughout the 14-day period were standard practice in acute oral toxicity studies and allowed detection of both immediate and delayed physiological changes (OECD, 2022).

3.7 Loperamide-Induced Constipation Model

3.7.1 Animal Selection

To maintain consistency across both the OECD-style limit/acute toxicity assessment and the subsequent laxative (GI motility) experiments, male mice were used throughout. A total of 50 mice were used for two experimental models. 25 sets of mice were used for fecal output analysis, while the other 25 mice were used for gastrointestinal motility testing.

3.7.2 Housing and Feeding Condition

Each mouse was housed individually in a labeled metabolic cage to prevent mixing of fecal samples. The custom-designed cages measured 20 × 20 × 20 cm and were fabricated from Stainless SUS304 Wire Mesh to ensure corrosion resistance. The walls and raised floor consisted of stainless-steel wire (1.0–1.2 mm diameter) arranged in a 12 × 12 mm grid, which prevented escape while allowing efficient passage of fecal matter. The mesh floor was positioned 3–5 cm above a removable tray lined with pre-weighed Whatman No. 1 filter paper to collect fecal samples and maintain cleanliness. Each cage featured a latched top cover for easy access, and a non-drip water bottle was mounted externally, with the drinking tube extending into the cage. During the study, mice were provided Integra 3000® rodent pellets and were allowed ad libitum access to water to maintain normal feeding conditions (Gao et al. 2022; Ayele and Kawet, 2024).

3.7.3 Loperamide-Induced Constipation Model Assay and Dose Administration

Constipation was induced in all groups by orally administering loperamide at a dose of 5 mg/kg body weight. The loperamide was dissolved in distilled water at a volume of 10 mL/kg per mouse, with an additional 10% volume prepared to account for losses during gavage. Mouse body weights were converted to kilograms to calculate the exact dose per animal, and the solution was freshly prepared each day to ensure stability and consistency (Arif et al. 2023).

During the six-day induction period, fecal output was recorded every day to assess the effectiveness of constipation induction. Successful induction was indicated by a reduction in both the number and weight of fecal pellets, reflecting delayed intestinal transit caused by loperamide (Ge et al. 2025). Handling was limited to dosing and observation to minimize stress that could affect gastrointestinal motility.

After induction, mice were assigned to negative control (distilled water), positive control (bisacodyl), and test groups receiving *Ipomoea triloba* extract per experiment.

3.8 Administration of Test Material

3.8.1 Selection and Administration of Doses

Dose levels for the *Ipomoea triloba* ethanolic leaf extract were set at 500 mg/kg (low), 1000 mg/kg (medium), and 2000 mg/kg (high) based on an acute oral toxicity limit test following the OECD 425 guideline (OECD, 2022). The 2000 mg/kg dose served as the limit dose used in acute toxicity testing, while lower doses were chosen as fractional proportions (½ and ¼ of the limit dose) to allow evaluation of dose-dependent effects while minimizing the risk to animals. (Ayele and Kawet, 2024; OECD, 2022).

After completing the six-day induction phase, the treatment period was conducted the following five days, during which all groups received their assigned treatment once daily by oral gavage. The negative control received distilled water at 10 mL/kg , the positive control received bisacodyl at 0.25 mg/kg (Ayele et al. 2022; Ayele and Kawet, 2024) , and the treatment groups received low, medium, and high doses of *Ipomoea triloba* extract All mice in the fecal output experiment underwent a 12-hour fasting period prior to receiving the fifth and final treatment dose, with water provided throughout the fasting period. After the final administration, fecal parameters—including pellet count, wet weight, and water content—were collected over a 24-hour period (Arif et al.2023)

A separate set of twenty-five mice was used for the gastrointestinal motility (charcoal meal) test. These mice followed the same acclimatization, constipation induction, and treatment schedule. (Arif et al. 2023).

3.8.2 Experimental Grouping Laxative Property

Table 6: Experimental grouping of mice for the evaluation of the laxative activity of Ipomoea triloba leaf extract.

Test Groups	Treatment/Dose	Number of Animals
Negative Control	Distilled water (vehicle), 10 mL/kg, p.o.	5
Positive Control	Bisacodyl 0.25 mg/kg, p.o.	5
Ipomoea triloba Low Dose	*Ipomoea triloba* leaf extract – 500mg/kg p.o.	5
Ipomoea triloba Mid Dose	*Ipomoea triloba* leaf extract – 1000mg/kg, p.o.	5
Ipomoea triloba High Dose	*Ipomoea triloba* leaf extract – 2000mg/kg, p.o.	5

Two separate sets of mice were used, with 25 animals per experiment. One set was used for fecal output analysis, while the other was used for gastrointestinal motility testing. Each set consisted of five groups of five mice, corresponding to the negative control, positive control, and three treatment doses of *Ipomoea triloba* extract.

3.8.3 Data Collection

After treatment administration, each mouse was placed individually in a clean metabolic cage lined with white Whatman No. 1 filter paper for hygienic collection and visualization of fecal pellets (Gao et al., 2022; Jeong et al., 2023). Fecal pellets from each mouse were collected at two-hour intervals over a span of 12 hours. After each collection, fresh labeled filter paper was placed in the cage for the next hour to prevent mixing of old and newly excreted pellets.

This duration aligned with established laxative study protocols that monitored acute defecation responses while minimizing animal stress (Kongdang et al. 2022; Ayele and Kawet, 2024). Fecal pellets were counted immediately to determine the total number per mouse and then weighed promptly using a calibrated digital balance to obtain fecal wet weight. Prompt weighing ensured accuracy by preventing moisture loss. The feces were then dried at room temperature for 24 hours before being reweighed to assess the fecal water content. Formula (formula-1) was used to compute the percentage of fecal water content, as follows:

$$\frac{weight\ of\ wet\ fecal\ matter - weight\ of\ dry\ fecal\ matter}{weight\ of\ wet\ fecal\ matter}$$

Data recorded included animal identification, treatment group, total pellet count, wet fecal weight, and water content. These parameters were used to evaluate the laxative activity of *Ipomoea triloba* leaf extract, where higher fecal output reflected improved intestinal motility and bowel evacuation Gao et al. 2022; Jeong et al. 2023).

3.9 Intestinal Motility Test

3.9.1 Gastrointestinal Motility Testing (Charcoal-Meal Assay)

The gastrointestinal motility test was carried out using the charcoal-meal method to evaluate the effect of the *Ipomoea triloba* ethanolic leaf extract. This method measured the distance traveled by a charcoal marker through the small intestine, serving as an indicator of intestinal propulsion (Tessema et al. 2020).

In a separate set of mice designated for this experiment, each animal underwent a 12 hour fasting period prior to receiving the fifth and final treatment dose. Twenty minutes after the treatment, a freshly prepared charcoal-meal suspension (10% w/v activated charcoal in 5% gum acacia) was administered orally at a volume of 1 mL/100 g body weight (10 mL/kg) (Arif et al. 2023). Twenty minutes after charcoal administration, the mice were humanely euthanized, and the entire small intestine was carefully excised from the pylorus to the cecum. The intestine was gently laid on a flat surface without stretching, and both the total length of the small intestine and the distance traveled by the charcoal marker were measured in centimeters (Arif et al. 2023; Ugwah-Oguejiofor et al. 2022; Dong et al. 2020).

3.9.2 Data Collection

Intestinal propulsion was assessed by measuring the distance traveled by the charcoal front from the pylorus to the cecum, which was expressed as percentage intestinal transit (Tessema et al. 2020; Ugwah-Oguejiofor et al. 2022; Jeong et al. 2023). The percentage intestinal transit was calculated using the formula

$$\text{Percentage intestinal transit (\%)} = \left(\frac{\textit{Distance traveled by charcoal}}{\textit{Total length of small intestine}}\right) \times 100\ \%$$

A higher percentage of intestinal transit will be interpreted as enhanced gastrointestinal motility, indicating the laxative activity of the extract. The collected data were encoded and analyzed to determine the laxative efficacy of *Ipomoea triloba* leaf extract, with comparisons made to the negative and positive control groups (Tessema et al. 2020). The analysis also assessed whether the extract exhibited dose-dependent effects on intestinal motility and verified the consistency of the responses.

3.10 Data Analysis

The data were expressed as mean (M) ± standard deviation (SD) to describe the average values and variability of each measured parameter across treatment groups. Comparisons among groups (negative control, positive control, and low, medium, and high doses of *Ipomoea triloba* extract) were performed using one-way analysis of variance (ANOVA). A *p*-value of less than 0.01 ($p < .001$) was considered statistically significant. When significant differences were detected (Arif et al. 2023), Tukey's Honestly Significant Difference (HSD) test was applied for post hoc multiple comparisons to determine which specific groups differed.

For outcomes involving multiple dependent variables, multivariate analysis of variance (MANOVA) was conducted using Pillai's Trace to assess the overall treatment effect. In addition, the Kruskal–

Wallis test, a non-parametric alternative to ANOVA, was performed as a sensitivity analysis to confirm the consistency and robustness of the findings.

Fecal pellet count, fecal water content, fecal wet weight, and gastrointestinal motility percentage were analyzed. An increase in these parameters relative to the constipation control (loperamide-treated group) indicated a laxative effect.

The interpretation of the statistical data focused on whether the ethanolic leaf extract of *Ipomoea triloba* produced an increase in intestinal transit and fecal output compared to the negative control, with effects compared to the positive control (bisacodyl) to evaluate the relative laxative activity of different extract doses.

All statistical analyses were performed using R version 4.5.2, ensuring accurate computation, proper data handling, and reproducibility of results. Graphical representations were presented in tables and figures. The selected statistical approach is consistent with current pharmacological motility studies in rodent models (Arif et al. 2023).

CHAPTER IV RESULTS AND DISCUSSION

This chapter presents and discusses the findings of the study on the laxative potential of the ethanolic leaf extract of *Ipomoea triloba* in loperamide-induced constipated Swiss albino mice. The results cover fecal-output parameters and intestinal transit using low, mid, and high doses of the extract, along with a positive control (bisacodyl) and a negative control. Data were analyzed using appropriate statistical tools, including ANOVA and post hoc tests, to determine significant differences among groups.

4.1 The Plant Extract

The crude ethanolic whole plant extract of *Ipomoea triloba* is dark greenish-black, semisolid in consistency, characterized by a distinct earthly odor and a slimy, sticky texture.

Figure 5. Crude ethanolic extract of *I. triloba*

4.2 Percentage Yield of *Ipomoea triloba*

Table 7. Percentage Yield of the Crude Ethanolic Extract from *Ipomoea triloba* Leaves

Trial	Initial Weight of Dried Leaves	Final Weight of Crude Extract	Total Percentage Yield
1	20 g	2.3 g	11.5%
2	20 g	2.8 g	14%
3	20 g	2.9 g	14.5%
Average	20 g	2.67 g	13.33%
		Mean SD	13.33 ± 1.61%.

Table 7 presents the results of the extraction process for *Ipomoea triloba*. From an initial weight of 20 g of dried leaves per trial, the extraction yielded an average weight of 2.67 g of crude extract, resulting in a mean percentage yield of 13.33% with a standard deviation of ±1.61%

4.3 Phytochemical Confirmatory Test

Qualitative phytochemical confirmatory tests were conducted at the AMCC Pharmacy Laboratory.

Table 8. Phytochemical Analysis of *Ipomoea triloba* Extract

Phytochemical Constituent	Result	Evidence
Flavoids (Lead Acetate Test)	Positive	

Saponins (Froth Test)	Positive	

Table 8 presents the phytochemical screening results of the *Ipomoea triloba* extract, which revealed the presence of flavonoids and saponins. The presence of flavonoids was indicated by the development of a yellow coloration during the test, while the presence of saponins was confirmed by the formation of stable froth observed after shaking. These positive reactions indicate that the extract contains the said phytochemical constituents.

4.4 Isolation and Health Assessment of Experimental Mice

A total of sixty (60) Swiss albino mice (*Mus musculus*) were obtained from a local breeder, CDO Mice Breeder, located at Indahag Habitat, Cagayan de Oro. The animals were bred and maintained under controlled conditions, with proper housing, adequate ventilation, appropriate cage density, and continuous access to balanced feed and clean water.

The health status and origin of the animals were verified through an official animal source and health declaration provided by the local breeder upon acquisition. The document indicated that the mice were healthy, active, and free from visible signs of disease based on routine monitoring, and were suitable for research purposes.

Throughout the acclimatization and pre-experimental period, the mice exhibited normal physical appearance, activity levels, feeding behavior, and responsiveness. No signs of distress, disease, or abnormality were observed.

Before beginning the experimental procedures, the study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), ensuring that all methods complied with established ethical guidelines for the humane use of laboratory animals.

Based on the documented health status and direct observation during acclimatization, all experimental animals were confirmed to be in good condition and suitable for use in the study. There was no evidence of any underlying condition that could influence the experimental outcomes.

4.5 OECD 425 Guided Experimental Framework Table 9. Necropsy Result

Test Subject	Liver (Weight in g)	Spleen (Weight in g)	Spleen (Length in mm)	Right Kidney (Weight in g)	Left Kidney (Weight in g)
M1	1.849g	0.19g	22 mm	0.205g	0.200g
M2	1.48g	0.14g	18 mm	0.269g	0.269g
M3	1.38g	0.19g	21 mm	0.226g	0.218g
M4	1.01g	0.15g	20 mm	0.291g	0.267g
M5	1.20g	0.17g	20 mm	0.248g	0.242g

The experimental framework followed the Organisation for Economic Co-operation and Development Guideline 425 for acute oral toxicity using the up-and-down procedure. Prior to dosing, all experimental animals were acclimatized under standard laboratory conditions with

adequate food and water to ensure stable physiological status.

A limit dose of 2000 mg/kg of *Ipomoea triloba* crude extract was administered orally to five mice over two days in a sequential manner, with doses calculated individually based on body weight. During the 14-day observation period, no mortality was observed in any of the animals.

All mice exhibited normal behavior, with no significant changes in movement, posture, respiration, or other clinical parameters. Body weights of all animals showed a gradual increase over time, indicating normal physiological condition. Necropsy findings revealed no visible lesions or abnormalities in major organs, including the liver, spleen, and kidneys.

4.5.1 Selection of Treatment Dose Levels

The selection of treatment doses was based on the results of the acute oral toxicity test conducted in accordance with OECD Guideline 425, where no mortality or signs of toxicity were observed at the limit dose of 2000 mg/kg, indicating an LDso greater than this value. The high dose (2000 mg/kg) was designated as the limited safe dose. The mid dose (1000 mg/kg) and low dose (500 mg/kg) were selected as fractions of the limit dose (50% and 25%, respectively), following standard dose-spacing practices in pharmacological studies to evaluate dose-dependent effects within the established safety margin.

4.6 Induction of Constipation Using Loperamide

Constipation was successfully induced in all loperamide-treated groups over the 6-day induction period, as shown by a gradual decrease in fecal pellet count as the days passed. In these induced groups, fecal output steadily dropped from the normal range of about 53–64 pellets (normal group) to approximately 30–35 pellets by the end of the induction period. In contrast, the normal group maintained a higher and stable fecal output throughout, showing regular bowel movement without any reduction.

4.7 Laxative Activity of the Extract

The laxative activity of *Ipomoea triloba* crude extract was evaluated through its effects on fecal output and gastrointestinal motility using two experimental setups. The results are presented and analyzed in the following subsections.

4.7.1 Effects on Fecal Output (Experiment 1)

Experiment 1 evaluated the laxative activity of *Ipomoea triloba* crude extract based on three fecal-output parameters: fecal pellet count, fecal wet weight, and fecal water content. These parameters represent bowel movement frequency, stool mass, and stool hydration, respectively. Overall results from descriptive, statistical, and post hoc analyses were used to determine the effect of the extract across different doses.

Table 10. Descriptive statistics for Experiment 1 fecal-output outcomes by treatment group

Group	Treatment	n	Pellet count	Wet weight (g)	Water content (%)
Negative Control	Distilled Water 10ml	5	27.80 (1.30)	0.83 (0.04)	42.68 (0.36)
Low Dose	*Ipomoea triloba* 500mg	5	26.40 (0.89)	0.79 (0.03)	48.20 (0.28)
Mid Dose	*Ipomoea triloba* 1000mg	5	47.60 (1.14)	1.44 (0.03)	51.38 (0.46)
High Dose	*Ipomoea triloba* 2000mg	5	67.20 (0.84)	2.02 (0.02)	55.08 (0.13)

Positive Control	Bisacodyl 0.25mg	5	74.80 (1.64)	2.28 (0.05)	54.44 (0.11)

Note: Values are presented as mean (SD). Experiment 1 outcomes included pellet count, wet weight, and water content.

Table 10 presents the descriptive statistics for fecal-output parameters across treatment groups. The data show differences among groups, indicating that fecal output varies depending on the treatment administered. Overall, higher values were generally observed in the treated groups compared with the negative control, particularly at the mid and high doses, indicating an increase in fecal-output parameters with increasing dose.

Among the extract-treated groups, the high-dose group showed the highest fecal-output values, while the mid-dose group showed moderate increases. In contrast, the low-dose group showed values closer to the negative control, indicating a weaker physiological effect at the lowest concentration.Overall, the descriptive statistics indicate a dose-dependent increase in fecal-output outcomes.

Note. Bars represent group means and error bars represent ±1 SD for fecal pellet count, fecal wet weight, and fecal water content. This figure complements the descriptive statistics reported in Table 9 by showing the mean profile of the three fecal-output indicators across treatment groups. Bisacodyl served as the positive control.

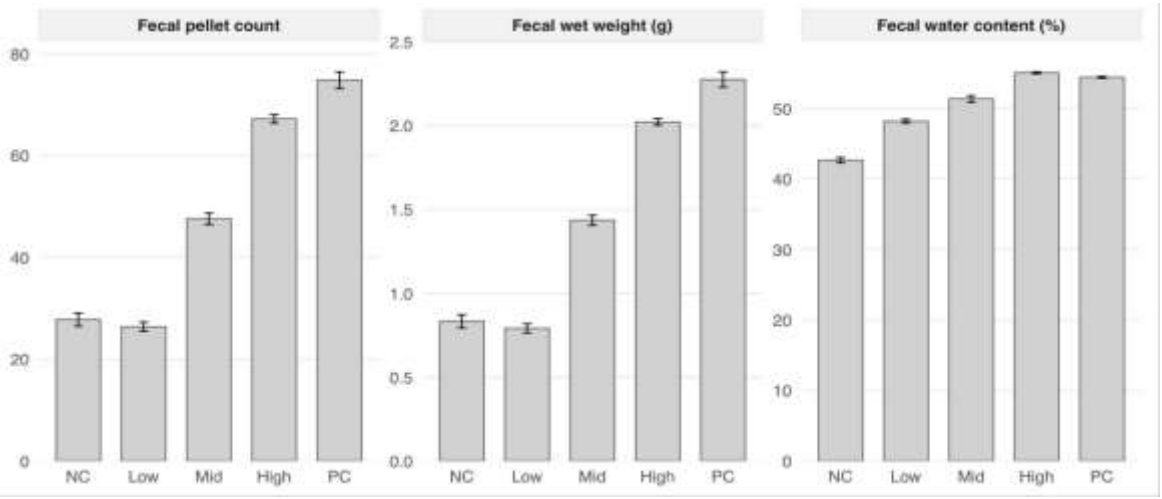


Figure 6. Mean fecal-output outcomes by treatment group in Experiment 1.

Table 10 shows clear differences among the treatment groups in all three fecal-output outcomes. For fecal pellet count and fecal wet weight, the positive control showed the highest mean values, followed by the high-dose group, mid-dose group, negative control group, and low-dose group. For fecal water content, the high-dose group showed the highest mean value, followed by the positive control, mid-dose group, low-dose group, and negative control group. Descriptively, these results suggest that the ethanolic leaf extract of *I. triloba* was associated with improved fecal output at the mid and high doses. Both groups showed higher pellet count, wet weight, and water content compared with the negative control group, indicating increased bowel movement, greater stool mass, and improved stool hydration. The positive control also showed high values across all parameters, consistent with the expected laxative effect of bisacodyl and supporting its role as the reference standard.

The low-dose group showed a weaker and less consistent response. Although its fecal water content

was higher than that of the negative control group, its pellet count and wet weight were slightly lower. This suggests that the lowest dose did not produce a clear improvement in overall fecal output under the present experimental conditions.

Overall, the descriptive results indicate a partial dose-dependent pattern, with the strongest effects observed at the mid and high doses. In particular, the high-dose group approached the positive control in pellet count and wet weight and slightly exceeded it in water content. These findings suggest that the laxative effect of *I. triloba* increases with dose, although statistical analysis is required to confirm significance.

Table 11. Multivariate omnibus test for Experiment 1 fecal-output outcomes.

Test statistic	Value	Approx. F	df1	df2	*p*
Pillai's trace	2.357	18.31	12	60.00	< .001

Note. Experiment 1 included pellet count, wet weight, and water content. df1 = hypothesis degrees of freedom; df2 = error degrees of freedom. Intestinal transit was analyzed separately in Experiment 2 (see Table exp2_omnibus).

As shown in Table 11, the treatment groups differed significantly when fecal pellet count, fecal wet weight, and fecal water content were considered jointly, Pillai's trace = 2.357, approximate $F(12, 60) = 18.31$, $p < .001$. This finding indicates that the overall fecal-output profile varied across the five treatment groups and that the observed descriptive differences were not due to random variation alone.

The significant multivariate result suggests that treatment condition was meaningfully associated with the combined indicators of bowel movement frequency, stool bulk, and stool hydration. In the context of the present study, this implies that the ethanolic leaf extract of *I. triloba* influenced fecal output as a set of related laxative outcomes rather than affecting only a single measure in isolation. This pattern is consistent with the expectation that an effective laxative intervention should improve multiple aspects of fecal excretion under loperamide-induced constipation.

Because the multivariate test was significant, it was appropriate to proceed to outcome-specific omnibus tests to determine which of the three fecal-output measures contributed to the overall group effect. These follow-up analyses are presented in the next subsection.v

Table 12. Outcome-specific omnibus tests for Experiment 1 fecal-output outcomes.

Outcome	Test	Statistic	*p*	ω²
Total pellets	One-way ANOVA	F(4, 20) = 1700.31	< .001	0.996
Total wet weight (g)	One-way ANOVA	F(4, 20) = 2038.15	< .001	0.997
Average water content (%)	One-way ANOVA	F(4, 20) = 1426.39	< .001	0.996

Note. Experiment 1 included pellet count, wet weight, and water content. ω² = omega squared.

Table 12 presents the outcome-specific omnibus tests for total pellet count, total wet weight, and average water content. The results show that the treatment group had a statistically significant effect on all three fecal-output outcomes. A significant group effect was found for total pellet count, $F(4,$

20) = 1700.31, $p < .001$, $\omega^2 = .996$; total wet weight, $F(4, 20) = 2038.15$, $p < .001$, $\omega^2 = .997$; and average water content, $F(4, 20) = 1426.39$, $p < .001$, $\omega^2 = .996$. These results indicate that the five treatment groups differed significantly across all fecal parameters when analyzed individually.

The effect sizes were large across all outcomes, indicating that most of the differences in fecal parameters were strongly influenced by the treatment condition. This means that the observed differences were not only statistically significant but also large in practical magnitude.

For total pellet count, the significant result indicates differences in bowel movement frequency among the groups. This is consistent with the descriptive findings in Table 1, where the mid-dose, high-dose, and positive control groups showed higher values than the negative control group. This suggests that the extract, particularly at higher doses, was associated with increased fecal output.

For total wet weight, the significant group effect indicates variation in stool mass across treatments. The pattern suggests that higher doses of *I. triloba* and the positive control produced greater fecal output, while the low-dose group showed a response closer to the negative control.

For average water content, the results indicate significant differences in stool hydration among groups. Higher fecal water content, particularly in the high-dose and positive control groups, suggesting improved stool softness and easier passage compared with the negative control group.

Overall, the outcome-specific analyses confirm that *I. triloba* affected all fecal-output parameters in a dose-dependent manner, with stronger effects observed at the mid and high doses. To further identify specific group differences, post hoc pairwise comparisons were conducted and are presented in the next subsection.

Table 13. Pairwise post hoc comparisons for Experiment 1 fecal-output outcomes.

Outcome	Group 1	Group 2	Mean difference	95% CI	Adjusted *p*	Remarks
Total pellets	Low Dose	Negative Control	-1.40	[-3.67, 0.87]	.377	Not significant
Total pellets	Mid Dose	Negative Control	19.80	[17.53, 22.07]	<.001	Significant
Total pellets	High Dose	Negative Control	39.40	[37.13, 41.67]	<.001	Significant
Total pellets	Positive Control	Negative Control	47.00	[44.73, 49.27]	<.001	Significant
Total pellets	Mid Dose	Low Dose	21.20	[18.93, 23.47]	<.001	Significant
Total pellets	High Dose	Low Dose	40.80	[38.53, 43.07]	<.001	Significant
Total pellets	Positive Control	Low Dose	48.40	[46.13, 50.67]	<.001	Significant
Total pellets	High Dose	Mid Dose	19.60	[17.33, 21.87]	<.001	Significant
Total pellets	Positive Control	Mid Dose	27.20	[24.93, 29.47]	<.001	Significant
Total pellets	Positive	High Dose	7.60	[5.33, 9.87]	<.001	Significant

	Control					
Total wet weight (g)	Low Dose	Negative Control	-0.04	[-0.11, 0.02]	.307	Not Significant
Total wet weight (g)	Mid Dose	Negative Control	0.60	[0.54, 0.67]	<.001	Significant
Total wet weight (g)	High Dose	Negative Control	1.19	[1.12, 1.25]	<.001	Significant
Total wet weight (g)	Positive Control	Negative Control	1.44	[1.38, 1.51]	<.001	Significant
Total wet weight (g)	Mid Dose	Low Dose	0.64	[0.58, 0.71]	<.001	Significant
Total wet weight (g)	High Dose	Low Dose	1.23	[1.17, 1.29]	<.001	Significant
Total wet weight (g)	Positive Control	Low Dose	1.48	[1.42, 1.55]	<.001	Significant
Total wet weight (g)	High Dose	Mid Dose	0.59	[0.52, 0.65]	<.001	Significant
Total wet weight (g)	Positive Control	Mid Dose	0.84	[0.78, 0.90]	<.001	Significant
Total wet weight (g)	Positive Control	High Dose	0.25	[0.19, 0.32]	<.001	Significant

Outcome	Group 1	Group 2	Mean difference	95% CI	Adjusted *p*	Remarks
Average water content (%)	Low Dose	Negative Control	5.52	[4.95, 6.09]	<.001	Significant
Average water content (%)	Mid Dose	Negative Control	8.70	[8.13, 9.27]	<.001	Significant
Average water content (%)	High Dose	Negative Control	12.40	[11.83, 12.97]	<.001	Significant
Average water content (%)	Positive Control	Negative Control	11.76	[11.19, 12.33]	<.001	Significant
Average water content (%)	Mid Dose	Low Dose	3.18	[2.61, 3.75]	<.001	Significant
Average water content (%)	High Dose	Low Dose	6.88	[6.31, 7.45]	<.001	Significant
Average water content (%)	Positive Control	Low Dose	6.24	[5.67, 6.81]	<.001	Significant
Average water content (%)	High Dose	Mid Dose	3.70	[3.13, 4.27]	<.001	Significant
Average water content (%)	Positive Control	Mid Dose	3.06	[2.49, 3.63]	<.001	Significant
Average water content (%)	Positive Control	High Dose	-0.64	[-1.21, -0.07]	.023	Not Significant

The pairwise comparisons in Table 13 identify the specific group differences underlying the significant omnibus findings. For total pellet count, the mid-dose, high-dose, and positive control groups showed significantly higher means than the negative control group, while the low-dose group did not differ significantly from the negative control group. A similar pattern was observed for total wet weight, where the mid-dose, high-dose, and positive control groups were significantly higher than the negative control group, but the low-dose group showed no significant difference.

For average water content, however, all four active-treatment groups differed significantly from the negative control group, including the low-dose group. These results indicate that the extract improved stool hydration even at the lowest dose, while clearer improvements in stool frequency and stool mass were observed at the mid and high doses.

Comparisons among the extract doses further suggest a dose-dependent response in Experiment 1. For pellet count and wet weight, the mid-dose group was significantly higher than the low-dose group, and the high-dose group was significantly higher than the mid-dose group. A similar increasing trend was observed for water content, where higher doses were associated with progressively higher mean values. Overall, these results indicate that the laxative effect of *I. triloba* increased with dose, particularly for fecal-output parameters.

Comparisons with the positive control provide additional insight into the relative efficacy of the extract. For pellet count and wet weight, the positive control showed significantly higher values than both the mid-dose and high-dose groups, indicating a stronger effect of bisacodyl for these outcomes. For water content, the high-dose group exceeded the positive control, while the positive control remained higher than the mid-dose group. This suggests that although the extract did not fully match bisacodyl in increasing stool frequency and mass, the high dose demonstrated strong activity in improving stool hydration.

Overall, the post hoc results refine the interpretation of the omnibus findings. The low dose showed limited laxative activity, with a significant effect only on water content. The mid-dose produced consistent improvements across all fecal-output measures compared with the negative control, while the high dose showed the strongest overall extract effect and, in some outcomes, approached or exceeded the positive control. These findings support a dose-dependent laxative effect of *I. triloba*, most evident at higher doses.

4.7.2 Effects on Gastrointestinal Motility (Experiment 2)

This section presents the effects of the ethanolic leaf extract of *Ipomoea triloba* on gastrointestinal motility, as measured by intestinal transit using the charcoal meal method in loperamide-induced constipated Swiss albino mice.

Table 14. Descriptive statistics for the Experiment 2 intestinal-transit outcome by treatment group.

Group	Treatment	n	Intestinal transit (%)
Negative Control	Distilled Water 10ml	5	62.59 (4.00)
Low Dose	*Ipomoea triloba* 500mg	5	92.79 (7.98)
Mid Dose	*Ipomoea triloba* 1000mg	5	93.16 (3.00)
High Dose	*Ipomoea triloba* 2000mg	5	99.11 (1.99)
Positive Control	Bisacodyl 0.25mg	5	96.25 (2.88)

Note. Values are M (SD). Experiment 2 included intestinal transit only and was conducted separately from Experiment 1 (see Table exp1_desc).

Table 14 presents the descriptive statistics for intestinal transit across treatment groups. The data show clear differences among groups, indicating that gastrointestinal motility varies depending on the treatment administered. The negative control group had the lowest intestinal transit, reflecting reduced intestinal movement under loperamide-induced constipation.

In contrast, all treated groups showed higher intestinal transit values. The groups that received *Ipomoea triloba* extract and the positive control (bisacodyl) all demonstrated increased intestinal transit compared with the negative control. Among the extract-treated groups, the high-dose group

showed the highest mean intestinal transit, followed by the mid-dose and low-dose groups. The positive control also showed a high value, consistent with its known effect as a laxative.

Because higher intestinal transit reflects greater movement of intestinal contents through the gastrointestinal tract, the results indicate that the treated groups experienced improved gastrointestinal motility. Compared with the negative control group, the treatment groups showed noticeably higher intestinal transit, suggesting that the extract improved the movement of contents along the intestines. This improvement implies that the extract was able to reduce or overcome the inhibitory effect of Loperamide on intestinal movement.

The marked difference between the negative control and the treated groups supports the possibility that the extract possesses laxative activity. Increased intestinal transit is commonly associated with enhanced bowel movement and faster passage of intestinal contents, both of which are indicators of improved digestive motility. The findings therefore suggest that the extract may help stimulate intestinal activity and alleviate slowed bowel movement.

In addition, the results showed a dose-dependent trend, wherein higher doses produced stronger effects on intestinal transit. This pattern further supports the effectiveness of the extract in enhancing gastrointestinal motility. Overall, the descriptive findings demonstrate that the extract consistently increased intestinal movement across all treatment groups, with the greatest improvement observed at higher concentrations.

Note. Bars represent group means and error bars represent ±1 SD for intestinal transit (%). This figure complements the descriptive statistics reported in Table 14 by showing the mean gastrointestinal-motility profile across treatment groups. Experiment 2 was conducted separately from Experiment 1. Bisacodyl served as the positive control.

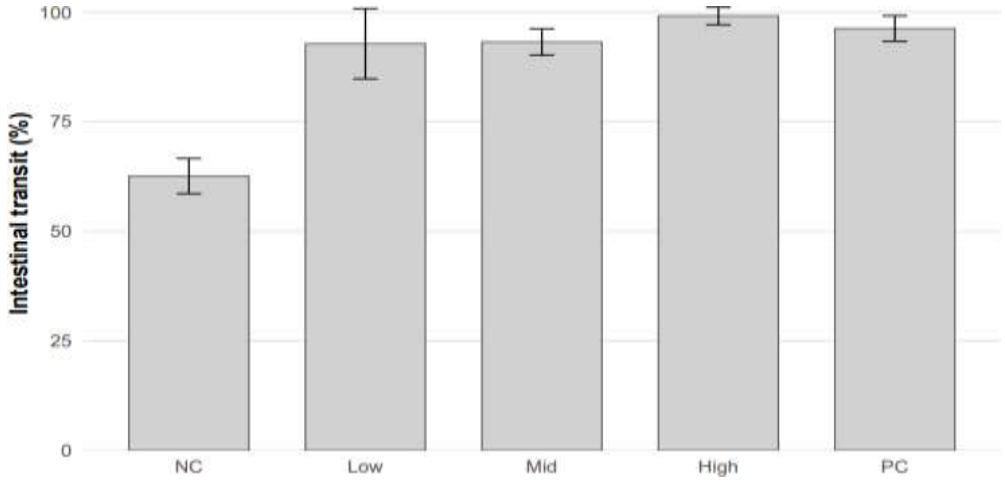


Figure 7. Mean intestinal transit by treatment group in Experiment 2.

Figure 7 presents the same results in terms of mean and standard deviation and is consistent with the data shown in Table 14. The negative control group recorded the lowest mean intestinal transit, while all groups treated with *I. triloba* extract and the positive control exhibited markedly higher mean values.

Although the high-dose group obtained the highest descriptive mean, the values among the extract-treated groups were relatively close to each other and distinctly higher than the negative control. This bar plot therefore supports the interpretation that *I. triloba* improves gastrointestinal motility across

all the tested dose, with the most evident contrast occurring between the negative control and the treatment groups.

In agreement with this, the descriptive statistics in Table 14 demonstrate clear variation among the experimental groups. The negative control consistently showed the lowest mean intestinal transit, whereas all treatment groups receiving *I. triloba* extract and bisacodyl showed considerably higher means. Within the extract-treated groups, the high-dose group obtained the highest mean, followed by the mid-dose and low-dose groups. The positive control also demonstrated a high mean intestinal transit value.

As increased intestinal transit reflects greater movement of the charcoal marker through the gastrointestinal tract, these results indicate enhanced gastrointestinal motility in all treated groups compared with the negative control. The substantial difference observed at the descriptive level suggests that *I. triloba* may have mitigated the reduced intestinal movement induced by loperamide, consistent with a laxative effect characterized by improved propulsion of intestinal contents.

Table 15. Omnibus test for the Experiment 2 intestinal-transit outcome

Outcome	Test	Statistic	*p*	ω^2
Intestinal transit (%)	One-way ANOVA	$F(4, 20) = 54.68$	< .001	0.896

Note. Experiment 2 included intestinal transit only and was conducted separately from Experiment 1 (see Table exp1_omnibus). ω^2 = omega squared.

Table 15 presents the omnibus test for intestinal transit. The results show that there is a significant difference among the treatment groups, $F(4, 20) = 54.68$, $p < .001$, $\omega^2 = .896$. This indicates that the treatment administered had a statistically significant effect on gastrointestinal motility in the loperamide-induced constipation model.

In terms of interpretation, this finding suggests that the treatments differed in their ability to improve the movement of the charcoal marker through the intestine. This indicates that the ethanolic leaf extract of *Ipomoea triloba*, similar to the positive control (bisacodyl), had an effect on gastrointestinal motility.

Because the omnibus test showed significant results, further analysis was conducted using post hoc comparisons to identify which specific groups differed from each other. These results are presented in Table 16.

Table 16. Pairwise post hoc comparisons for the Experiment 2 intestinal-transit outcome.

Outcome	Group 1	Group 2	Mean difference	95% CI	Adjusted p	Remarks
Intestinal transit (%)	Low Dose	Negative Control	30.20	[21.69, 38.70]	< .001	Significant
Intestinal transit (%)	Mid Dose	Negative Control	30.57	[22.06, 39.07]	< .001	Significant
Intestinal transit (%)	High Dose	Negative Control	36.52	[28.01, 45.03]	< .001	Significant

Intestinal transit (%)	Positive Control	Negative Control	33.66	[25.15, 42.17]	< .001	Significant
Intestinal transit (%)	Mid Dose	Low Dose	0.37	[-8.14, 8.87]	1.000	Not Significant
Intestinal transit (%)	High Dose	Low Dose	6.32	[-2.19, 14.83]	.212	Not Significant
Intestinal transit (%)	Positive Control	Low Dose	3.46	[-5.04, 11.97]	.741	Not Significant
Intestinal transit (%)	High Dose	Mid Dose	5.95	[-2.55, 14.46]	.261	Not Significant
Intestinal transit (%)	Positive Control	Mid Dose	3.09	[-5.41, 11.60]	.810	Not Significant
Intestinal transit (%)	Positive Control	High Dose	-2.86	[-11.36, 5.65]	.850	Not Significant

Note. Experiment 2 included intestinal transit only and was conducted separately from Experiment 1 (see Table exp1_posthoc). CI = confidence interval; HSD = honestly significant

The post hoc comparisons in Table 16 show that all active treatment groups had significantly higher intestinal transit than the negative control group. The low-dose, mid-dose, and high-dose groups of *Ipomoea triloba* extract all showed significant increases in intestinal transit compared with the negative control group.

The positive control group (bisacodyl) also showed significantly higher intestinal transit than the negative control group. These results indicate that both the extract and bisacodyl improved gastrointestinal motility compared with the negative control group.

In contrast, no significant differences were found among the three extract doses. The low-dose and mid-dose groups did not differ significantly, and the high-dose group was also not significantly different from either low-dose or mid-dose groups. In addition, none of the extract-treated groups showed significant differences when compared with the positive control group. This suggests that although the highest mean value was observed in the high-dose group, the statistical results do not provide clear evidence of a dose-dependent increase in intestinal transit within the tested doses.

Overall, these findings indicate that the extract improved gastrointestinal motility at all administered doses, but the level of improvement did not significantly differ among the low-, mid-, and high-dose groups. The lack of significant difference between the extract-treated groups and the positive control further suggests that the extract produced a comparable effect to bisacodyl in terms of intestinal transit. Therefore, the main difference observed in this experiment was between the negative control group and all treatment groups, rather than differences among the treated groups themselves.

4.8 Integrated Discussion of Findings

The integrated findings of this study demonstrate that the ethanolic leaf extract of *Ipomoea triloba* possesses significant laxative activity in a loperamide-induced constipation model in Swiss albino mice. These findings directly address the general objective of evaluating the laxative potential of the plant extract, as evidenced by improvements in both fecal output and intestinal motility.

In relation to the first objective, the extraction process yielded a measurable amount of crude extract, confirming the efficiency of the method used. This supports the adequacy of the extract for pharmacological testing.

For the second objective, a phytochemical confirmatory test confirmed the presence of saponins and flavonoids in the *Ipomoea triloba* leaf extract. With respect to the third and fourth objectives, the acute toxicity test established a safe range for administration, allowing the classification of the extract into low-, mid-, and high-dose groups. The application of OECD guidelines ensured that the selected doses were both safe and scientifically justified (OECD 2020).

In addressing the fifth objective, the extract demonstrated significant laxative activity based on both fecal parameters and intestinal transit. However, the results revealed two distinct response patterns. In Experiment 1, which assessed fecal pellet count, fecal wet weight, and fecal water content, a clear dose-dependent effect was observed. The low-dose group showed minimal activity, while the mid- and high-dose groups exhibited progressively greater improvements. The observed dose-response relationship suggests that higher concentrations of the extract are required to produce substantial effects on fecal output parameters, consistent with findings that plant-based laxatives exhibit increased efficacy at higher doses (Nafees et al. 2020).

In contrast, Experiment 2, which evaluated intestinal transit, showed that all extract-treated groups significantly increased gastrointestinal motility compared to the negative control, but no significant differences were observed among the doses. This indicates that the extract is capable of stimulating intestinal movement even at low concentrations.

When both experiments are considered together, the results suggest that *Ipomoea triloba* extract exerts a dual mechanism of laxative action. The stimulation of intestinal motility appears to occur even at lower doses, while significant improvements in fecal output require higher concentrations. This indicates that the extract can initiate bowel movement at low doses but achieves a more complete laxative effect, including increased stool bulk and hydration, at higher doses.

In relation to the sixth objective, the comparison with the standard drug, Bisacodyl, showed that the extract produced comparable effects, particularly at higher doses. While the standard drug remained more effective in increasing fecal pellet count and fecal wet weight, the high-dose extract demonstrated strong activity and even exceeded the standard in terms of fecal water content. This suggests that although the extract may differ in potency, it exhibits substantial laxative potential and may act through mechanisms that enhance stool hydration.

The observed pharmacological effects can be explained by the phytochemical constituents of the extract. Saponins may contribute by increasing intestinal secretion and mildly irritating the mucosal lining, thereby stimulating peristalsis. Flavonoids, on the other hand, may enhance smooth muscle contraction and regulate intestinal motility. The combined action of these compounds likely accounts for the improvements in both fecal output and intestinal transit observed in this study (Uka and Okeke 2020; Borlongan et al. 2024).

The consistency of results across both experimental models strengthens the validity and reliability of the findings. The extract demonstrated its ability to improve multiple aspects of bowel function, indicating that its laxative effect is not limited to a single parameter.

These findings support the alternative hypothesis (H_1), which states that the ethanolic leaf extract of *Ipomoea triloba* significantly increases fecal pellet count, fecal wet weight, and intestinal transit in constipated mice. Therefore, the null hypothesis (H_0) is rejected.

Overall, the integrated findings confirm that *Ipomoea triloba* leaf extract possesses significant laxative activity, likely mediated by the effects of saponins and flavonoids. The results provide scientific evidence supporting its traditional use and highlight its potential as a natural alternative for the management of constipation.

CHAPTER V

This chapter presents a summary of the findings of the study assessing the laxative potential of *Ipomoea triloba* ethanolic leaf extract in a loperamide-induced constipation model in Swiss albino mice. The conclusions are based on the results of fecal output parameters and gastrointestinal motility. Recommendations for the future research and potential applications as a natural laxative are also provided.

5.1 Summary and Conclusion of the Study

This study evaluated the laxative potential of the ethanolic leaf extract of *Ipomoea triloba* using a loperamide-induced constipation model in Swiss albino mice. The findings demonstrated that the extract possesses significant laxative activity, improving both fecal output and gastrointestinal motility, with a generally dose-dependent effect on fecal parameters and consistent enhancement of intestinal transit. The extract was also found to be safe within the tested dose range, supporting its experimental use and potential therapeutic application.

In relation to the specific research objectives, the findings are summarized as follows:

Percentage Yield of Extract

- The ethanolic extraction of *Ipomoea triloba* leaves produced a measurable amount of extract, indicating that the process was effective in obtaining sufficient material for the study. The percentage yield indicates how much extract was obtained, where a higher value means more extract was collected.

Phytochemical Confirmatory Tests

- The extract tested positive for flavonoids and saponins, indicating the presence of phytochemical properties associated with laxative activity.

Acute Toxicity Test (OECD Model)

- The extract exhibited no mortality and no severe toxic effects within the tested dose range, establishing its safety and enabling the determination of appropriate experimental doses.

Determination of Extract Doses

- Based on toxicity results, the extract doses were categorized into low (500 mg), mid (1000 mg), and high (2000 mg) levels, which were used to assess dose-dependent effects.

Laxative Activity of *I. triloba* Based on Fecal Parameters (Experiment 1)

- Significant differences were observed among treatment groups in fecal pellet count, fecal wet weight, and fecal water content ($p < 0.001$).
- Mid- and high-dose groups showed a significant increase in fecal pellet count and wet weight compared to the negative control.
- The low-dose group showed no significant effect on pellet count and wet weight.
- All extract-treated groups showed significantly higher fecal water content.
- The high-dose group showed the highest fecal water content among the extract groups and slightly exceeded the positive control (bisacodyl).
- The positive control showed higher fecal pellet count and wet weight compared with the extract-treated groups.

Gastrointestinal Motility *I. triloba* Based on Intestinal Transit (Experiment 2)

- A significant difference in intestinal transit was observed among treatment groups ($p < 0.001$).
- All extract-treated groups showed significantly increased intestinal transit compared to the negative control, indicating enhanced gastrointestinal motility.

- No significant differences were found among the different extract doses.
- No significant differences were observed between extract-treated groups and the positive control (bisacodyl), indicating comparable efficacy.

Comparison with Control Groups (Positive and Negative Controls) Compared to the positive control (bisacodyl-treated group):

- The extract did not exceed bisacodyl in fecal pellet count and fecal wet weight in Experiment 1.
- The high-dose extract showed relatively higher fecal water content, suggesting improved stool hydration.
- The extract showed comparable effectiveness to bisacodyl in intestinal transit in Experiment 2, indicating similar motility-enhancing activity.

Compared to the negative control (distilled water-treated group):

- All extract-treated groups showed a significant increase in fecal pellet count, fecal wet weight, and fecal water content in Experiment 1.
- In Experiment 2, all doses significantly enhanced intestinal transit compared with the negative control group.

In conclusion, the ethanolic leaf extract of *Ipomoea triloba* demonstrated significant laxative activity by improving fecal output and enhancing gastrointestinal motility in loperamide-induced constipated mice. The findings support the alternative hypothesis, indicating that the extract significantly increases fecal pellet count, fecal wet weight, fecal water content, and intestinal transit percentage compared with the standard drug (bisacodyl); thus, the null hypothesis is rejected. Overall, *Ipomoea triloba* shows potential as a plant-based alternative for constipation management, with stronger effects observed at higher doses.

5.2 Recommendations

Based on the findings of this study, several recommendations are proposed for future research and related investigations.

5.2.1 For Future Research

Future studies may explore other extraction methods and plant preparations of *Ipomoea triloba* to determine whether different formulations yield stronger or more consistent laxative effects. Additional research may also assess other physiological parameters, such as electrolyte balance and intestinal fluid regulation, to further elucidate its mechanism of action. Furthermore, studies using different validated experimental models of constipation may be conducted to confirm the consistency of its laxative effects under varying conditions.

5.2.2 For Methodology Improvement

Future studies should consider increasing the sample size per group to enhance statistical reliability and reduce variability in fecal-output measurements. Improved randomization is also recommended to minimize variation among samples and ensure consistency across groups. The inclusion of additional control groups, such as a vehicle control and an alternative standard laxative, may also strengthen baseline comparisons and improve evaluation of the extract's relative efficacy.

5.2.3 For Practical Application

The findings of this study support further exploration of *Ipomoea triloba* as a potential source for herbal laxative development. Future applied research may focus on the formulation of standardized dosage forms, such as capsules or oral preparations, to assess its suitability for practical therapeutic use. Furthermore, the extract may be evaluated for development into plant-based laxative agents, considering

its observed effects on stool frequency, stool mass, and stool water content. Finally, the establishment of standardized extraction and quality control protocols is recommended to ensure consistency, safety, and reproducibility for potential practical applications.

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