

Evaluation of Anti-diarrheal Activity of 80 % Methanol Crude Root Extract and Solvent Fractions of *Ficus Thonningii* B.(Moraceae) in Swiss Albino Mice

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Research Article

Keywords: Antidiarrheal, *Ficus thonningii* roots, crude extract, solvent fractions

Posted Date: July 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10192365/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background: Diarrhea is a leading cause of childhood mortality worldwide. Traditional medicine in Ethiopia employs *Ficus thonningii* roots for diarrhea treatment, but scientific validation is limited.

Methods: Antidiarrheal activity of 80% methanol crude root extract and solvent fractions was evaluated in Swiss albino mice using castor oil-induced diarrhea, enteropooling, and charcoal meal transit models.

Results: The crude extract and aqueous fraction significantly delayed diarrhea onset, reduced stool frequency and weight, and inhibited intestinal fluid accumulation and motility in a dose-dependent manner. At 400 mg/kg, the aqueous fraction surpassed loperamide in reducing fecal output.

Conclusion: Findings provide pharmacological evidence supporting the ethnomedicinal use of *Ficus thonningii* roots for diarrhea management and highlight the need for chemical fingerprinting and compound isolation.

Keywords: Antidiarrheal, *Ficus thonningii* roots, crude extract, solvent fractions

Background

Diarrhea is clinically characterized by the passage of loose or watery stools three or more times within a 24-hour period, or at a frequency greater than what is normal for the individual [8]. Globally, it remains one of the leading causes of childhood mortality, second only to respiratory illnesses. Each year, hundreds of millions of cases are reported, with nearly half a million deaths occurring among children under five [2,15]. The burden is disproportionately high in low-income regions, particularly Sub-Saharan Africa and South Asia, which together account for the majority of these fatalities. In Ethiopia, national demographic surveys indicate that diarrheal disease contributes to more than 13% of child deaths [3].

The condition is most often associated with gastrointestinal infections caused by bacteria, viruses, or parasites. Common bacterial agents include *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus*, and *Salmonella typhi*, while fungal pathogens such as *Candida albicans* may also be implicated [6,19]. Acute episodes are typically linked to enteric microorganisms, whereas chronic forms are often related to inflammatory bowel disorders or malabsorption syndromes. Persistent diarrhea is frequently associated with protozoa such as *Giardia lamblia* and *Cryptosporidium* [9]. In developing countries, rotavirus and pathogenic *E. coli* are recognized as the primary causes of moderate to severe diarrheal illness [1].

Pathophysiologically, diarrhea results from an imbalance between intestinal absorption and secretion. Mechanisms include increased luminal osmolality, excessive electrolyte secretion, impaired absorption, and accelerated intestinal transit [4]. Clinically, diarrheal disorders are categorized into secretory, osmotic, inflammatory, and functional types.

Medicinal plants have long been employed in traditional medicine for the management of gastrointestinal disorders. In many developing nations, up to 80% of the population relies on plant-based remedies for primary healthcare [10]. These natural products are rich in bioactive compounds and continue to serve as valuable sources for the discovery of novel therapeutic agents.

Medicinal Plants and *Ficus thonningii* Blume (Moraceae) in Diarrhea Management

In Ethiopia, traditional healing practices remain deeply embedded in culture, with nearly four-fifths of the population relying on herbal remedies [12,13]. A wide range of plants are reported to be used against diarrheal illnesses, including *Conyza pyrrhoppapa*, *Ficus thonningii*, *Tamarindus indica*, *Brucea antidysenterica*, *Clutia abyssinica*, *Capsicum annum*, *Ficus sur*, *Leonotis ocymifolia*, *Lepidium sativum*, *Ocimum gratissimum*, *Amaranthus caudatus*, *Croton macrostachyus*, *Ensete ventricosum*, *Grewia villosa*, and *Nicotiana tabacum* [11,18].

Ficus thonningii Blume (Moraceae), locally known as “Chibha” in Amharic, is an evergreen tree reaching 6–12 meters in height, commonly found in forests, grasslands, savannahs, and riverbanks [14]. Different parts of the plant are traditionally employed to treat gastrointestinal and systemic disorders. In African folk medicine, its leaves are used for stomach complaints, while in Kenya boiled leaves are consumed to manage diarrhea [16,17]. In Ethiopia, communities in the Zegie Peninsula chew its roots or use its bark for similar purposes [18].

Pharmacological investigations have confirmed that extracts of *F. thonningii* possess antimicrobial, antidiarrheal, antiprotozoal, antihelminthic, antioxidant, and anti-inflammatory properties [20–25]. The plant is also used as a galactagogue to stimulate lactation [26]. Traditional preparations include infusions, decoctions, and macerations, with roots employed for conditions such as malaria, fever, hepatitis, and dental problems [27]. Reports from Zimbabwe describe its use in preventing miscarriages and nosebleeds, while in Ethiopia it is applied for diarrhea, pneumonia, and chest pain [29]. Analgesic and anti-inflammatory effects comparable to aspirin have been documented, alongside wound-healing and hypoglycemic activities in animal models [28,30].

Rationale for the Study

Although modern pharmacological agents are available for the treatment of diarrhea, many are associated with undesirable adverse effects. For instance, racecadotril has been linked to bronchospasm and vomiting [31], while loperamide may cause constipation and intestinal obstruction [32]. Long-term use of opioids such as morphine and its analogues can also result in central nervous system complications [33]. These limitations highlight the need for safer and more effective alternatives.

Despite the widespread ethnomedicinal use of *Ficus thonningii*, its therapeutic potential cannot be fully validated without scientific investigation. Growing concerns about antimicrobial resistance, drug-related side effects, and the emergence of secondary infections have further increased interest in plant-based remedies [34,35]. Traditional medicine offers practical, affordable, and culturally acceptable approaches that remain relevant in many communities. Among the numerous plants traditionally employed for diarrhea management, *F. thonningii* is particularly noteworthy. This study was designed to provide experimental evidence supporting its antidiarrheal properties by evaluating the activity of its crude methanol root extract and solvent fractions in vivo. The findings are expected to contribute to the search for novel antidiarrheal agents that overcome the limitations of conventional drugs. Moreover, the results establish a foundation for future research aimed at isolating bioactive compounds, clarifying their mechanisms of action, and developing standardized formulations for clinical application.

Materials and Methods

Chemicals and Reagents

The study employed distilled water, absolute methanol (99.9%, Hregco Chemical), *n*-hexane (Alpha Chemika, India), ciprofloxacin hydrochloride (Cadila Pharmaceuticals, Ethiopia), loperamide hydrochloride (Medochemie Ltd., Cyprus), atropine sulfate injection (Humanwell Pharmaceutical, Ethiopia PLC), castor oil (Amman Pharmaceutical Industries, Jordan), ethyl acetate (Alpha Chemika, India), activated charcoal (SDFCL, India), Tween 80 (Atlas Chemical Industries, India), sulfuric acid (HiMedia Laboratories Pvt. Ltd., India), lead acetate (Guangdong Guanghua Chemicals, China), acetic anhydride (May and Baker Ltd., England), Dragendorff's reagent (May and Baker Ltd., England), hydrochloric acid (BDH Laboratory Supplies, England), glacial acetic acid (BDH Laboratory Supplies, England), and benzene (Fisher Scientific, UK).

Instruments and Equipment

Experimental procedures utilized a digital electrical balance (EPH-400, Abron Exports, India), hot air oven (Medit-Medizin Technik, Germany), rotary evaporator (Yamato Scientific Co. Ltd., Japan), Whatman filter paper No. 1 (Schleicher and Schuell Microscience GmbH, Germany), oral gavage tubes, sterile surgical gloves, examination gloves, gauze bandages, surgical blades (Sterilance Suzhou, China), absorbent cotton, syringes with needles, and PVC rulers (Crytstle, Ethiopia).

Plant Collection and Identification

Roots of *Ficus thonningii* Blume (Moraceae) were collected from Dirmara kebele, Dembia woreda, located 25 km from Gondar city in Northwest Ethiopia. The plant was authenticated by a botanist from the Department of Biology, University of Gondar. The plant name was checked against The World Flora Online (accessed 09 June 2026) and MPNS.” A voucher specimen (Ref. No. 0002/STG/2021) was deposited in the university herbarium for future reference.

Experimental Animals

A total of 320 adult Swiss albino mice (both sexes), aged 8–12 weeks and weighing 20–30 g, were used. The animals were bred and maintained at the animal house of the Department of Pharmacology, College of Medicine and Health Sciences, University of Gondar. They were housed in plastic cages (5–8 mice per cage) with wood-chip bedding, provided with free access to food and water, and kept under standard laboratory conditions (12-hour light/dark cycle). All animals were acclimatized for one week prior to experimentation. Care and handling followed OECD guidelines [36].

Methods

Preparation of 80% Methanol Crude Extract

Roots of *Ficus thonningii* were thoroughly washed with tap water to remove adhering soil and debris, then shade-dried at room temperature. A total of 600 g of dried roots were coarsely powdered using a steel mortar and pestle. The powdered material was subjected to maceration with 80% methanol in a 1:5 (w/v) ratio for 72 h at room temperature, with occasional stirring. The extract was separated from the marc using gauze and subsequently filtered through Whatman No. 1 filter paper under suction. This procedure was repeated twice with fresh solvent under identical conditions. The combined filtrates were concentrated using a rotary evaporator at 40°C to remove methanol. The resulting extract was frozen at –18°C overnight and lyophilized. The dried crude extract was stored at –20°C until further use.

Fractionation

Fifty-two grams of the crude extract were suspended in 260 ml of distilled water (1:5 w/v) and transferred into a separatory funnel. An equal volume of hexane was added, and the mixture was shaken intermittently. Two distinct layers formed: hexane (upper) and aqueous (lower). Each layer was collected separately. The aqueous layer was re-extracted with fresh hexane three times, and all hexane fractions were pooled. Subsequently, the aqueous layer was partitioned three times with 260 ml of ethyl acetate, yielding ethyl acetate fractions and an aqueous residue. The

hexane and ethyl acetate fractions were evaporated at 40°C, while the aqueous fraction was lyophilized. All dried fractions were weighed, and the yields calculated. Fractions were stored in airtight containers at 4°C until used for antidiarrheal activity evaluation.

Yields of Extracts

From 600 g of powdered root, 66 g of crude extract was obtained (11% w/w). Fractionation of 52 g of crude extract yielded:

- Hexane fraction: 20.5 g (39.42% w/w)
- Ethyl acetate fraction: 10 g (19.23% w/w)
- Aqueous fraction: 21.5 g (41.35% w/w)

Phytochemical Screening

The crude extract and solvent fractions were screened for secondary metabolites including flavonoids, tannins, anthraquinones, glycosides, steroids, phenols, terpenoids, alkaloids, and saponins using standard qualitative phytochemical tests.

Animal Grouping and Dosing

Three experimental models were employed to evaluate both the crude extract and solvent fractions (*n*-hexane, ethyl acetate, and aqueous). Mice were randomly assigned into five groups, each consisting of six animals per model.

Group I (Negative Control): Received 2% Tween 80 or 10% dimethyl sulfoxide (10 mL/kg).

Group II (Positive Control): Treated with loperamide (3 mg/kg) for castor oil-induced diarrhea and enteropooling models, or atropine sulfate (5 mg/kg) for the gastrointestinal motility test.

Groups III–V (Test Groups): Administered 100, 200, and 400 mg/kg doses, respectively.

This dosing protocol was applied to both the 80% methanol crude extract and the solvent fractions of *Ficus thonningii* roots. Doses were selected based on acute oral toxicity studies. For administration, dried plant extracts were reconstituted in 2% Tween 80 (crude extract) or 10% dimethyl sulfoxide (solvent fractions).

Acute Oral Toxicity Studies

Acute toxicity testing was performed in healthy, non-pregnant female Swiss albino mice, as they are more sensitive to toxic effects [41]. Mice aged 8–12 weeks were acclimatized for seven days

prior to experimentation. Animals were fasted (food but not water) for 4 hours before and 2 hours after extract administration [42].

The initial mouse received 2 g/kg of the methanolic crude extract. It was observed continuously for 24 hours, with particular attention during the first 4 hours. Subsequent mice were treated sequentially based on the initial outcome. All animals were monitored daily for two weeks for signs of toxicity, including loss of appetite, piloerection, lacrimation, tremors, convulsions, salivation, diarrhea, or death [43].

Antidiarrheal Activity Determination

Castor Oil-Induced Diarrhea

The procedure described by Shoba and Thomas [44] was followed. Thirty mice were fasted for 18 hours and randomly divided into five groups (n = 6). One hour after dosing (as per grouping above), each mouse received 0.5 mL of castor oil via oral gavage.

Animals were then placed individually in cages lined with non-wetting transparent paper, which was replaced after each defecation. Over a 4-hour observation period, the following parameters were recorded:

Onset time of diarrhea

Total number of fecal outputs

Consistency of feces (wet vs. dry)

Results were expressed as the percentage inhibition of diarrhea relative to controls.

% inhibition of *Diarrhea*

$$= \frac{\text{Mean number of wet stools of (control group - treated group)}}{\text{Mean number of wet stools of control group}}$$

% inhibition of *defecation*

$$= \frac{\text{Total num. of feces in the (negative control - treated group)}}{\text{Total number of feces in the negative control}} \times 100$$

This procedure was followed for both the crude extract and solvent fractions.

Castor Oil-Induced Enteropooling

The method described by Chitme et al. [45] was employed to assess the effect of *Ficus thonningii* root extract on castor oil-induced intestinal fluid accumulation. The activity of the extract in reducing intraluminal fluid was determined by measuring the volume of fluid collected from the small intestine.

Thirty mice were randomly divided into five groups (n = 6 per group) and fasted for 18 hours (food withheld, water allowed) prior to the experiment. One hour after treatment with the respective doses (as outlined in the grouping and dosing section), each mouse received 0.5 mL of castor oil orally. After one hour, the animals were sacrificed by cervical dislocation.

The small intestine was carefully isolated by ligating the pyloric and cecal ends. The intestine was weighed, and its contents were expelled into a graduated cylinder to measure fluid volume. Following evacuation, the intestine was reweighed. The percentage inhibition of enteropooling was then calculated using the following formula:

$$\% \text{ of inhibition by using MWIC} = \frac{MWICC - MWICT}{MWICC} \times 100$$

Where, MWIC – Mean Weight of Intestinal Content MWICC - Mean Weight of Intestinal Content of Control Group MWICT - Mean Weight of Intestinal Content of test Group

$$\% \text{ of inhibition MVIC} = \frac{MVICC - MVICT}{MVICC} \times 100$$

Where, MVIC – Mean Volume of Intestinal Content MVICC - Mean Volume of Intestinal Content of Control Group and MVICT - Mean Volume of Intestinal Content of investigation Cluster.

Normal Gastrointestinal Transit in Mice

Normal gastrointestinal transit was assessed following the procedure described by others [46].

Mice were fasted for 18 hours (food withheld, water available ad libitum). One hour after administration of the respective treatments—vehicle (distilled water, 10 mL/kg) for Group I, *Ficus thonningii* extract for test groups, or atropine sulfate (5 mg/kg) as the reference drug—each mouse received 1 mL of a charcoal meal (10% activated charcoal suspension in 2% Tween 80).

After one hour, the animals were sacrificed, and the abdomen was opened to remove the small intestine. The total length of the intestine (from pylorus to cecum) and the distance traveled by the charcoal marker were measured.

The **peristaltic index (PI)** was calculated for each mouse as:

$$\text{Peristaltic index} = \frac{\text{length of charcoal meal}}{\text{length of the small intestine}} \times 100$$

$$\% \text{ of inhibition} = \frac{\text{Average distance traveled by charcoal marker (controls - treated) group}}{\text{Average distance traveled by the charcoal marker of negative controls}} \times 100$$

237 **Castor Oil-Induced Gastrointestinal Motility Test**

238 The effect of *Ficus thonningii* root extract on gastrointestinal motility was evaluated using the
 239 castor oil-induced motility model, as described previously [47]. Thirty mice were fasted for 18
 240 hours (food withheld, water available ad libitum) and randomly divided into five groups (n = 6
 241 per group).

242 One hour after administration of the respective treatments (vehicle, reference drug, or extract
 243 doses), each mouse received 0.5 mL of castor oil orally to induce diarrhea. One hour later,
 244 animals were given 1 mL of charcoal meal (5% activated charcoal suspension in purified water)
 245 by oral gavage.

246 Thirty minutes after charcoal administration, the mice were sacrificed by cervical dislocation.
 247 The abdomen was opened, and the small intestine was carefully removed. The total length of the
 248 intestine (from pylorus to cecum) and the distance traveled by the charcoal marker were
 249 measured using a ruler.

250 The gastrointestinal transit was expressed as a percentage of the distance traveled by the charcoal
 251 relative to the total length of the small intestine:

$$252 \quad \text{Peristalsis index (PI)} = \frac{\text{distance traveled by the charcoal meal}}{\text{total length of small intestine}} \times 100$$

$$253 \quad \% \text{ of inhibition} = \frac{\text{PI of negative control} - \text{PI of drug or extract treated}}{\text{PI of negative control}} \times 100$$

254
 255 The *in vivo* antidiarrheal index (ADI *in vivo*) was estimated agreeing to the formula established by
 256 Aye-Than et al [47]

$$257 \quad \text{ADI invivo} = \sqrt[3]{Dfreq * Gmeq * Pfreq}$$

258 Where D freq is the postponement in evacuation time or diarrhea beginning obtained from castor
 259 oil diarrhea test, calculated as

$$260 \quad Dfreq$$

$$261 \quad = \frac{\text{mean onset of diarrhea (in the treated group - in the negative control group)}}{\text{mean onset of diarrhea in the negative control group}}$$

$$262 \quad \times 100$$

G meq is the gut meal travel decrease (as % of control) attained from charcoal meal test (% inhibition), and P freq is the decrease in the number of wet stools (as % of the negative control) from the castor oil-induced diarrhea model (% I. of Diarrhea).

Ethical Clearance

All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals [48]. Ethical approval for the study was obtained from the Department of Pharmacology Ethical Committee, University of Gondar, under clearance letter number SOP4/100/2013.

Statistical Analysis

Data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using SPSS software version 26.0. Differences within and between groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. A p-value of less than 0.05 was considered statistically significant.

Results

Preliminary Phytochemical Screening

The initial phytochemical analysis of the 80% hydro-methanol crude extract and solvent fractions of *Ficus thonningii* roots revealed the presence of alkaloids, anthraquinones, saponins, tannins, phenols, terpenoids, and flavonoids. Steroids and glycosides were absent in both the crude extract and solvent fractions. Among the solvent fractions, the aqueous fraction tested negative for alkaloids, whereas anthraquinones, saponins, tannins, phenols, terpenoids, and flavonoids were consistently detected in both *n*-hexane and ethyl acetate fractions (Table 1).

Chemical constituents	80% of methanol crude extract	Fractions		
		Hexane	Ethyl acetate	Aqueous residue
Alkaloids	+	+	+	--
Anthraquinones	+	+	+	+
Glycoside	--	--	--	--
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Saponins	+	+	+	+
Steroids	--	--	--	--
Tannins	+	+	+	+
Terpenoids	+	+	+	+

Key : (+) = present (--) = absent

Acute Oral Toxicity Test

Administration of the crude root extract of *Ficus thonningii* at a single oral dose of 2000 mg/kg produced no mortality or visible signs of toxicity. Animals were closely observed during the first four hours (at 30-minute intervals) and monitored daily for 14 days thereafter. No behavioral changes, reduction in food or water intake, or other toxic manifestations were noted throughout the observation period.

Effects on Castor Oil-Induced Diarrhea

The **80% methanol crude extract** of *F. thonningii* roots significantly delayed diarrhea onset and reduced stool frequency and weight at doses of 100 mg/kg ($p<0.05$), 200 mg/kg ($p<0.05$), and 400 mg/kg ($p<0.001$) compared to the negative control.

A notable difference was observed between 100 mg/kg and 200 mg/kg, but not between 200 mg/kg and 400 mg/kg, nor between 400 mg/kg and the standard drug, except in delaying onset.

Diarrheal inhibition rates were **52.94%, 58.82%, and 64.70% ($p<0.01$)** at 100, 200, and 400 mg/kg, respectively. The highest dose (400 mg/kg) produced the greatest inhibition of defecation and lowest fecal output, surpassing the positive control (71.76% at 3 mg/kg).

Aqueous fraction: Significantly prolonged diarrhea onset and reduced stool weight and frequency at 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.01$), and 400 mg/kg ($p < 0.01$). Inhibition rates were **45.26%, 56.84%, and 68.42%**, respectively.

Ethyl acetate fraction: Reduced stool frequency and weight in a dose-dependent manner, with significant effects at 200 mg/kg ($p < 0.05$) and 400 mg/kg ($p < 0.05$). The highest inhibition was **63.2% ($p < 0.05$)** at 400 mg/kg.

n-Hexane fraction: Showed minimal inhibition compared to other fractions.

All extracts showed **dose-dependent reductions** in wet and total fecal output.

The **aqueous fraction at 400 mg/kg** produced the strongest effect (31.6% and 35% reduction), outperforming the standard drug (21% and 21.43%).

Both the methanol crude extract and ethyl acetate fraction showed moderate reductions, while the aqueous fraction consistently demonstrated the **highest efficacy**.

The **n-hexane fraction** was the least effective. (Table 2)

Effects on Castor Oil-Induced Enteropooling

In the enteropooling model, the 80% methanol crude extract of *F. thonningii* significantly reduced both the mean volume (except at 100 mg/kg) and weight of intestinal contents at all tested doses compared with the negative control ($p < 0.05$). No significant differences were observed between the extract at 200 mg/kg and 400 mg/kg and the standard drug (loperamide, 3 mg/kg).

The percentage inhibition of intestinal content weight was:

- 65.3% at 100 mg/kg ($p < 0.01$)
- 71.42% at 200 mg/kg ($p < 0.01$)

333 • 75.51% at 400 mg/kg ($p < 0.01$)

334 (Table 3).

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Table 2. Anti-diarrheal effects of 80% methanol extract and solvent fractions of the roots of *F. thonningii* on castor oil-induced diarrheal model in mice.

Extracts/ Solvent fractions	Dose administered	Onset of Diarrhea	Number of wet feces in 4 hrs	Total No of defecations	Purging frequency in number of wet stools (Pfreq) (%)	The average weight of wet feces(g)	Weight of total feces(g)	% wet fecal out put	% of total fecal out put	% of reducti on
Crude	2% tween 80	50.00±8.32	5.83±0.75	10.00±1.3	----	0.85±0.21	1.18±0.3	--	--	----
	Lop 3	165±14.46 ^{a3}	2.67±0.33 ^{a3}	4.33±0.5 ^{a3}	54.2	0.24±0.05 ^{a2}	0.33±0.06 ^{a2}	28.24	28	71.76
	80 ME100mg	86.83±8.4 ^{d1}	4.17±0.17	6.7±0.33 ^{a1}	28.5	0.40±0.05 ^{a1}	0.54±0.06 ^{a1}	47	45.8	52.94
	80 ME 200mg	128.50±13.2 ^{a2}	3.33±0.33 ^{a2}	5.83±0.4 ^{a2}	42.9	0.35±0.06 ^{a1}	0.48±0.08 ^{a1}	41.2	40.7	58.82
	80 ME 400mg	155.00±24.9 ^{a3}	3.00±0.26 ^{a2}	5.00±0.36 ^{a3}	48.54	0.30±0.06 ^{a2}	0.41±0.07 ^{a2}	35.3	34.75	64.70
n-hexane	10% DMSO	54.67±2.70	7.67±0.92	14.83±1.5	---	0.95±0.09	1.40±0.08	--	--	---
	Lop 3	163.67±17 ^{a3}	2.0±0.34 ^{a3}	4.67±0.5 ^{a3}	74	0.2±0.01 ^{a3}	0.30±0.02 ^{a3}	21	21.43	78.94
	HF 100mg	93±14 ^{b1}	6.33±0.42 ^{a1}	9.67±0.76 ^{a1}	40.50	0.72±0.08 ^{a2}	0.9±0.12 ^{a1}	75.8	64.3	24.2
	HF 200mg	101.17±4.9 ^{a2}	4.67±0.56 ^{a1}	8.17±1.14 ^{a2}	49.11	0.58±0.02 ^{a3}	0.72±0.02 ^{a3}	61	51.4	39
	HF 400mg	130±5.36 ^{a3}	2.5.00±0.82 ^{a3}	5.67±1.3 ^{a3}	67.9	0.32±0.06 ^{a3}	0.38±0.08 ^{a3}	33.7	40	66.3
Ethyl acetate	EAF100mg	71.33±12.2 ^{d2}	3.83±0.17 ^{a2}	6.83±0.4 ^{a1}	50.06	0.50±0.08	0.8±0.12	52.6	57.1	47.4
	EAF 200mg	100.83±7.8 ^{a3}	3.33±0.21 ^{a3}	6.17±0.6 ^{a2}	56.6	0.38±0.04 ^{a1}	0.62±0.04	40	44.3	60
	EAF 400mg	129.50±12.0	3.00±0.52 ^{a2}	5.83±0.95 ^{a2}	60.9	0.35±0.06 ^{a1}	0.54±0.01 ^{a1}	36.8	38.6	63.2
Aqueous	AqF 100mg	91.83±12.3	4.00±1.24	10.00±2.5	47.8	0.52±0.17 ^{a1}	0.83±0.3 ^{a1}	54.74	59.3	45.26
	AqF 200mg	140.00±24 ^{a1}	3.00±0.89 ^{a1}	7.50±1.65 ^{a2}	60.9	0.41±0.09 ^{a2}	0.63±0.15 ^{a2}	43.2	45	56.84
	AqF 400mg	160.33±25 ^{a2}	2.00±0.77 ^{a2}	5.17±1.5 ^{a2}	73.9	0.30±0.06 ^{a2}	0.49±0.1 ^{a2}	31.6	35	68.42

Values are expressed as mean ± CI₉₅ (n = 6); analysis was performed using one way ANOVA followed by Tukey's Post hoc test; ^a compared with negative control values; ^b compared with standard drug ; ^c compared with 100 mg/kg, ^d compared with 200 mg/kg; ^e compared with 400 mg/kg, ¹p<0.05, ²p<0.01, ³p<0.001; 80% ME = 80% methanolic extract, HF = n-hexane fraction, EAF = Ethyl acetate fraction, AF = aqueous fraction, Lop3 = Loperamide 3 mg , DMSO= Dimethyl Sulfoxide CI₉₅ = 95% confidence interval. Controls are 10 ml/kg 2% tween -80 (80 % ME) and dimethyl sulfoxide (n-hexane,ethyl acetate and aqueous fractions)

Table 3. Effects of 80 % methanol crude extract and solvent fractions of the roots of *F. thonningii* roots on castor oil-induced enteropooling in mice

Extraction/solvent fractions	Dose administered	Mean-weight of small intestinal content (gm)	% inhibition	Mean-volume of small intestinal content(ml)	% inhibition
80% MeOH	2% tween 80	0.49±0.12	--	0.4±0.02	---
	Lop 3	0.10±0.00 ^{a2}	79.59	0.1±0.02 ^{a2}	75
	80 ME100 mg	0.17±0.03 ^{a2}	65.30	0.2±0.02	50
	80 ME 200 mg	0.14±0.03 ^{a2}	71.42	0.15±0.02 ^{a1}	62.5
	80 ME 400 mg	0.12±0.01 ^{a2}	75.51	0.12±0.02 ^{a2}	70
Solvent fractions	10% DMSO	0.49±0.03	--	0.44±0.02	--
	Lop 3	0.15±0.01 ^{a3}	69.38	0.13±0.02 ^{a3}	70.45
	HF 100 mg	0.28±0.02 ^{a2}	42.86	0.4±0.03 ^{c1d2}	9.1
	HF 200 mg	0.24±0.05 ^{a3}	51.02	0.3±0.03 ^{a1}	31.82
	HF 400 mg	0.2±0.02 ^{a3}	59.2	0.15±0.02 ^{a3}	66
	EAF100 mg	0.22±0.04 ^{a2}	55.1	0.25±0.03	43.2
	EAF 200 mg	0.18±0.03 ^{a3}	63.3	0.15±0.02 ^{a3}	66
	EAF 400 mg	0.14±0.03 ^{a3}	71.4	0.13±0.02 ^{a3}	70.5
	AqF 100 mg	0.25±0.03 ^{a2}	49	0.22±0.03 ^{a1c1d1}	50
	AqF 200 mg	0.19±0.02 ^{a3}	61.2	0.13±0.02 ^{a3}	70.5
	AqF 400 mg	0.14±0.02 ^{a3}	71.4	0.12±0.02 ^{a3}	72.7

Values are expressed as mean ± CI₉₅ (n = 6); analysis was performed using one way ANOVA followed by Tukey's Post hoc test; ^a compared with negative control values; ^b compared with standard drug ^c compared with 100 mg/kg; ^d compared with 200 mg/kg; ^e compared with 400 mg/kg, ¹p<0.05, ²p<0.01, ³p<0.001; 80% ME = 80% methanolic extract, HF = n-hexane fraction, EAF = Ethyl acetate fraction, AF = aqueous fraction, Lop3=loperamide 3mg, DMSO=Dimethyl Sulfoxide CI₉₅ = 95% confidence interval. Controls are 10 ml/kg 2% tween 80 (for 80% ME) and dimethyl Sulfoxide (n-hexane, Ethyl acetate and aqueous fractions).

Effects on Normal Gastrointestinal Transit in Mice

The 80% methanol crude extract and solvent fractions of *Ficus thonningii* significantly inhibited normal intestinal propulsion compared with the negative control. The crude extract reduced the percentage intestinal transit of the charcoal meal in a dose-dependent manner, producing 19.85%, 25.27%, and 31.6% inhibition at 100, 200, and 400 mg/kg, respectively ($p < 0.01$). Similarly, the aqueous fraction inhibited intestinal transit by 17.7%, 31.7%, and 38.34% at the same graded doses ($p < 0.01$). Atropine sulfate (5 mg/kg) produced the highest inhibition (56.70%, $p < 0.001$). Among the fractions, the aqueous extract at 400 mg/kg showed maximal inhibition of intestinal propulsion, while the 100 mg/kg dose did not produce a significant effect compared with the negative control (Table 4).

377 **Table 4. Effect of *Ficus thonningii* Extracts and Fractions on Normal Gastrointestinal**
378 **Transit in Mice**

Extracts/ solvents	Dose administered	The total length of the small intestine (cm)	Distance moved by the charcoal meal (cm)	Peristalsis index (%)	%Inhib ition
80 % MeOH	2% Tween 80	57.77±1.09	40.74±1.25	70.51	---
	ATR 5	55.96±1.28	18.33±.56 ^{a3e1}	32.76	53.54
	80 ME100 mg	53.58±0.93	30.28±2.15 ^{a3}	56.51	19.85
	80 ME 200 mg	54.47±1.17	28.70±1.18 ^{a3}	52.69	25.27
	80 ME 400 mg	55.30±1.21	26.69 ±.55 ^{a3 e3}	48.26	31.6
Solvent fractions	DMSO	52.17±2.31	36.5±1.25	69.96	--
	ATR 5	53.00±2.20	16.23±.46 ^{a3e1}	30.32	56.7
	HF 100 mg	57.67±2.38	35.4±2.15 ^{a2}	61.4	12.2
	HF 200 mg	53.67±3.21	30.35±1.18 ^{a3}	56.55	19.2
	HF 400 mg	52.50±2.17	25.62±.55 ^{a3 e3}	48.8	30.25
	EAF100 mg	50.17±2.29	30.51±1.23 ^{a2d1}	60.81	13.07
	EAF 200 mg	52.50±2.51	28.45±1.07 ^{a3}	54.2	22.5
	EAF 400 mg	54.50±3.26	26.6±1.07 ^{a3}	48.8	30.24
	AqF 100 mg	49.50±1.67	28.54±1.25 ^{a3d1}	57.6	17.7
	AqF 200 mg	54.83±2.15	26.22±0.92 ^{a3}	47.8	31.7
	AqF 400 mg	52.50±2.50	22.65±.65 ^{a3 e3}	43.14	38.34
Values are expressed as mean ± CI₉₅ (n = 6); analysis was performed using one way ANOVA followed by Tukey's Post hoc test; ^a compared with negative control values; ^bcompared with standard drug ^ccompared with 100 mg/kg ; ^dcompared with 200 mg/kg; ^e compared with 400 mg/kg, ¹p<0.05, ²p<0.01, ³p<0.001; ATR5= 5mg atropine 80% ME = 80% methanolic extract, HF = n-hexane fraction, EAF = Ethyl acetate fraction, AF = aqueous fraction, CI₉₅ = 95% confidence interval. Controls are 10 ml/kg 2% tween-80 (for 80% ME) and DMSO= Dimethyl Sulfoxide (solvent fractions).					

379

380 **Effects on Castor Oil-Induced Gastrointestinal Motility**

381 The 80% methanol crude extract of *Ficus thonningii* significantly inhibited intestinal transit of
382 the charcoal meal at all tested doses. The percentage reduction in gastrointestinal transit was
383 28.4% (p < 0.05), 55.5% (p < 0.001), and 65.7% (p < 0.001) at 100, 200, and 400 mg/kg,

384 respectively. The effect at 200 mg/kg was comparable to atropine sulfate (57.0% inhibition at 5
385 mg/kg).

386 All three solvent fractions (aqueous, ethyl acetate, and *n*-hexane) also significantly inhibited
387 gastrointestinal motility at all tested doses compared with the negative control. The aqueous
388 fraction at 400 mg/kg produced the greatest inhibition (54.86%, $p < 0.001$) (Table 5).

389
390 **Table 5.** Effects of 80% MeOH extract and solvent fractions on castor oil-induced
391 gastrointestinal motility in mice

Extracts/solvents	Dose administered	The total length of the small intestine (cm)	Distance moved by the charcoal meal (cm)	Peristalsis index (%)	%Inhibition
80 % MeOH	2% tween 80	54.17±2.37	28.67±0.88	53.0 ±1.9	--
	ATR 5	52.67±1.22	12.00±0.58 ^{a3}	22.8±1.6	57.0
	80 ME100 mg	54.33±1.62	20.67±1.48 ^{a1d2}	38.0±1.7	28.4
	80 ME 200 mg	55.83±2.00	14.33±1.28 ^{a3}	25.7±1.1	55.5
	80 ME 400 mg	56.00±0.93	10.17±0.65 ^{a3}	18.2±0.9	65.7
Solvent fractions	DMSO	48.17±2.31	30.67±2.12	63.58±2.6	---
	ATR 5	54.00±2.20	15.83±0.98 ^{a3}	29.40±1.7	53.76
	HF 100 mg	55.67±2.38	30.33±1.54 ^{a1}	54.5±1.3	14.3
	HF 200 mg	54.67±3.21	28.17±2.24 ^{a2}	51.0±3.0	19.7
	HF 400 mg	55.50±2.17	26.33±1.40 ^{a1}	47.5.0±3.0	25.3
	EAF100 mg	51.17±2.29	20.50±1.23 ^{a2d1}	40.14±1.9	37
	EAF 200 mg	53.50±2.51	18.83±1.07 ^{a3}	35.2±1.5	44.6
	EAF 400 mg	52.50±3.26	18.0±1.07 ^{a3}	34.3±1.1	54.4
	AqF 100 mg	49.50±1.67	22.17±1.25 ^{a3d1}	44.8±2.3	29
	AqF 200 mg	55.83±2.15	19.50±0.92 ^{a3}	35±1.9	43
	AqF 400 mg	50.50±2.50	14.50±0.73 ^{a3}	28.7 ±1.6	54.86

Values are expressed as mean ± CI₉₅ (n = 6); analysis was performed using one way ANOVA followed by Tukey's Post hoc test; ^a compared with negative control values; ^b compared ^c compared with 100mg/ kg; ^d compared with 200 mg/kg; ^e compared with 400 mg/kg, ¹ $p < 0.05$, ² $p < 0.01$, ³ $p < 0.001$; ATR=5 mg atropine 80% ME = 80% methanolic extract, HF = *n*-hexane fraction, EAF = Ethyl acetate fraction, AF = aqueous fraction, CI₉₅ = 95% confidence interval. Controls are 10 ml/kg 2% tween-80 (for 80% ME) and DMSO=dimethyl Sulfoxide (*n*-hexane, ethyl acetate and aqueous fractions).

In Vivo Anti-Diarrheal Index (ADI)

The anti-diarrheal index (ADI) was calculated by considering three parameters: delay in defecation (Dfreq), reduction in gastrointestinal transit (Gmeq), and purging frequency (Pfreq). The 80% methanol crude extract at 400 mg/kg produced a high ADI (87.5%), comparable to the standard drug loperamide hydrochloride (89.23% at 3 mg/kg). All solvent fractions demonstrated dose-dependent increases in ADI values, with the aqueous fraction showing the highest activity (90.80% at 400 mg/kg).

Regression analysis confirmed strong dose-response relationships:

- 80% methanol extract ($R^2 = 0.973$)
- *n*-hexane fraction ($R^2 = 0.954$)
- Ethyl acetate fraction ($R^2 = 0.996$)
- Aqueous fraction ($R^2 = 0.986$)

Table 6. *In vivo* anti-diarrheal indices of 80 % methanol extract and solvent fractions of the roots of *F. thonningii*

Fractions/ solvents	Dose administered	Delay in defecation (Dfreq %)	Gut meal travel distance, (Gmeq %)	Purging frequency in number of wet feces (%)	Antidiarrhea l index(ADI)
80% MeOH	2 % tween 80	---	--	--	---
	Positive con.	230	57.0	54.2	89.23
	80 ME100 mg	73.7	28.4	28.5	39
	80 ME 200 mg	157	55.5	45.9	73.7
	80 ME 400 mg	210	65.7	48.54	87.5
Solvent fractions	DMSO	--		--	--
	Positive. cont.	157.3	53.76	74	85.5
	HF 100 mg	70.11	14.3	17.50	26
	HF 200 mg	101.64	19.7	39.11	42.8
	HF 400 mg	137.8	25.3	67.9	61.86
	EAF100 mg	30.5	37	50.06	38.4
	EAF 200 mg	84.4	44.6	56.6	59.7
	EAF 400 mg	137	54.4	60.9	76.85
	AqF 100 mg	68	29	47.8	45.5
	AqF 200 mg	150	43	58.9	72.4.
	AqF 400 mg	192.3	54.86	70.9	90.8
80 ME = 80% methanolic extract, HF = n-hexane fraction, EAF = Ethyl acetate fraction, AqF = aqueous fraction					

Discussion

Study Purpose

This research investigated the antidiarrheal potential of the 80% methanol crude root extract of *Ficus thonningii* and its solvent fractions in Swiss albino mice.

Phytochemical Contributions

Phytochemical screening confirmed the presence of alkaloids, tannins, saponins, phenols, terpenoids, and flavonoids in the crude extract. These compounds are known to influence gastrointestinal physiology:

Tannins and flavonoids act as astringents, suppress intestinal fluid secretion, and block prostaglandin synthesis.

Alkaloids and terpenoids regulate smooth muscle activity, thereby reducing excessive motility.

Saponins and phenols provide anti-inflammatory and antimicrobial effects.

The antidiarrheal activity of *F. thonningii* roots is therefore likely due to the combined action of these phytoconstituents.

Safety Profile

Acute toxicity testing revealed that the extract was safe up to 2000 mg/kg, with no mortality or behavioral changes observed. Since the LD₅₀ value is more than three times higher than the lowest effective dose (100 mg/kg), the extract demonstrates a wide therapeutic margin. According to WHO classification, compounds with LD₅₀ values above 2000 mg/kg are considered “unlikely to be hazardous.” This suggests that the extract is safe for further pharmacological studies.

Mechanism of Action in Castor Oil–Induced Diarrhea

Castor oil produces diarrhea via its metabolite ricinoleic acid, which irritates the intestinal lining and stimulates prostaglandin release. Prostaglandins, particularly PGE₂, enhance intestinal motility, increase secretion, and reduce NaCl and water absorption. They also impair glucose uptake, leading to fluid accumulation in the lumen.

The significant reductions in stool frequency, stool weight, intestinal fluid, and motility observed with *F. thonningii* extracts indicate that the plant may act through:

Inhibition of prostaglandin synthesis or activity, thereby reducing secretion and fluid retention.

Regulation of intestinal motility, as shown by delayed gastrointestinal transit in both normal and castor oil-induced models.

Improved absorption of fluids and electrolytes, which decreases purging frequency.

Comparative Efficacy

At 400 mg/kg, the aqueous fraction exhibited the strongest antidiarrheal index (ADI = 90.8%), slightly exceeding the effect of loperamide (89.23%). This suggests that water-soluble phytochemicals may be the most potent contributors to the activity. The consistent dose-dependent responses across all fractions reinforce the pharmacological relevance of *Ficus thonningii*.

Overall, the study confirmed that the 80% methanol crude root extract of *F. thonningii* possesses marked antidiarrheal properties in castor oil-induced diarrhea models.

Castor Oil–Induced Diarrhea Model

The castor oil model is a standard approach for assessing antidiarrheal agents, as it reflects both motility and secretory mechanisms. In this model, parameters such as onset of defecation, stool frequency, and stool weight—particularly wet stools—are critical indicators. The crude extract of *F. thonningii* significantly reduced both the frequency and weight of wet and total stools, confirming its therapeutic potential.

This observation is consistent with earlier findings on medicinal plants that reduce wet stool output. Since castor oil causes diarrhea mainly by impairing fluid and electrolyte absorption and stimulating intestinal peristalsis, one plausible mechanism of *F. thonningii* is its ability to enhance fluid and electrolyte uptake in the gut.

Dose-Dependent Activity

The extract delayed the onset of diarrhea and reduced stool parameters at all tested doses, with the strongest inhibition at 400 mg/kg ($p < 0.001$). The weaker effect at 100 mg/kg compared to 200 and 400 mg/kg suggests that 100 mg/kg may be below the threshold for optimal activity. This dose-dependent pattern mirrors findings from other medicinal plants, where higher doses are required to achieve significant antidiarrheal effects.

Mechanistic Insights

The reduction in wet fecal output points to an anti-secretory mechanism, likely involving suppression of prostaglandin synthesis. Castor oil–induced diarrhea is mediated by prostaglandins, nitric oxide, and platelet-activating factors, which drive intestinal secretion and

motility. The ability of *F. thonningii* extract to counteract these mediators indicates that its phytoconstituents may act by:

Inhibiting prostaglandin pathways, thereby reducing fluid accumulation.

Modulating intestinal motility, delaying transit time.

Enhancing fluid and electrolyte absorption, lowering stool frequency.

Fractionation Studies

To pinpoint the active constituents, the 80% methanol crude root extract of *Ficus thonningii* was separated using solvents of different polarities. Among the fractions, the aqueous extract displayed the strongest antidiarrheal effect, particularly at 400 mg/kg, where its antidiarrheal index (ADI) exceeded that of loperamide. This indicates that the most potent bioactive compounds are likely water-soluble phytochemicals such as tannins, flavonoids, and saponins, which are known to reduce intestinal secretion and regulate motility.

Fractional Efficacy and Phytochemical Contributions

The aqueous fraction showed the highest efficacy, followed by the ethyl acetate and n-hexane fractions. This order of activity reflects the polarity of the solvents and the distribution of secondary metabolites. Polar constituents—including tannins, flavonoids, and saponins—are concentrated in aqueous and semi-polar fractions, explaining their stronger effects. These compounds are recognized for their antidiarrheal actions through mechanisms such as:

Enhancing Na⁺/K⁺-ATPase activity, which promotes water and electrolyte absorption.

Antisecretory effects, reducing ricinoleic acid-induced fluid accumulation.

Antimotility actions, achieved by inhibiting prostaglandins, autocoids, and calcium influx.

Thus, the pronounced activity of *F. thonningii* roots can be attributed to the synergistic effects of these phytochemicals, with the aqueous fraction retaining the most active constituents.

Enteropooling Model

In the enteropooling assay, the crude extract, aqueous fraction, and ethyl acetate fraction significantly reduced intestinal fluid volume and weight at all tested doses, supporting an antisecretory mechanism. This effect is likely mediated by tannins and flavonoids, which enhance colonic absorption of water and electrolytes. In contrast, the n-hexane fraction showed notable activity only at higher doses (200–400 mg/kg), underscoring its weaker contribution to

fluid regulation. These findings are consistent with previous reports linking anti-enteropooling activity to overall antidiarrheal efficacy.

Gastrointestinal Motility

Both the crude extract and its fractions significantly suppressed charcoal meal transit. At 400 mg/kg, the 80% methanol extract achieved 65.7% inhibition, which was greater than atropine (57% at 5 mg/kg). This demonstrates strong antimotility activity, likely attributable to phytochemicals such as flavonoids, tannins, and terpenoids. Flavonoids are known to block spasmogen-induced contractions and prostaglandin release, tannins reduce calcium influx into smooth muscle cells, and terpenoids act as calcium antagonists—all contributing to reduced intestinal motility.

Dual Mechanism of Action

The antidiarrheal efficacy of *F. thonningii* root extract appears to result from a dual mechanism: suppression of motility and inhibition of intestinal secretion. Unlike conventional agents such as loperamide, which often cause constipation, the plant extract may provide a safer alternative by balancing motility reduction with fluid regulation.

Anti-Diarrheal Index (ADI)

The in vivo ADI, which integrates delay in defecation, inhibition of motility, and reduction in purging frequency, confirmed dose-dependent activity. The aqueous fraction at 400 mg/kg produced the highest ADI (90.8%), slightly exceeding loperamide (89.23%). In contrast, the n-hexane fraction consistently showed the lowest ADI, reflecting its limited contribution. This validates ADI as a reliable parameter for grading antidiarrheal agents.

Mechanistic Confirmation via Enteropooling and Motility Models

Effective antidiarrheal drugs typically act by reducing motility and/or secretion. To verify these mechanisms, both enteropooling and motility assays were employed. The extracts inhibited castor oil-induced intestinal transit more strongly than normal transit, suggesting that they specifically counteract ricinoleic acid-induced hypermotility.

This finding is consistent with previous reports that antidiarrheal agents reduce gastrointestinal contractions, slow transit, and allow more time for water and electrolyte absorption. Importantly, the extracts achieved motility inhibition without causing excessive constipation—a common drawback of standard drugs like loperamide—highlighting their therapeutic promise.

Role of Polar Secondary Metabolites

The comparable inhibitory effects observed with the crude extract and the aqueous fraction emphasize the importance of polar phytochemicals in mediating antimotility activity. Compounds such as polyphenols, flavones, alkaloids, saponins, and terpenoids—identified in the 80% methanol extract and its fractions—are likely responsible for these effects. At 400 mg/kg, the crude extract inhibited charcoal meal transit by 65.7% ($p < 0.001$), exceeding the inhibition produced by atropine sulfate (57% at 5 mg/kg). This superior activity suggests that the extract effectively modulates peristaltic movement, thereby reducing diarrheal output. By slowing gastrointestinal motility, the extract enhances water and electrolyte absorption, which explains the observed reduction in wet stool weight and frequency.

Antispasmodic Properties of Fractions

Both the aqueous and ethyl acetate fractions exhibited significant antispasmodic activity, with the aqueous fraction at 400 mg/kg producing the strongest inhibition (54.86%, $p < 0.001$). In contrast, the n-hexane fraction showed activity in the charcoal meal test but failed to significantly reduce wet stool number and weight, particularly at lower doses. This indicates that antimotility effects alone are insufficient to control diarrhea unless accompanied by antisecretory activity. Similar findings have been reported in other studies, where extracts with limited efficacy in castor oil-induced models still demonstrated notable antispasmodic properties.

Mechanistic Insights: Autocoids and Prostaglandins

The crude extract and all fractions significantly slowed charcoal meal transit, suggesting inhibition of autocoid and prostaglandin release. Flavonoids are known to block spasmogen-induced contractions, suppress autocoid and prostaglandin discharge, and reduce intestinal transit. Tannins, also present in the extracts, contribute to muscle relaxation by limiting intracellular calcium influx or stimulating calcium pumps. These combined actions explain the extracts' ability to reduce hypermotility.

Synergistic Role of Phytochemicals

The coexistence of flavonoids, tannins, terpenoids, alkaloids, and saponins in *F. thonningii* roots likely accounts for the dual antimotility and antisecretory effects observed. Flavonoids and tannins reduce intestinal contractions and fluid secretion, while terpenoids act as calcium antagonists to further suppress motility. Together, these phytochemicals act synergistically, providing a mechanistic basis for the broad antidiarrheal efficacy of the plant.

Role of Tannins and Terpenoids

Tannins are well-established antidiarrheal agents because they can denature intestinal mucosal proteins, forming protein–tannate complexes. These complexes strengthen mucosal resistance and reduce peristaltic activity, thereby limiting fluid loss. Terpenoids, also detected in the 80% methanol crude extract and its fractions, were shown to counteract castor oil-induced hyperpropulsion. Their antispasmodic effects are largely attributed to calcium antagonism, which relaxes intestinal smooth muscle and suppresses abnormal motility.

Inhibition of Nitric Oxide and Secretory Pathways

Flavonoids, tannins, and terpenoids are also known to inhibit nitric oxide synthesis by downregulating inducible nitric oxide synthase (iNOS). Since nitric oxide contributes to intestinal secretion and motility, its suppression further supports the antidiarrheal activity of *F. thonningii*. Castor oil-induced enteropooling involves cyclic AMP/GMP signaling and CFTR channel phosphorylation, which drive chloride and sodium secretion. The ability of the extract and fractions to reduce intestinal fluid accumulation suggests interference with these secretory pathways.

Anti-Diarrheal Index (ADI)

The ADI, which integrates delay in defecation, intestinal motility, and purging frequency, provides a comprehensive measure of antidiarrheal efficacy. Both the crude extract and solvent fractions showed dose-dependent increases in ADI, with the highest values recorded at 400 mg/kg. The aqueous fraction consistently produced the strongest ADI, while the n-hexane fraction showed the weakest activity. This confirms ADI as a reliable parameter for evaluating and ranking antidiarrheal agents.

Ethnomedicinal Validation

Overall, the findings demonstrate that the 80% methanol crude root extract of *F. thonningii* possesses significant antidiarrheal activity through dual mechanisms: inhibition of gastrointestinal propulsion and suppression of fluid secretion. These results validate the traditional use of *F. thonningii* roots as a non-specific antidiarrheal remedy in folk medicine. Importantly, the combined antimotility and antisecretory effects suggest that the plant could serve as a promising candidate for managing diarrheal diseases of varied origins.

Conclusion

This study demonstrated that the 80% methanol crude root extract of *Ficus thonningii* and its solvent fractions possess notable antidiarrheal activity in vivo. The extract was safe at doses up to 2000 mg/kg, showing no signs of acute toxicity, which supports its suitability for further pharmacological investigation.

Across multiple experimental models—including castor oil-induced diarrhea, enteropooling, and gastrointestinal motility—the extract and fractions produced dose-dependent effects. The aqueous fraction consistently exhibited the strongest efficacy, even surpassing loperamide in terms of antidiarrheal index (ADI). The activity is likely mediated by phytoconstituents such as tannins, flavonoids, terpenoids, alkaloids, saponins, and phenols, which act synergistically through dual mechanisms:

Antimotility effects via inhibition of prostaglandins, autocoids, and calcium influx.

Antisecretory effects through enhanced absorption of water and electrolytes and suppression of nitric oxide and CFTR-mediated secretion.

The dual action of *F. thonningii*—reducing both intestinal propulsion and fluid secretion—provides a mechanistic basis for its broad antidiarrheal efficacy. These findings validate its ethnomedicinal use as a non-specific antidiarrheal remedy and highlight its potential as a source of novel therapeutic agents.

Future research should aim to isolate and characterize the specific bioactive compounds responsible for the activity, while also assessing chronic toxicity and conducting clinical trials to establish its therapeutic relevance in humans.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

Isolation of Active Compounds: Future research should aim to isolate and characterize the specific phytoconstituents—such as tannins, flavonoids, terpenoids, alkaloids, and saponins—responsible for the antidiarrheal activity of *Ficus thonningii*.

Mechanistic Studies: Detailed investigations are needed to confirm the involvement of prostaglandin inhibition, nitric oxide suppression, and Na⁺/K⁺-ATPase activation in mediating the observed pharmacological effects.

Toxicological Evaluation: While the crude extract was safe in acute toxicity tests, sub-chronic and chronic toxicity studies are recommended to establish long-term safety profiles.

Clinical Trials: Controlled clinical studies should be conducted to validate the efficacy and safety of *F. thonningii* extracts in humans, particularly in regions where diarrheal diseases are prevalent.

Formulation Development: Given its superior activity, the aqueous fraction should be prioritized for development into standardized herbal formulations or phytomedicines

651 **ABBREVIATIONS**

652 ADI – Antidiarrheal Index

653 ANOVA - Analysis of Variance

654 MVIC – Mean Volume of Intestinal Content

655 MVICC - Mean Volume of Intestinal Content of Control Group

656 MVICT - Mean Volume of Intestinal Content of Test Group

657 MWIC – Mean Weight of Intestinal Content

658 MWICC - Mean Weight of Intestinal Content of Control Group

659 MWICT - Mean Weight of Intestinal Content of Test Group

660 OECD: Organization for Economic Cooperation and Development

661 SEM - Standard Error of the Mean

662 SPSS: Statistical Package for the Social Sciences

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672 **Declarations**

673 **Acknowledgment**

674 The authors acknowledge University of Gondar for allowing the laboratory setup to conduct this
675 experimental research.

676 **Ethical Approval**

677 Ethical issues and the study protocol were approved by the Institutional review board of University
678 of Gondar.

679 **Competing interests**

680 There is no conflict of interest

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687 **Authors' Contributions**

688 Sisay Tarekegn, Asefa Belay and Mestayet Geta designed and conducted the laboratory
689 experiment. Muluken Adela is involved in manuscript writing, study design and analysis of the
690 results. All authors had read and approved the final manuscript.

691 **Data Availability**

692 The datasets used and/or analyzed during the current study are available from the corresponding
693 author on reasonable request.

694 **Funding**

695 No funding

696 **Consent for publication**

697 Not applicable

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