Preliminary Phytochemical Screening and Pharmacological Evaluation of Ethanolic Extract of *Simarouba glauca* bark for Anti-Diarrhoeal Activity in Rats

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ABSTRACT

Diarrhoea is a leading cause of morbidity and mortality worldwide. The present study evaluated the ethanolic bark extract of Simarouba glauca for anti-diarrhoeal activity in rats. Bark was shade dried, powdered and extracted with ethanol using a Soxhlet apparatus. Wistar albino rats (100–150 g) were divided into five groups (n=5): normal,control , loperamide (5 mg/kg), and extract at 200 and 400 mg/kg. Anti-diarrhoeal activity was evaluated using castor oil-induced diarrhoea, charcoal meal gastrointestinal motility and castor oil-induced enteropooling models. The extract reduced wet stools, total stools and charcoal transit, with the 400 mg/kg dose showing an effect comparable to loperamide. The activity may be associated with tannins, flavonoids and quassinoids. The ethanolic bark extract showed significant anti-diarrhoeal activity and supports its traditional use.

Keywords: *Simarouba glauca;* anti-diarrheal; castor oil; loperamide; ethanolic bark extract; charcoal meal; atropine sulphate; CMC; tragacanth

1. INTRODUCTION

Diarrhoea is defined as the passage of three or more loose or liquid stools per day. It is one of the most common gastrointestinal disorders and remains a major public health problem in developing countries. According to the World Health Organization, diarrhoea is a major cause of morbidity and mortality among children under five years of age [1]. It results from an imbalance between absorptive and secretory functions of intestinal tract along with increased intestinal motility. The major causative agents include enteric infections, bacterial toxins, viruses and parasites [2]. Various mechanisms are involved in pathogenesis of diarrhoea[3]. Castor oil is widely used to induce experimental diarrhea. It contains ricinoleic acid, which irritates the intestinal mucosa, stimulates peristalsis and alters electrolyte permeability, leading to increased secretion and motility [4]. Many anti-diarrheal drugs such as Loperamide and Atropine are available in market. Loperamide acts by inhibiting intestinal motility, but prolonged use may cause adverse effects such as constipation, drowsiness, nausea and dry mouth [5]. Hence, search for safe and effective herbal drugs with anti-diarrheal activity is still continued. Medicinal plants have been used since ancient times for treatment of various diseases. Herbal medicines are considered relatively safe with less side effects and cost effective. Several plants with anti-diarrhoeal activity have been reported in the literature [6]. *Simarouba glauca* DC is an important medicinal plant belonging to family Simaroubaceae. It is commonly known as Paradise Tree, Bitter wood, Aceituno and Laxmi taru. It is a medium sized evergreen tree, native to Central and South America and now extensively cultivated in India, particularly in Andhra Pradesh, Karnataka and Maharashtra. The tree can grow up to 15 meters height with bright green leaves. In traditional system of medicine, different parts of *Simarouba glauca* are used for various ailments. Bark is the most important part. Traditionally bark decoction is used for treatment of diarrhoea, dysentery, amoebiasis and malaria. In Brazil, it is popularly known as most effective remedy for dysentery. Leaves are used for fever and malaria. Seeds contain edible oil which is used for cooking purpose. Phytochemical studies on *Simarouba glauca* have reported a wide range of secondary metabolites[34]. Bark contains mainly quassinoids, triterpenoids, alkaloids, flavonoids, phenolic compounds and tannins. Important quassinoids isolated from bark are glaucarubinone, glaucarubine, glaucarubin, simarolide, simaroubolide, Ailanthionone and holacanthone. Tannins and flavonoids are known to possess anti-diarrheal activity through various mechanisms. Tannins precipitate proteins in intestinal mucosa, make mucosa more resistant to chemical alteration and reduce secretion. Flavonoids may inhibit intestinal motility and secretion induced by castor oil [13]. Pharmacologically, *Simarouba glauca* bark has been reported to have antibacterial, anti-amoebic, anti-protozoal, antimalarial, antiulcer and anticancer activities. Bark extract has shown potent activity against *Entamoeba histolytica, Escherichia coli* and *Staphylococcus aureus* which are common pathogens causing diarrhoea. Ethanolic extract is preferred because ethanol can extract both polar compounds like

tannins and flavonoids and moderately non-polar compounds like quassinoids effectively compared to aqueous extract. Though bark is traditionally claimed for anti-diarrheal activity, only few scientific studies have been carried out on Ethanolic bark extract using experimental models like castor oil induced diarrhoea and charcoal meal transit test. Hence, the present study was undertaken to provide scientific validation for traditional use of *Simarouba glauca* bark as anti-diarrheal agent. The aim of this study was to evaluate the phytochemical profile and anti-diarrhoeal activity of the ethanolic extract of *Simarouba glauca* bark in rats. The objectives were to collect and authenticate the bark, prepare the ethanolic extract by Soxhlet extraction, conduct preliminary phytochemical screening, evaluate activity using castor oil-induced diarrhoea, charcoal meal-induced gastrointestinal motility and castor oil-induced enteropooling models, and conducting *in-silico* studies [14].

2. MATERIALS AND METHODS

2.1 Ethical consideration

Experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Sri Krishnadevaraya University Anantapuramu, Andhra Pradesh, India.(Approval No. IAEC/02/09/SKU/2025 Registration No 1889/GO/Re/S/16/CCSEA) and performed as per guidelines of CCSEA

2.2 Instruments

Soxhlet apparatus, Rotary vacuum evaporator, Digital weighing balance, Mechanical grinder

2.3 Chemicals and Reagents

Distilled water, Loperamide, Castor oil, Activated charcoal, Atropine sulfate, Tragacanth, Ethanol, CMC (Carboxy Methyl Cellulose), and chloroform were obtained from the Sri Krishnadevaraya university college of pharmaceutical sciences, Anantapur.

2.4 Plant material: collection and extraction

2.4.1 Plant collection and identification

Fresh bark of *Simarouba glauca* DC were collected from Polymer Science Department, S.K University, Anantapur and then spread on a clean surface and left for about 21 to 30 days to dry indoors, after they grounded into a fine powder by using electrical grinder. The plant has been identified by Dr B. RAVI PRASAD RAO, Professor of Botany in S.K.University, Botanical Garden, Anantapuramu-515003, Andhra Pradesh, India. The plant authentication voucher No is 57455.

Figure 1. ***Simarouba glauca*** **leaves**

2.4.2 Preparation of extract

The bark of *Simarouba glauca* was shade-dried and subsequently pulverized using an electric grinder. The resultant coarse powder was sieved through No.22 mesh and an exact quantity of 30 g was taken for extraction. Soxhlet extraction was performed using 300 ml of absolute ethanol (99%) at a ratio of 1:10 (feed: solvent). The flask was sealed with paraffin wax and extracted for 24 hours. After extraction, the extract was filtered via Whatsman No.1 filter paper to eliminate debris. The filtrate was then dried under vacuum using a rotary evaporator, the extract is about 4.4 g, equivalent to 14.6% yield. For in vivo studies, the extract was given to rats orally at two dose levels i.e., 200 mg/kg and 400 mg/kg [34].

2.4.3 Experimental animals

Healthy male albino rats of Wistar strain (100-150 g) were procured from Biogen Laboratory Animal Facility, Bangalore. The animals were maintained in polypropylene cages under standard laboratory conditions with a temperature of 27±2°C, relative humidity of 44-56% and 10 h light/14 h dark cycle. They were provided with a standard pellet diet and water and *ad libitum* and were acclimatized to the laboratory environment for a period of one week before initiating the experiment. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Faculty of Pharmacy, S.K. University with Approval No. IAEC/02/SKU/09/2026 and were conducted in accordance with CCSEA guidelines for the care and use of laboratory animals.

2.5 Phytochemical screening of *Simarouba glauca*

The ethanolic bark extract of *Simarouba glauca DC*. (EESG) was subjected to preliminary qualitative phytochemical screening to identify major secondary metabolites, including alkaloids, flavonoids, terpenoids, steroids, phenols, tannins, saponins, carbohydrates, glycosides, coumarins, proteins, amino acids and quinones, using standard pharmacognostic procedures [35].

2.6 *In-silico* evaluation of anti-diarrhoeal activity using molecular docking

Molecular docking and pharmacokinetic prediction were performed using PubChem, the RCSB Protein Data Bank (PDB), Molsoft, Swiss ADME, PyRx, Open Babel, Auto Dock Vina and BIOVIA Discovery Studio Visualizer. The selected protein structure (PDB ID: 4DKL) and phytoconstituent ligand structures were retrieved from the respective databases and prepared for docking. The docking workflow followed established molecular-docking procedures using Auto Dock Vina and PyRx [40, 41].

A working directory was created for the target protein and ligand files. The target protein structure (PDB ID: 4DKL) was downloaded in PDB format from the RCSB Protein Data Bank, while the selected ligands were retrieved from PubChem in SDF format [42].The protein structure was prepared by removing water molecules, adding polar hydrogens and assigning charges, after which the macromolecule was saved in PDBQT format for docking. Ligand structures were imported into PyRx, energy-minimized and converted to Autodock-compatible PDBQT files using the available ligand-preparation tools [40, 41].

The prepared protein and ligand files were loaded into PyRx and the Autodock Vina wizard was used to define the docking setup. The active-site region and grid-box dimensions were specified, and the docking run was executed to estimate ligand-binding poses and affinities. The resulting docking data were exported as a CSV file for further analysis. The protein–ligand complexes were then examined in BIOVIA Discovery Studio Visualizer, where two-dimensional interaction diagrams and three-dimensional complex views were generated [40, 41].

2.6.1 SwissADME analysis

Swiss ADME was used to estimate the physicochemical properties, pharmacokinetic characteristics, drug-likeness and medicinal-chemistry friendliness of the selected compounds. The BOILED-Egg model was consulted to predict passive gastrointestinal absorption and blood–brain barrier permeation from the compounds' lipophilicity and topological polar surface area [44].

2.7 *In-vivo* evaluation of anti-diarrhoeal activity of *Simarouba glauca*

- **Castor oil induced diarrhoea :**

Wistar rats weighing 100–150 g were fasted for 18 h with free access to water and housed individually in cages lined with white blotting paper. The animals were divided into five groups of five: Group I received normal saline orally, Group II received castor oil, Group III received loperamide (5 mg/kg, orally), and Groups IV and V received EESG at 200 and 400 mg/kg orally, respectively. One hour after treatment, castor oil (0.1 mL/rat) was administered orally, and the animals were observed for diarrhoeal output. The number of wet stools and the weight of fresh faeces were recorded and compared with the control group. Castor oil-induced diarrhoea and its inhibition were assessed using established methods.

Percentage inhibition of diarrhoea was calculated as

$$\% \text{ Inhibition of diarrhoea} = \frac{\text{Mean number of wet stools of (control group - treated group)}}{\text{Mean number of wet stools in the control group}} \times 100$$

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

$$\text{Inhibition of weight} = \frac{\text{Total weight of fresh feces (negative control - treatment group)}}{\text{Total weight of fresh feces of negative control}} \times 100$$

- **charcoal meal induced gastrointestinal motility test:**

Wistar rats weighing 100–150 g were fasted for 18 h with free access to water and allocated to five groups. Group I received normal saline, Group II received tragacanth solution , Group III received atropine sulphate (1 mg/kg, intraperitoneally), and Groups IV and V received EESG at 200 and 400 mg/kg orally, respectively. One hour after treatment, each animal received 1 mL/kg of a 5% charcoal suspension. Thirty minutes later, the animals were euthanized according to the study protocol, and the small intestine was removed from the pylorus to the caecum. The distance travelled by the charcoal meal and the total length of the small intestine were measured to assess intestinal transit using the charcoal-meal method [8,13].

The peristalsis index was calculated as

$$\text{Peristalsis Index} = \frac{\text{Distance travelled by Charcoal meal}}{\text{Length of Small Intestine}} \times 100$$

$$\% \text{ Inhibition} = \frac{\text{Mean length of the intestine - Mean distance traveled by meal}}{\text{Mean length of intestine}} \times 100$$

- **castor oil induced enteropooling test**

Wistar rats weighing 100–150 g were fasted for 18 h with free access to water and housed individually. Five animals were assigned to each of five groups: Group I received normal saline orally, Group II receives vehicle and castor oil, Group III received Loperamide (3 mg/kg, orally), and Groups IV and V received EESG at 200 and 400 mg/kg orally, respectively. One hour after treatment, castor oil (0.1 mL/rat) was administered orally. Thirty minutes later, the animals were euthanized according to the study protocol, and the intestine from the pylorus to the caecum was removed. The intestinal contents were collected in a measuring cylinder and their volume was recorded to evaluate intestinal fluid accumulation [3, 4].

Percentage inhibition of intestinal fluid accumulation was calculated as

$$\% \text{ Inhibition} = \frac{\text{Mean of Vc - Mean of Vt}}{\text{Mean of Vc}} \times 100$$

Vc = Mean intestinal fluid volume of the control group

Vt = Mean intestinal fluid volume of the test/standard group

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening of *Simarouba glauca*

The preliminary phytochemical screening test of ethanolic bark extract of *S.glauca* revealed the following phytochemicals

Table 1. Phytochemical screening results

S. No.	Phytochemical test	Ethanolic extract (Soxhlet)
1	Alkaloids	(+)
2	Flavonoids	(+)
3	Terpenoids	(+)
4	Steroids	(-)
5	Phenols	(+)
6	Tannins	(+)
7	Saponins	(-)
8	Glycosides	(+)
9	Coumarins	(-)
10	Carbohydrates	(+)
11	Proteins	(-)
12	Amino acids	(-)
13	Quinones	(-)

"(+)" indicates = presence of compound

"(−)" indicates = absence of compounds

3.2 Molecular docking and *in-silico* analysis

Molecular docking analysis was carried out to evaluate the binding affinity and interaction pattern of selected phytoconstituents of *Simarouba glauca* against the anti-diarrheal target protein 4DKL. The docking study revealed that all the selected bioactive compounds could effectively accommodate within the active binding pocket of the target protein with favourable binding energies. The major quassinoids of *S. glauca* showed promising binding scores. The docking score was found to be approximately -7.3 kcal/mol for Glaucarubinone, - 8.0kcal/mol for Loperamide and -7.2 for Ailanthinone. The results indicated stable binding via hydrogen bonds and hydrophobic interactions with key amino acid residues.

The complete docking results should be presented in a separate table along with target protein name, PDB ID, binding energy, type of interaction, interacting amino acid residues and 2D/3D visualization figures.

3.2.1 Ligand interactions with receptor (4DKL)

Table 2. Ligand interactions with receptor (4DKL)

S. No.	Property / compound	Binding energy	Ligand efficiency	Inhibition constant
1	Loperamide	-8.0 kcal/mol	0.24 kcal/mol	1.35 µM
2	Glaucarubinone	-7.3 kcal/mol	0.21 kcal/mol	4.4 µM
3	Ailanthinone	-7.2 kcal/mol	0.21 kcal/mol	5.3 µM

3.2.2 Molecular properties and drug-likeness score

Table 3. Molecular properties and drug-likeness score

S. No.	Properties	Loperamide	Glaucarubinone	Ailanthinone
1	Molecular weight	477.04 g/mol	494.53 g/mol	478.53 g/mol
2	No. of HBA	3	10	9
3	No. of HBD	1	4	3
4	Log P	5.1	0.21	1.04
5	Log S (moles/L)	7.26e-04 mol/L	4.71e+02 mol/L	5.77e+00 mol/L
6	Log PSA	43.76	159.82	139.59
7	Molecular volume	470	335	320
8	BBB score	No	No	No
9	Drug likeness	Yes	Yes	Yes
10	Water solubility	Moderately soluble	Good soluble	Good soluble

3.2.3 Pharmacokinetics

Table 4. Pharmacokinetics

S. No.	Properties	Loperamide	Glaucarubinone	Ailanthinone
1	GI absorption	High	Low	High
2	BBB permeability	Yes	No	No
3	Skin permeability	-5.65 cm/s	-9.22 cm/s	-8.47 cm/s
4	Lipinski rule of 5	Follows (0 violations)	Follows (0 violations)	Follows (0 violations)
5	No. of rotatable bonds	8	4	4

LOPERAMIDE (Std)

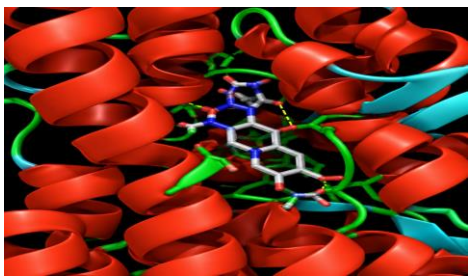

Fig 2: 3D Conformation of Loperamide with 4DKL

The modeled tertiary structure of the protein receptor utilized for molecular docking simulations. The architecture displays the spatial arrangement of folding domains, with distinct secondary structural elements color-coded to differentiate helices, sheets, and coils against a dark background (Fig: 2).

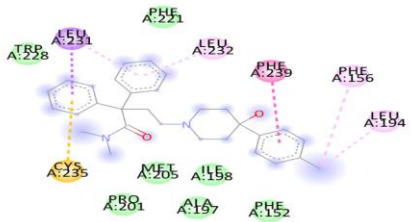

Fig 3: 2D Conformation of Loperamide with 4DKL

The diagram illustrates the specific intermolecular interactions between the ligand and the surrounding amino acid residues of Chain A. The residues involved in different interaction types are color-coded: green circles represent Van der Waals interactions; pink and purple dashed lines indicate Pi-Alkyl/Alkyl interactions (with LEU231, LEU232, PHE239, PHE156, and LEU194); the yellow dashed line denotes a Pi-Sulfur interaction (with CYS235); and the orange dashed line shows a Pi-Pi stacked/T-shaped interaction (with TRP228). The solvent accessibility of the ligand atoms is indicated by the blue/purple shading surrounding the structure (Fig: 3).

GLAUCARUBINONE

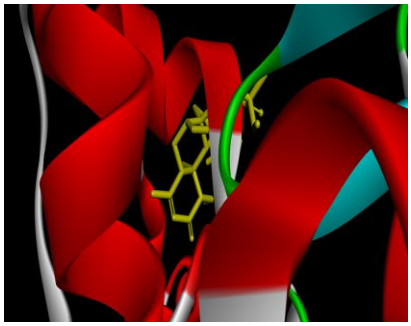

Fig 4: 3D Conformation of Glaucarubinone with 4DKL

The ribbon representation illustrates the secondary structure of the target protein, highlighting alpha-helices (red) and beta-sheets/loops (cyan and green). The docked ligand molecule is depicted as a yellow stick structure, securely embedded within the binding pocket of the protein. This visualization highlights the spatial orientation and conformational fit of the ligand inside the active site (Fig: 4).

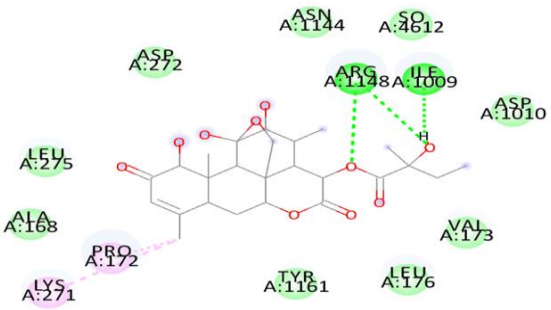

Fig 5: 2D Conformation of Glaucarubinone with 4DKL

The green dashed lines represent conventional hydrogen bonds formed between the ligand and the active site residues ARG1148 and ILE1009, indicating strong binding affinity. The light green circles signify residues involved in Van der Waals interactions (including ASN1144, ASP272, LEU275, ALA168, TYR1161, LEU176, VAL173, and ASP1010). The pink dashed lines represent hydrophobic interactions, specifically Alkyl/Pi-Alkyl interactions with PRO172 and LYS271. The light blue/purple contours around the ligand atoms represent the solvent accessibility surface (Fig: 5).

AILANTHINONE

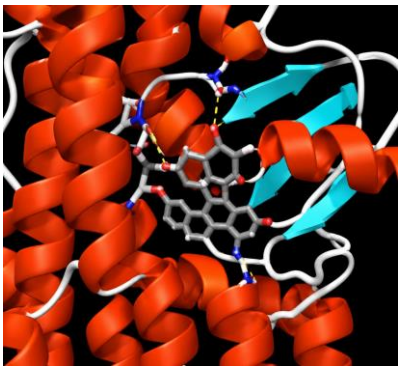

Fig 6: 3D Conformation of Ailanthinone with 4DKL

The secondary structure of the entire target protein receptor is rendered in a solid ribbon representation against a dark background, showing predominantly alpha-helices (red), beta-sheets (cyan), and loops/turns (green and white). The docked ligand molecule is shown as a grey/element-colored stick model bound to its specific pocket on the protein surface, demonstrating the overall global orientation and binding topography of the complex (Fig:6)

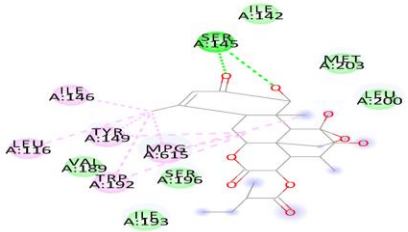

Fig 7: 2D Conformation of Ailanthionone with 4DKL

The green dashed lines indicate conventional hydrogen bonding interactions formed with the key catalytic residue SER145, stabilizing the ligand-receptor complex. The light green circles represent residues contributing to Van der Waals stability (including ILE142, MET203, LEU200, SER196, ILE193, and VAL189). The pink/purple dashed lines illustrate hydrophobic interactions, specifically Alkyl and Pi-Alkyl configurations involving residues ILE146, TYR149, LEU116, and TRP192. The blue contour outlines around specific ligand atoms indicate their relative solvent exposure (Fig: 7)

3.3 *In-vivo* anti-diarrhoeal activity

3.3.1 Castor oil-induced diarrhoea

Effect of ethanolic bark extract of *Simarouba glauca* for castor oil induced diarrhea in rats

Table 5: Castor oil-induced diarrhea

Group	Total no. of stools	Total wt. of stools	Total wt. of dry stools	Total wt. of wet stools	% inhibition
Normal	45.80±0.52	14.25±0.28	2.15±0.18	0.18±0.06	--
Control	92.40±0.76	31.80±0.52	4.25±0.32	25.50±0.62	--
Standard drug	38.60±0.94	25.40±0.48	3.85±0.25	10.92±0.28	57.18
Simarouba glauca Test-I (200 mg/kg)	61.50±0.82	27.36±0.55	4.10±0.29	14.13±0.42	44.59
Simarouba glauca Test-II (400 mg/kg)	50.75±0.88	24.60±0.46	3.65±0.27	13.24±0.38	48.08

There was a marked anti-diarrhoeal effect of the ethanolic bark extract of *Simarouba glauca* against castor oil-induced diarrhoea in rats. The castor oil-treated control group showed an increase in the total number of stools and wet stool weight compared with the normal group. Treatment with *Simarouba glauca* extract resulted in a reduction in stool output and wet stool weight compared with the control group. The maximum percentage inhibition of diarrhoea produced by the extract was 48.08% at 400 mg/kg, whereas

the 200 mg/kg dose produced 44.59% inhibition .The standard drug showed 57.18% inhibition. Thus, the ethanolic bark extract of *Simarouba glauca* demonstrated a dose-related anti-diarrhoeal effect in the castor oil-induced diarrhoea model (Table :5).

Bar chart of Castor oil induced diarrhoea

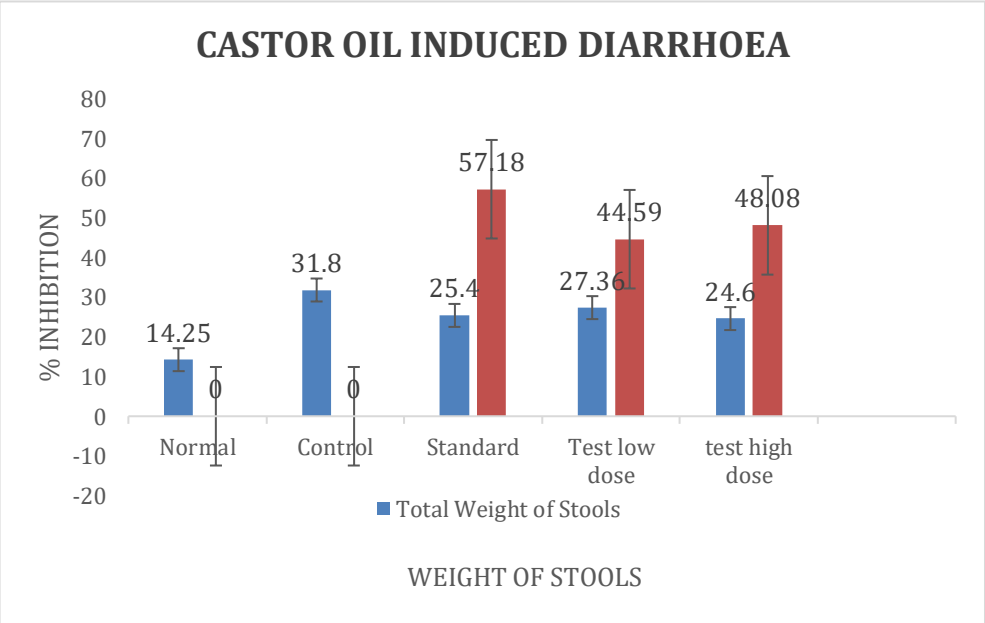

Figure 8: Comparison between % inhibition and Weight of stools

3.3.2 Charcoal meal-induced gastrointestinal motility

Effect of *simarouba glauca* ethanol bark extract on intestinal transit time in rats

Table 6. Charcoal meal-induced gastrointestinal motility

Group	Mean distance travelled by charcoal (cm)	Mean length of small intestine(cm)	Peristalsis index
Normal	55.00±0.07	154.60±0.14	35.58±0.01
Control	55.32±0.016	153.8±0.241	65.42±0.24
Standard drug	54.86±0.015	84.6±0.128	36.92±0.16
Simarouba glauca Test-I (200 mg/kg)	55.16±0.015	128.4±0.182	55.74±0.21
Simarouba glauca Test-II (400 mg/kg)	55.08±0.014	107.8±0.151	47.32±0.18

The ethanolic bark extract of *Simarouba glauca* was evaluated for its effect on gastrointestinal motility using the charcoal meal-induced gastrointestinal motility test in rats. The normal group showed a peristalsis index of 65.42 ± 0.24%, whereas the standard drug-treated group showed a reduced peristalsis index of 36.92 ± 0.16%. Treatment with *Simarouba glauca* at 200 mg/kg (Test-I) produced a peristalsis index of 55.74 ± 0.21%, while the 400 mg/kg (Test-II) dose reduced the peristalsis index to 47.32 ± 0.18%. The distance travelled by charcoal was 128.4 ± 0.182 cm and 107.8 ± 0.151 cm at 200 and 400 mg/kg, respectively, compared with 153.8 ± 0.241 cm in the normal group. The higher dose showed a greater reduction in charcoal transit and peristalsis index, indicating an inhibitory effect of the extract on intestinal motility. Values are expressed as mean ± SEM; n = 5 animals/group (Table:6).

Bar chart of charcoal meal induced gastrointestinal motility test

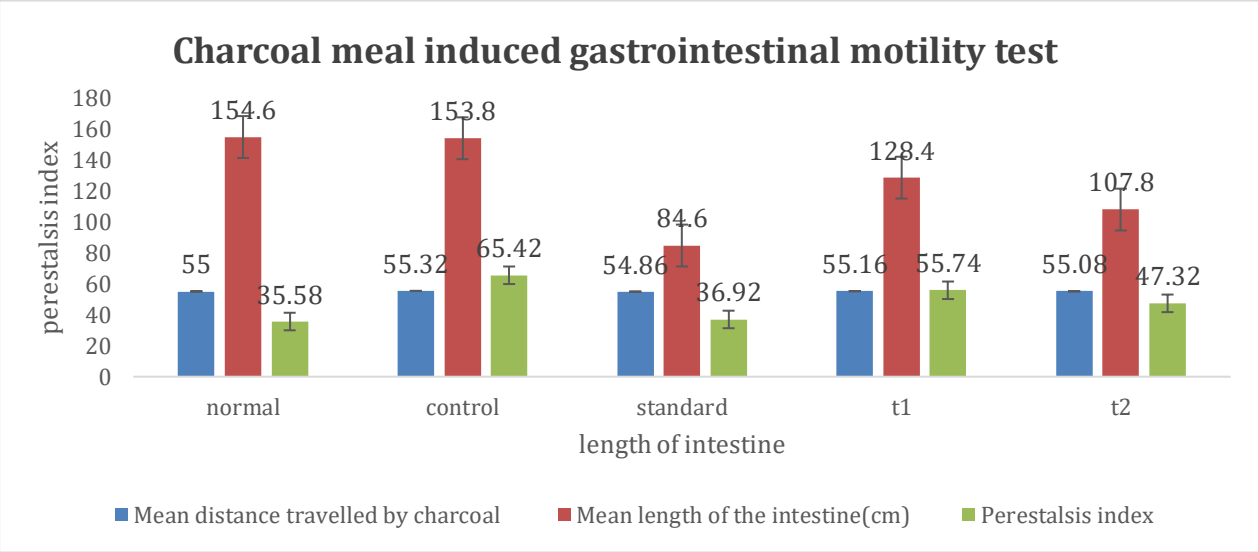

Figure 9: Comparison between % peristalsis index and Length of intestine

3.3.3 Castor oil-induced enteropooling

Table7. Castor oil-induced enteropooling

Group	Weight of intestinal content (ml)	% inhibition
Normal	1.28±0.02	--
control	7.42±0.21	--
Standard drug	5.18±0.27	30.19
Simarouba glauca Test-I (200 mg/kg)	5.94±0.22	19.95
Simarouba glauca Test-II (400 mg/kg)	5.63±0.18	24.12

The ethanolic bark extract of *Simarouba glauca* was evaluated for its anti-secretory effect using the castor oil-induced enteropooling test in rats. The standard drug-treated group showed a reduction in the weight of intestinal contents (5.18 ± 0.27 ml) with 30.19% inhibition .Treatment with *Simarouba glauca* at 200 mg/kg (Test-I) reduced the intestinal content weight to 5.94 ± 0.22 ml, showing 19.95% inhibition. At 400 mg/kg (Test-II), the intestinal content weight was further reduced to 5.63 ± 0.18 ml, with 24.12% inhibition.The higher dose showed greater inhibition of intestinal fluid accumulation compared with the lower dose, indicating a dose-dependent anti-enteropooling effect of the extract. Values are expressed as mean ± SEM; n = 5 animals/group (Table 7).

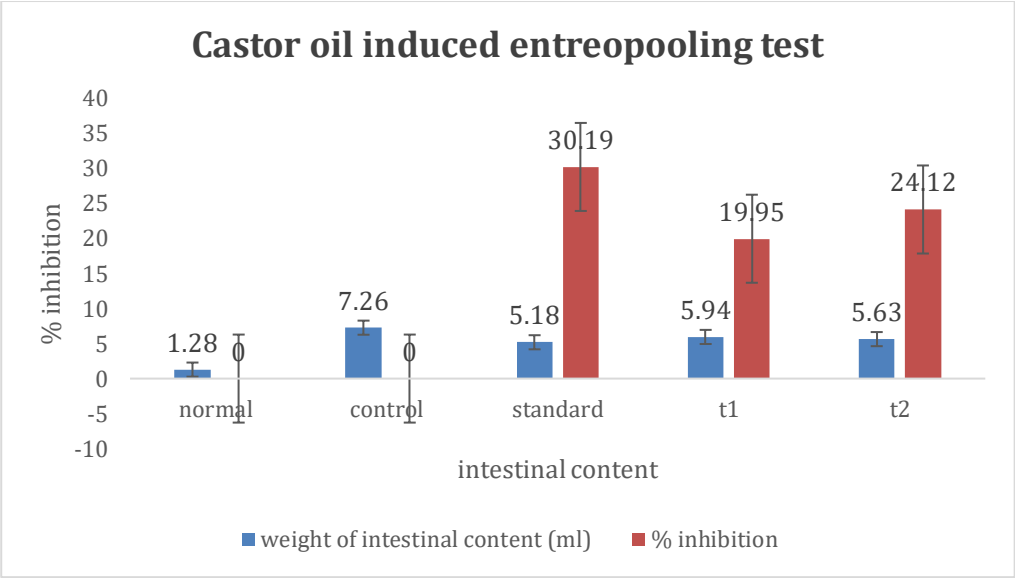

Figure 10: Comparison between % inhibition and weight of intestinal content

4. DISCUSSION

The present study investigated the phytochemical constituents and preliminary anti-diarrhoeal potential of the ethanolic bark extract of *Simarouba glauca* using experimental rat models. The extract was evaluated through castor oil-induced diarrhoea, charcoal meal-induced gastrointestinal motility, and castor oil-induced enteropooling assays.

In the castor oil-induced diarrhoea model, the extract at 200 and 400 mg/kg exhibited reported inhibition of 44.59% and 48.08%, respectively, compared with 57.18% for the standard drug. These findings indicate a potential reduction in diarrhoeal output. Since castor oil induces diarrhoea through increased intestinal secretion and gastrointestinal motility, the observed activity may involve modulation of these processes, although the underlying mechanism remains undetermined [45].

In the charcoal meal-induced gastrointestinal motility test, the reported peristalsis index decreased from 55.74% at 200 mg/kg to 47.32% at 400 mg/kg, suggesting a possible dose-related reduction in intestinal transit. Reduced gastrointestinal motility may contribute to the anti-diarrhoeal effect by allowing greater time for intestinal fluid absorption.

The extract also demonstrated reported inhibition of intestinal fluid accumulation of 19.95% and 24.12% at 200 and 400 mg/kg, respectively, compared with 30.19% for the standard drug. This finding suggests a possible antisecretory effect, although the relative contributions of reduced secretion and enhanced absorption require further investigation.

Preliminary phytochemical screening revealed alkaloids, flavonoids, terpenoids, phenols, tannins, and glycosides. Tannins and flavonoids are of particular interest because certain compounds belonging to these groups have demonstrated biological effects relevant to intestinal secretion and motility. Nevertheless, the contribution of individual constituents to the observed activity cannot be established without further phytochemical characterization and experimental validation.

Molecular docking analysis yielded binding energies of −7.3 and −7.2 kcal/mol for glaucarubinone and ailanthinone, respectively, compared with −8.0 kcal/mol for loperamide. Although these scores suggest predicted binding under the selected computational conditions, docking alone cannot establish biological activity or a mechanism of action. The protein target 4DKL, ligand structures, and predicted interactions should be validated, followed by appropriate experimental studies [46].

5. CONCLUSION

The present study reports preliminary phytochemical findings and experimental observations suggesting that the ethanolic bark extract of *Simarouba glauca* may possess anti-diarrhoeal activity in the rat models employed. The higher 400 mg/kg dose generally demonstrated greater effects than the 200 mg/kg dose. However, these findings should be interpreted cautiously due to the small sample size and the need to verify the numerical results, units, statistical analyses, and molecular docking details. Further studies employing validated experimental protocols, appropriate statistical methods, and comprehensive characterization of the active phytochemical constituents are necessary to confirm these findings and establish the anti-diarrhoeal potential of *Simarouba glauca* bark extract.

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7. AUTHOR CONTRIBUTIONS

K. Prathyusha -writing, original draft. Formal analysis, data curation and Smt.V.Rajani: conceptualization, methodology investigation, writing – review & editing. Supervision, acquisition. Both authors approved the final manuscript.

8.DECLARATION OF INTEREST

The authors declare that there is no conflict of interest.

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