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RESEARCH ARTICLE

In-vitro and *in-vivo* Antibacterial Effects of *Plumeria rubra* Extract: A Novel Approach for Treating Infectious and Non-Infectious Diarrhoea

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ABSTRACT

Scientific rationale behind the medical application of *Plumeria rubra* in both infected and non-infectious diarrhoea is evaluated in the present manuscript. *In-vivo* and *in-vitro* experiments were conducted to examine the effect of a crude extract of *P. rubra* leaf on castor oil-induced diarrhoea, gastrointestinal movement, and fluid accumulation in the intestines. Using an *in-vitro* assays, the antibacterial effectiveness of *P. rubra* was evaluated against several enteric and non-enteric harmful bacteria, and its phytochemical and antioxidant potential was also assessed. *P. rubra* significantly decreased the severity of diarrhoea in rats with reductions of 39.20, 47.30 and 70.30%, which was observed at doses of 150, 300 and 450mg/kg, respectively. Intestinal secretions revealed decreases of 24.14, 28.09 and 38.90, respectively, while intestinal motility decreased by 32.11, 45.78 and 54.80% at dosages of 150, 300 and 450mg/kg, respectively. *P. rubra* exhibited bactericidal activity against various bacteria, including *Escherichia coli* (71.80%), *Staphylococcus aureus* (91.60%), *Bacillus subtilis* (84.24%), *Pseudomonas aeruginosa* (76.83%), at a concentration of 12.5mg/mL. The 10mg/mL exhibited bactericidal effects against all previously indicated pathogens. HPLC analysis revealed the presence of rutin, quercetin, kaempferol, plumericin and isoplumericin, while significant antioxidant activity was observed in DPPH, SOD and NO scavenging assays, with inhibition ranging from 78 to 86%. *P. rubra* demonstrated efficacy against both enteric and non-enteric pathogens that cause diarrhoea and justified its therapeutic usage and applications for both forms of diarrhoea.

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INTRODUCTION

Diarrhoea is characterized by an escalation in the frequency, liquidity, or quantity of excrement and is a prevalent issue in the veterinary sector (Kim *et al*., 2021; Wang *et al.*, 2025). Diarrhoea can result from conditions that affect either the small or large intestines or both (Amin *et al.*, 2016; Terefe *et al.,* 2023). Localization to the small or large bowel may be challenging in diffuse illness. It may not be necessary in cases of severe diarrhoea, as biopsies are unlikely, and symptomatic treatment may not vary. Diarrhoea can be manifested as either acute or chronic. Diarrhoea that lasts for less than two weeks is typically referred to as acute (Li *et al.*, 2024) and often resolves without veterinary care (Bukhari *et al*., 2024; Javed *et al*., 2025). Acute diarrhoea is a frequently seen ailment in primary care for small animals in veterinary practice. Diarrhea strikes 70% of the

global population, particularly in developing nations, where hygienic conditions are deeply concerning (Bihani, 2021). An infectious agent typically causes the most common type of diarrhoea, known as acute diarrhoea.

However, medications, toxins, or acute inflammation can also be contributing causes (Solcan *et al.*, 2015). Rotavirus is the primary culprit responsible for causing inf ectious diarrhoea. However, it is important to note that other viral agents such as *norovirus, parvovirus, feline torovirus, coronavirus, circovirus, canine adenovirus, enteroviruses, feline astrovirus, enterovirus, reovirus, norovirus,* and *adenovirus* can also be involved (Ahmad *et al.,* 2012). Bacterial agents like *Clostridium perfringens*, *Salmonella* spp., *Escherichia coli*, *Yersinia* spp, *Shigella* spp, *Campylobacter jejuni, Clostridium difficile,* and *Vibrio cholera,* and parasite agents like *Uncinaria stenocephala*, *Toxocara* spp, *Taenia* spp., *Trichuris vulpis*, *Dipylidium caninum,* are less likely involved. While protozoa causing infectious diarrhea, such as *Giardia, Isospora, Coccidia,* also play an important role, including *Tritrichomonas foetus*, *Cryptosporidium* (Dong *et al.*, 2019).

Diarrhoea continues to be a prevalent and economically detrimental health issue in global animal production, impacting livestock, including cattle, pigs, and poultry, as well as juvenile animals in both intensive and smallholder systems globally (Zhang *et al.,* 2024; Wang *et al.*, 2025). Resulting from a multifaceted interplay of infectious agents (bacteria, viruses, and parasites) and non-infectious factors (stress, inadequate nutrition, and environmental conditions), it precipitates severe dehydration, stunted growth, diminished feed efficiency, reduced milk and egg production, compromised reproduction and significantly increased mortality rates, responsible for up to 57% of fatalities in weaned calves in certain dairy operations and contributing to substantial losses in poultry and swine populations (Sen *et al.,* 2025). These effects result in billions of dollars in annual production losses from reduced productivity, increased veterinary expenses, and decreased market output, ultimately jeopardizing food security and the sustainability of animal husbandry globally. Consequently, effective prevention and management measures are crucial to protect animal welfare and the global livestock economy (Zhang *et al*., 2025).

Plumeria rubra, Linn. (Apocynaceae) It is a well-known plant with medicinal and therapeutic effects, and is grown in tropical and subtropical regions of the world. In South Asian languages, it is known as "Lal Champa," whereas in English, it is recognized as Frangipani (Fathiazad *et al.*, 2012). Different parts of *P. rubra* has been used as potential medicinal agents against various disease conditions in humans and animals (Khare, 2004; Hussain *et al.*, 2013).

In the traditional medicine this plant has been utilized to treat many diseases of animals and public health importance (Khan *et al.*, 2024). Pharmaceutical studies have determined its antipyretic, anti-cancer, analgesic, lipid-lowering, anti-diabetic, antibacterial, antioxidant and insecticidal properties (Khan *et al.*, 2022). Antibiotics are often recommended as a component of its therapeutic management strategy but due to emergence of drug resistance, toxicity alternative novel agents like botanicals can be best one and cost effective to treat and lower the pathogenesis of diarrhea (Khan *et al.*, 2022). According to the 'Small Animal Veterinary Surveillance Network' (SAVSNET), only 16% of incidents with diarrhoea presented in general practice underwent diagnostic testing (Khan *et al.*, 2018a). Keeping in view the importance of infectious and non-infectious diarrhoea this research and its treatment challenges current research was designed to explore the rationale of use of *P. rubra* in the treatment of infectious and non-infectious diarrhea.

MATERIALS AND METHODS

Drugs: Atropine sulphate was bought from Xian Janssen Pharmaceutical Ltd. Antibiotic discs utilized for antibiotic tests were bought from Sigma Chemicals. Acacia powder, hydrolyzed starch, and veggie charcoal were bought from Shanghai Aladdin Biochemical Technology Co., Ltd. All drugs utilized were of a chemical grade.

Animals: Rats weighing between 200 and 240 g of either sex were acquired from Shandong Experimental Animal Center. The animals were given unrestricted access to regular food and tap water and were kept in a temperature-controlled environment (23 to 25°C). The rats were euthanized by cervical dislocation. The tests received approval from the Academic Ethics Committee of Qilu Normal University, with reference number xsllsc2025-032 following the National Institute of Health guidelines (NIH, 2023).

Bacterial isolates: Bacterial isolates utilized in this study included Escherichia *coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa, Bacillus cereus*, and *Enterococcus faecalis.* Bacteria were scientifically separated. In Luria-Bertani soup (16 h) strains of bacteria were normally grown up aerobically at 37°C. Bacterial strains were in microbiology lab under temp 4°C.

Crude extract preparation: Fresh leaves of the *P. rubra* plant were obtained and picked leaves were dehydrated in a covered area at a temperature of 25°C and any contaminants or foreign substances were physically eliminated through meticulous selection. Through the utilization of a specialist herbal grinder, the dried leaves were ground into a coarse powder. *P. rubra* leaf powder weighing 250 grams was submerged in a mixture of methanol solvent and distilled water at a volume-to-volume ratio of 30:70 for a period of twelve days. The process of soaking was carried out in laboratory jars that were meticulously sealed. In order to strain the soak, pudding cloth and Whatman-1 filter paper were utilized, and a rotary evaporator was important in the process. As shown in Fig. 1, the percolate was subjected to evaporation at a temperature of 37 degrees Celsius while the pressure was reduced (Manzoor *et al.*, 2022a).

The % vintage of the aqueous methanolic extract of leaves was determined utilizing the following formula:

$$\% \text{ age yield} = \text{Theoretical yield (g)}/\text{Actual yield (g)} \times 100 \quad (1)$$

Working stocks: For *in vitro* and *in vivo* tests, the crude extract was dissolved in a solution containing 10% DMSO and Tween-80 (5%) on the day of the experiment. Further

concentrations were then prepared using distilled water. To conduct antibacterial studies in a controlled environment, a solution of 1 mL of 2.08 g of *P. rubra* extract was dissolved in methanol (50%) and after that subjected to centrifugation at 14,000 g for 60 minutes. The liquid portion, the supernatant, was gathered and passed through a microfilter (10µM). Meanwhile, the solid residue, the pellet, was weighed, and its weight was deducted from the initial amount of extract (2.08 g) obtained before the centrifugation process. The concentration of *P. rubra* extracts reached a final value of 180µg/µL (Khan *et al.*, 2018b).

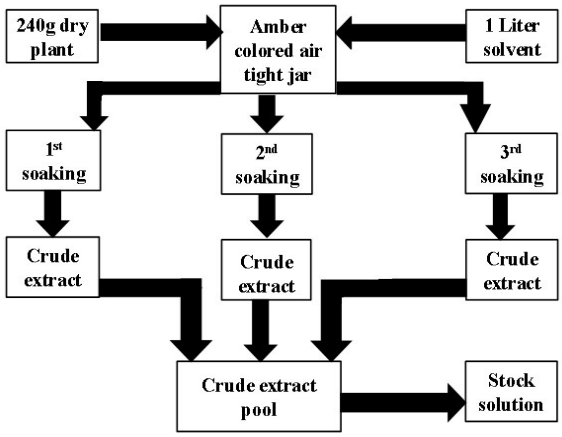

Fig. 1: Flow diagram of extract and dose preparation.

HPLC Analysis: An HPLC analysis was performed to determine the amount of phenolic acids in the leaf extract of *P. rubra.* (Hussain *et al.*, 2013). For the high-performance liquid chromatography (HPLC) analysis, a double-ramp solvent gradient and a C-18 column (250 × 4.6mM) were used. In a period of thirty-six minutes, this arrangement makes it possible to separate eight to nine phenolics. The leakage rate was determined to be 0.0008 microliters per minute, whereas the column had a film width of 5 micrometers. In the oven, the temperature was maintained at 30°C. Not only was the copiability of component departure great, but it also maintained consistency from run to run. Rutin, quercetin, kaempferol, isoplumericin and plumericin were utilized as reference substances. These substances were obtained from Aldrich in St. Louis, Missouri, United States of America, and had a purity greater than 99%. To achieve a concentration of 50µg/mL, solutions were prepared in methanol. A comparison was made between the maintenance periods of the *P. rubra* sample and those of the standards to differentiate the samples at hand. Both the separation factor and resolution were used to assess the efficiency of HPLC in separating components.

Antioxidant activity: Antioxidant activity was accomplished using 2,2-diphenylpicrylhydrazyl (DPPH), Superoxide Dismutase (SOD) Assay, and Nitric oxide (NO) Scavenging Assay.

DPPH assay: As detailed in a previous publication, the DPPH assay was carried out in the same manner. An aqueous methanolic extract of *P. rubra* leaves was mixed with a DPPH solution prepared with 5mL of methanol and then left in the dark for 40 minutes. The concentrations of the extract ranged from four milliliters to four milliliters. The absorbance of the hatching solution was measured at 517nM using a spectrophotometer (Hussain *et al.*, 2013). These studies were carried out on three separate occasions, and the percentage inhibition was maintained in the vitamin C counterparts. Calculations for the DPPH scavenging outcome are determined as follows:

$$1\% = A\ (\text{blank}) - B\ (\text{sample})/A\ (\text{blank}) \times 100$$

No scavenging assay: At a concentration of 10mg/mL, *P. rubra* was extracted by employing an aqueous-ethanolic solution. To attain concentrations of 1000 and 2000µg/mL, ascorbic acid and the extract were diluted with distilled water. At 4 degrees Celsius, the experimental solutions were kept. During the process, a freshly manufactured Griess reagent was used. 0.5mL of sodium nitroprusside (10mM) in phosphate-buffered saline was added to different extract dilutions (1mL), and the mixture was incubated at 25 degrees Celsius for 3 hours. This was done in order to find the ideal concentration of extract, which was 1000 and 2000µg/mL. A freshly prepared Griess reagent of the same volume was added to the extract to introduce a spike. Although the extracts were not included in the control samples, the amount of buffer used remained the same throughout the experiment. Although the various colored tubes had sufficient quantities of extracts, there was no sodium nitroprusside present in any of them. A total of 150 liters of the reaction mixture was poured into a plate with 96 wells. To determine the optical density at 546nM, a UV-Vis microplate reader manufactured by Alibaba in Hangzhou, China, was used. The following equation was used in this investigation to determine the percentage of NO scavenging activity exhibited by ascorbic acid and extracts, as well as the percentage of inhibition observed using the usual method.

$$\%\ \text{NO scavenging activity} = \text{Blank} - \text{sample} / \text{blank} \times 100$$

Superoxide dismutase assay: The herb's ability to scavenge superoxide anion radicals was evaluated using a slightly modified version of the published approach. A combination of the PMS, NADH, and NBT systems was used to produce superoxide radicals. The materials being evaluated were placed into a test tube containing a Tris-HCl buffer at 625µL, 16mM and pH 8.0. Additionally, 125µL of NBT (300M) and 125µL of NADH (468M) were added to the test tube. Upon the addition of 125µL (60mM) PMS to the mixture, the reaction started. The absorbance was measured using a Hitachi U-1900 UV-Vis spectrophotometer before examination. Superoxide dismutase assay was performed according to the method described by Isik *et al.* (2018) and Khan *et al.* (2025).

***In-vitro* experiment**: The *in vitro* antibacterial efficacy was conducted according to the methods previously outlined by Satoh and Nishida (2004). In summary, a 10mL sample of inoculum with an optical density of 0.22 and containing approximately 10^6CFU was mixed with different amounts of *P. rubra* extract, such as 1.0 and 2.5mg, in 200mL. The mixture was then incubated under aerobic conditions for 16

hours at 37°C. Following the maturation period, the bacterial growth underwent a decimal dilution process and was then spread onto nutrient agar plates. These plates were subsequently incubated at 37°C for 24 hours. The negative control consisted of bacteria cultured with LB alone. To achieve complete eradication, germs were cultured with specific antibiotics at concentrations of gentamicin (100µg/mL) for *B. cereus, E. coli*, *P. aeruginosa,* and *S. typhi*. On the following day, the number of colonies was tallied. For growing inoculated bacteria, the figures were reported as CFU. The bactericidal effects were quantified as a percentage inhibition using

100 – [(cfu in extract / original inoculums) X 100]

***In-vivo* experiment:** For the *in-vivo* trial, 25 rats were partitioned into 5 equal groups, each consisting of 5 animals and each rat orally administered with 1mL of castor oil caused diarrhoea. Group I was designated as the control group and received 2mL/kg of saline solution by oral gavage. Group II was given atropine (3mg/kg) intraperitoneally as ordinary treatment and served as a positive control. Groups III, IV and V were administered *P. rubra* at oral dosages of 150, 300 and 450mg/kg, respectively, 1h before castor oil administration which were selected after pilot study and with reference to previous studies (Khan *et al*., 2016; Khan *et al*., 2022). The frequency of both liquid and solid fecal excretions was recorded hourly for 4 hours. The average amount of feces excreted by the treatment groups was compared to that of the control group (Khan *et al.*, 2022).

% reduction = [Mean diarrheal score (control) – mean score of treatment group] X 100 mean diarrheal score (control)

Enteropooling induced by castor oil: The methodology of Khan *et al*. (2020) was employed to ascertain the presence of fluid buildup within the lumen. The rats that had not eaten overnight were separated into 5 groups (n = 5). Group I was given a control treatment of oral 2 ml/kg normal saline; group II was given (i.p.) 3 mg/kg atropine; and groups III, IV, and V were given *P. rubra* at doses of 150, 300, and 450mg/kg, respectively, one hour before receiving castor oil orally. After 2 hours, rats were euthanized, and the small intestine was extracted by ligating the split ends with a filament and weighing. The intestinal innards were extracted into a tube and their dimensions quantified. The intestine was weighed again, and the discrepancy between the entire intestine weight and the empty intestine weight was determined using the following formula.

Wi / Wa × 1000
Where Wi is intestine weight and Wa is animal weight in grams.

Small intestinal transit: Rats were fasted for 18 hours and then divided into six groups, each consisting of 5 animals. Group I was administered normal saline (2mL). Group II was administered castor oil (2mL) along with saline. Group III received intraperitoneal atropine (3mg/kg). Groups IV, V and VI were administered *P. rubra* at doses of 150, 300 and 450mg/kg, respectively, 1 hour prior to castor oil administration, based on the pilot study. A volume of 1mL of marker, consisting of a suspension of 10% charcoal in a solution of 5% gum acacia, was provided orally for 1hour following the castor oil. After 1h, the animals were euthanized, and the pylorus area traveled by charcoal meal was restrained and represented as % of the entire intestine length (Pylorus caecum) (Khan *et al.*, 2018a; Khan *et al.*, 2020). The subsequent equation computes the percentage of inhibition.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Acute oral dose toxicity study: The toxicity of *P. rubra* was assessed in 25 rats. Rats were divided into five groups, with 5 animals per group. Prior to administration, rats were fasted for 24 hours. Subsequently, they were orally administered doses of 2000-4000mg/kg via oral gavage. The rats were monitored for 14 days after administration of a high dose of *P. rubra* extract to detect any signs of abnormality (Manzoor *et al.*, 2022b).

Statistical analysis: The statistical analysis for *in-vivo* investigations was done using a one-way ANOVA following unpaired t-test (two-tailed) and Tukey's post-test. For *in-vitro* investigations, a paired t-test (two-tailed) was employed.

RESULTS

HPLC analysis: The HPLC results and examination confirmed the presence of several phytoconstituents, including isoquercetin, kaempferol, rutin and plumericin, which were recognized by their retention time (Fig. 2).

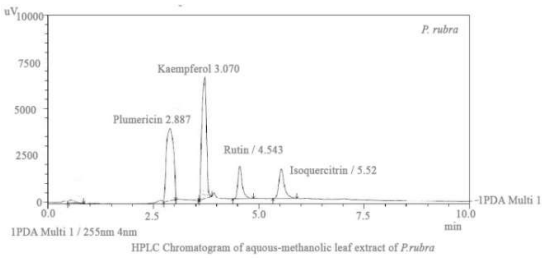

Fig. 2: HPLC Chromatogram Graph showing different Phytochemical constituents.

DPPH assay: DPPH experiment, *P. rubra*'s aqueous methanolic extract demonstrated virtuous antioxidant capacity concerning ascorbic acid (Fig. 3).

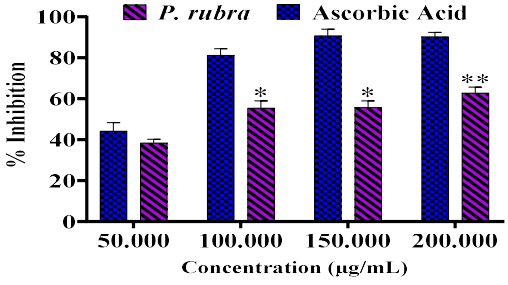

Fig. 3: Anti-oxidant effect of *P. rubra* measured by the DPPH assay in comparison to ascorbic acid.

NO scavenging assay: Antioxidant effect *P. rubra* showed significant (87%) NO scavenging potential in comparison with standard (ascorbic acid) as presented (Fig. 4).

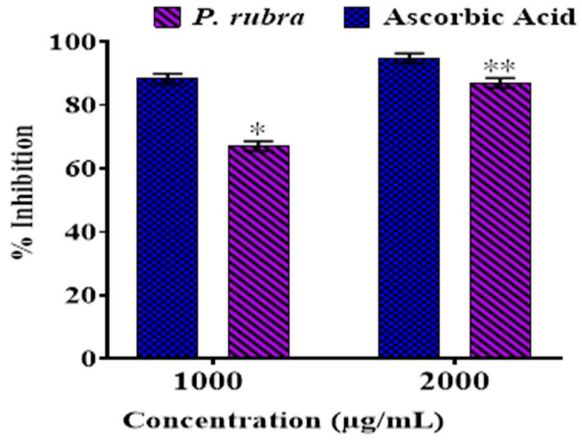

Fig. 4: Antioxidant effect of *P. rubra* measured by the NO scavenging assay in comparison to ascorbic acid.

Superoxide dismutase assay: *P. rubra* showed maximum 85% SOD inhibition in comparison with standard (ascorbic acid) as presented (Fig. 5).

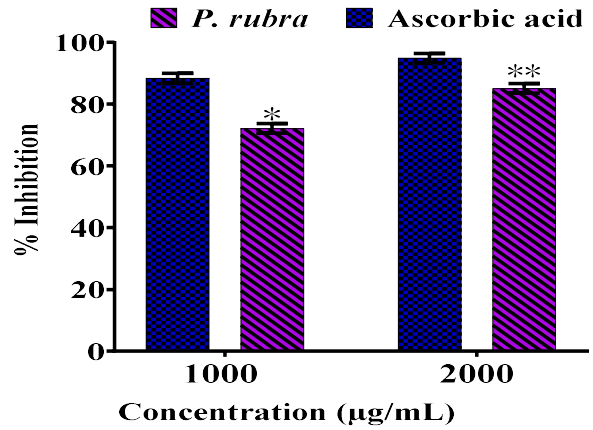

Fig. 5: Antioxidant effect of *P. rubra* measured by the SOD assay in comparison to ascorbic acid.

***In vitro* antibacterial efficacy:** To assess the antibacterial efficacy of *P. rubra* against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus* a total of 10^6 CFU were exposed to varying doses of *P. rubra* extract. *P. rubra* demonstrated bactericidal activity in comparison to all tested bacteria at concentrations equivalent to 10 and 12.5mg/mL, with the exception of *S. aureus* and *P. aeruginosa.* In these cases, the *P. rubra* extract showed a bacteriostatic effect at a concentration of 1mg/200µL. When subjected to *E. coli*, a volume of 2.5mg/200µL of *P. rubra* exhibited bactericidal activity of 66.86–71.80%. Though there was no observed outcome of evaluated capacity at a dose of 1mg/200µL of evaluated capacity (Fig. 6) while the *P. rubra* extract, at concentrations of 1 and 2.5mg/200µL in the evaluated capacity, exhibited bactericidal activity of 78.88 and 91.60% against *S. aureus*, respectively. When testing *P. aeruginosa,* a volume of 2.5mg/200µL of *P. rubra* extract resulted in a bactericidal activity of 76.83–76.22 percent (Fig. 6).

***In vivo* Antibacterial effects:** All rats in control group showed clinical signs of diarrhoea within 30 minutes of receiving castor oil, and this continued for the next 4 hours. The intraperitoneal administration of atropine (3 mg/kg) resulted in a significant reduction of 69.84%. *P. rubra* administered orally at doses of 150, 300, or 450 mg/kg brings about a momentous diminution in the frequency of bowel movements after four hours. *P. rubra* in a dose-dependent (150, 300 and 450mg/kg) suggestively reduced defecation, in a dose-dependent manner, with respective reductions of 39.2, 47.3 and 70.3% (Fig. 7). Administration of the extract resulted in a delay in the initiation of diarrhoea, with animals (30%) exhibiting diarrhea within 1 hour (P<0.001).

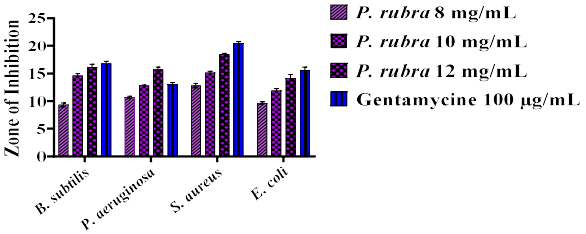

Fig. 6: Antibacterial activity of *P. rubra* against different strains of bacteria.

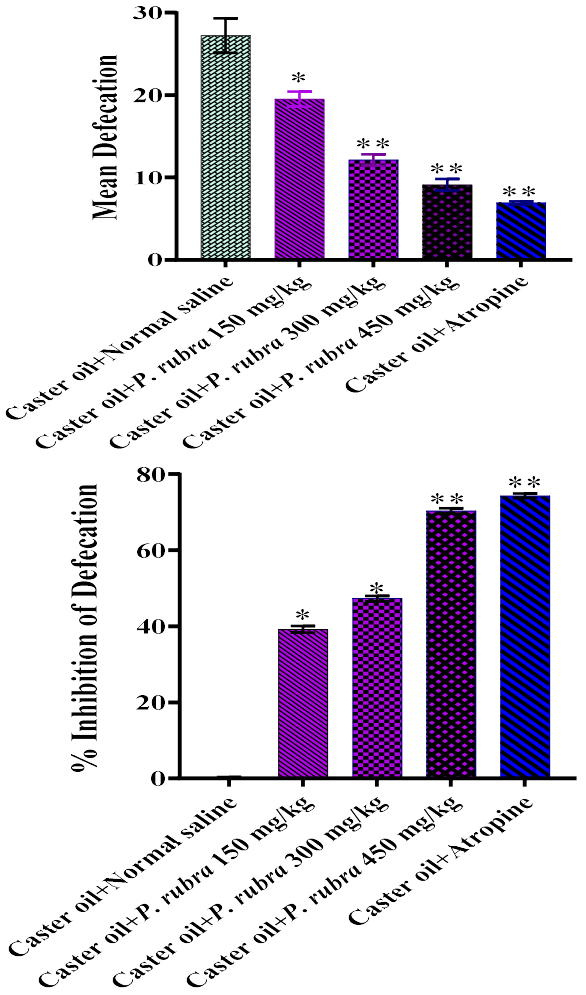

Fig. 7: Mean defecation in castor oil induced diarrhoea (a), and % inhibition of defecation cause by *P. rubra* (b) with respect to control.

Enteropooling effect: The administration of castor oil resulted in a substantial rise in secretions of the intestine from 77.35 ± 1.52g to 149 ± 5.90g. The extract of P. rubra decreased the intestinal secretions generated by castor oil in rats by 24, 28 and 38% at dosages of 150, 300 and 450mg/kg, respectively (Fig. 8). The inhibitory effect showed a dose-dependent response, with dosages of 150.

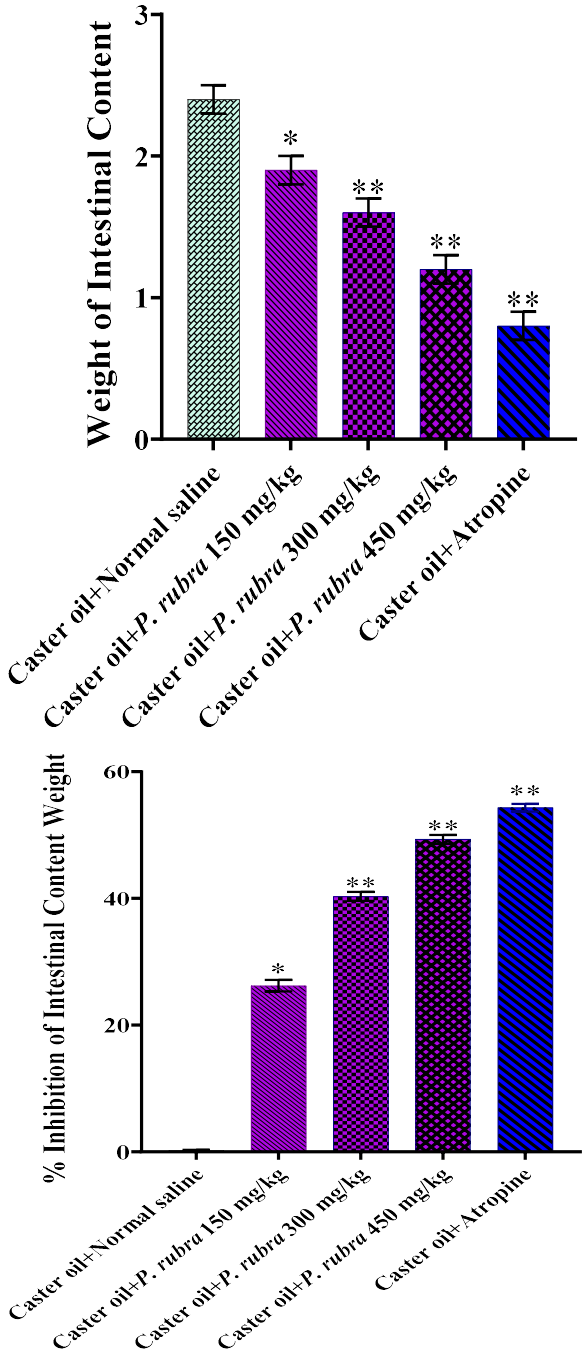

Fig. 8: Weight of intestinal content (a) and % inhibition of intentional content (b) caused by *P. rubra* against castor oil induced enterpooling in rats.

Intestinal motility: The application of castor oil resulted in a momentous intensification in intestinal motility, as indicated by the increase in the distance traveled by the charcoal meal from 44.54 ± 1.66cM to 66.21 ± 3.88cM (P<0.01). The extract of *P. rubra* caused a decrease in intestinal motility in rats by 32% (42.96 ± 3.38cM; P<0.01), 45% (37.11 ± 3.73cM; P<0.001) and 54% (29.97 ± 4.30cM; P<0.001) at dosages of 150, 300 and 450mg/kg, respectively (Fig. 9). The effectiveness of the treatment varied according to the dosage, with a clear relationship up to 300mg/kg. However, no additional effects were seen at the higher dosage of 450mg/kg.

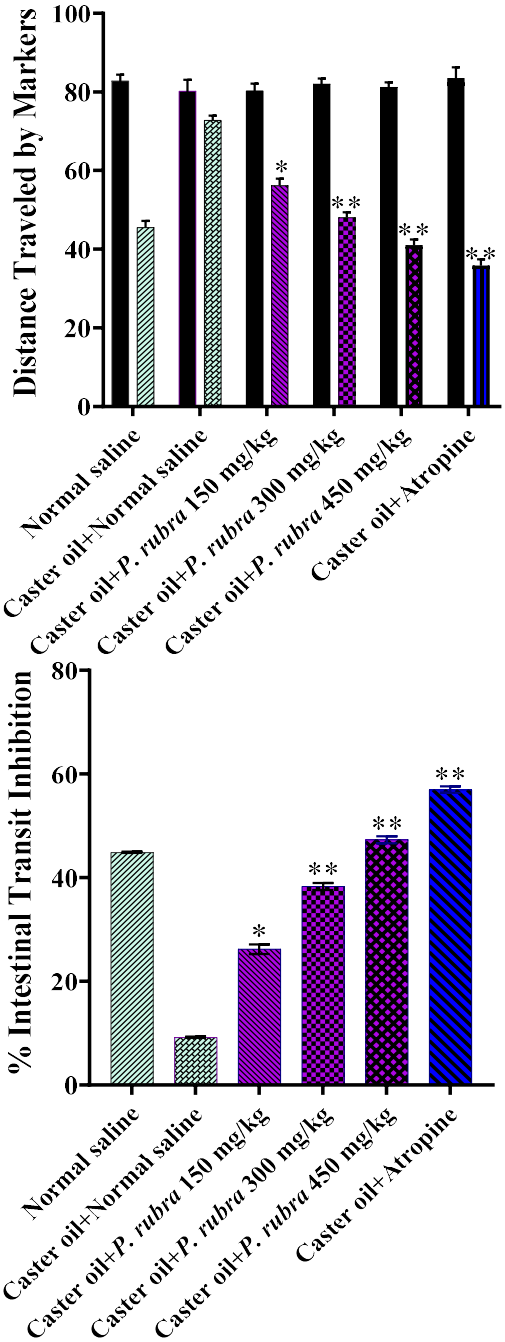

Fig. 9: Effect of various doses of *P. rubra* on intestinal motility (a) distance traveled by the marker, (b) % inhibition of intestinal transit.

Acute oral dose toxicity: In acute oral dose toxicity study; no animal showed any toxic effects up to dose 4000mg/kg.

DISCUSSION

Diarrhoea is caused by bacterial infections as well as other functional abnormalities (Yi *et al.,* 2025). Antibiotics have been effective in treating these infections and avoiding consequences (Singh *et al.*, 2018). However, there is a growing need for new antimicrobial drugs due to the increasing development of microbial resistance to those currently available (Satoh and Nishida, 2004). Botanical-driven compounds, products, and formulations play an important role in curing infectious and non-infectious diseases (Murugan *et al.,* 2023). Likewise, in the current experiment, depending on the doses, *P. rubra* demonstrated a wide-ranging protective effect against diarrhoea. *P. rubra* demonstrated a considerable dose-dependent inhibition of intestinal fluid buildup induced by castor oil and the bulk of intestinal content, surpassing the effects of atropine (Fig. 7; Fig. 8). *P. rubra* markedly decreased the intestinal transit generated by castor oil (Fig. 9). Atropine, due to its anticholinergic effects, caused a notable decrease in bowel movement frequency and an increase in the time it takes for food to pass through the intestines (Omer *et al.*, 2021). Nevertheless, it failed to prevent the accumulation of liquid in the intestines induced by castor oil and the increase in the weight of intestinal material (Schuster, 2001). This suggests that other mediators, in addition to acetylcholine, play a role in castor oil-induced enteropooling. Atropine can delay the movement of food through the intestines, possibly by slowing the rate of stomach emptying. Castor oil can cause diarrhoea by increasing the volume of intestinal contents and preventing water reabsorption (Içen *et al.*, 2015). The production of ricinoleic acid causes mucosal inflammation and intestinal irritation, leading to increased prostaglandin release. This stimulates discharge and hinders NaCl and water resorption. *P. rubra* likely enhanced the NaCl and water resorbence by reducing gut peristalsis, as seen by the diminution in intestinal transportation caused by the charcoal meal (Khan *et al.*, 2020). Diarrhoeal episodes are linked to the stimulation of Cl- channels, leading to the release of Cl- from the cell (Singleton *et al.,* 2025). This release of Cl- causes a significant water outflow into the intestinal lumen, resulting in excessive, liquid diarrhea (Kozat and Çelik, 2025). In these mechanisms and in diarrhoeal occurrence, *P. rubra* may impede the excretion of fluid in the inner cavity by altering the process. The antidiarrheal effect of botanicals is attributed to flavonoids, alkaloids, tannins, saponins, reducing sugars, triterpenes, and sterols (Khan *et al.*, 2020).

The analysis of *P. rubra* showed the existence of triterpenoids, alkaloids, tannins, gums, flavonoids, mucilage, phenols, and carbohydrates (Khan *et al.*, 2018b; Khan *et al.*, 2022). The phytochemicals in *P. rubra* may be responsible for its antidiarrhoeal effects (Fig. 2). The antidiarrhoeal activities of the active terpenoids mentioned have been thoroughly demonstrated. Diterpenes, sesquiterpenes, flavonoids, terpenoids, and terpenes derivatives have been found to block autocoid and prostaglandin excretion (Fathiazad *et al.*, 2012; Liu *et al*., 2024). As a result, they effectively suppress the peristalsis and discharge caused by castor oil. *P. rubra* has demonstrated efficacy in numerous antimicrobial trials in contradiction to the bacteria and fungus responsible for causing infectious diarrhoea (Hussain *et al.*, 2013).

Previous studies have shown that *P. rubra* possesses antibacterial characteristics (Khan *et al.*, 2018b). Antimicrobial effects of *P. rubra* extract were observed against *P. aeruginosa*, *E. coli*, *S. aureus, S. typhi*, and *B. cereus*, among other bacteria (Fig. 6). It is well-known that these microbes can cause infections in the intestines and elsewhere in the body (Schuster, 2001). *P. rubra* showed to serve as potential source for new and improved antimicrobials due to antioxidant action of its phytochemical compounds (rutin, quercetin, kaempferol, plumericin, and isoplumericin) and its effectiveness against drug-resistant infections and both Gram-positive and Gram-negative bacteria. Results from recent studies (Murugan *et al.,* 2020; Terefe *et al.,* 2023; Wang *et al.*, 2025) have complemented the antidiarrhoeal effect observed in animals with the *P. rubra* extract. An acute oral dose toxicity study showed drug tolerability up to 4000mg/kg in rats, complementing the *P. rubra* toxicity study we reported earlier in rabbits (Khan *et al.*, 2020). *P. rubra* proved to be a safe household remedy for treating infectious or noninfectious diarrhea without noticeable toxicity.

Conclusions: The antidiarrheal effect of *Plumeria rubra* leaf extract has been scientifically justified through *in-vivo* and *in-vitro* assays. The presence of various phytoconstituents may be responsible for its antidiarrheal effect. Efficacy in infectious diarrhea is likely mediated by its antibiotic activity against various bacteria, which may explain the traditional use of *P. rubra* for abdominal cramps, diarrhea, and dysentery.

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Authors Contribution: TS, JR and JC Designed Study and Research protocols; HD, RG, XX and YL Executed Experiment and Compiled Data and Results; IAK, AA, MKK drafted Research Manuscript; MK, WAQH and MM analyzed the data and proof readed the Final Manuscript.

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