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Research

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# Efficacy of tamarind leaf infusion (*Tamarindus indica* L.) as an antidiarrheal agent in mice

**Ratna Kristiani Nababan[1], Lina Noviyanti Sutardi[2], Anisa Rahma[2], Aulia Andi Mustika[3]**

[1] Study Program of Veterinary Medicine, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[2] Sub-division of Veterinary Pharmacy, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[3] Division of Pharmacology and Toxicology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia

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## Abstract

**Background** Bacground Tamarind (*Tamarindus indica* L.) is an herbal plant that is used to treat various diseases, but there has been no scientific research on the activity of tamarind leaf infusion as an antidiarrheal.

**Objectives** This study aimed to analyze the effectiveness of tamarind leaf infusion preparation as an antidiarrheal agent through the intestinal protection method and determine the effective concentration for providing antidiarrheal effects in mice.

**Methods** This study used 30 mice were divided into five groups consisting of negative control (1% Tween 80), positive control (loperamide hydrochloride), and three groups treated with tamarind leaf infusion with graded concentrations (25%, 50%, and 100%). The parameters used in the intestinal protection method were frequency of defecation, stool consistency, time of onset of diarrhea, and duration and recovery time of diarrhea.

**Results** The phytochemical test results of tamarind leaf infusion contained flavonoids, steroids, and terpenoids. In vivo testing of tamarind leaf infusion in mice at concentrations of 25%, 50%, and 100% demonstrated the potential antidiarrheal activity of tamarind leaf infusion.

**Conclusion** Tamarind leaf infusion has antidiarrheal activity, with The best effectiveness at a concentration of 50%.

**Keywords:** antidiarrheal | infusion | tamarind leaves | mice | intestinal protection method

## Introduction

Diarrhea is an infectious gastrointestinal disorder that is a major global health concern. According to the 2018 Basic Health Research Report by the Ministry of Health of the Republic of Indonesia, the prevalence of diarrhea across all age groups reached 8%, with 12.3% among children under five and 10.6% among infants (Balitbangkes, 2019). In animals, particularly cattle, the incidence of diarrhea has also been reported at high rates: 86.6% in calves aged 0–8 months, 95.8% in cows aged 24–36 months, and 100% in those aged 48–60 months (Putri *et al*., 2023).

Diarrhea in animals may result from both infectious and noninfectious factors. Infectious causes include bacteria, viruses, and parasites transmitted through food, air, or

*Corresponding author Email: linans@apps.ipb.ac.id

direct contact. Non-infectious causes involve changes in feeding frequency, stress, drugs, and toxins (Humphries & Linscott, 2015). Sudden, unusual changes in diet can also trigger diarrhea in animals. Environmental stress and exposure to drugs or toxins can irritate the gastrointestinal tract, ultimately leading to diarrhea.

Diarrhea can be treated through both modern and traditional medicine. Modern therapies typically involve antibiotics, anti-motility agents, and non-steroidal anti-inflammatory drugs (NSAIDs). However, these treatments may produce adverse effects such as allergic reactions, antibiotic resistance, and an increased risk of gastrointestinal bleeding (Carter *et al*., 2015). Consequently, many people opt for traditional herbal remedies over conventional pharmaceuticals (Agustini *et al*., 2023). Traditional medicines are considered a preferred first-line approach for managing diarrhea owing to their minimal side effects, holistic action, and antimicrobial properties.

The use of medicinal plants offers several advantages: they are readily available, relatively inexpensive, efficient, and are believed to have fewer side effects (Sumayyah & Salsabila, 2017). Tamarind (*Tamarindus indica* L.) is a plant traditionally used to treat diarrhea. The leaves of *T. indica* contain various secondary metabolites including flavonoids, tannins, steroids, alkaloids, terpenoids, and saponins (Husain *et al*., 2022; Dahiru *et al*., 2023). In Indonesia, tamarind leaves have long been used in folk medicine to manage diarrhea by boiling them in decoctions (Risfianty & Indrawati, 2020). However, no scientific study has validated this traditional approach. This study aimed to evaluate the antidiarrheal efficacy of tamarind leaf infusion and determine the most effective concentration.

# Methods

### Time and Location

The study was conducted between September 2023 and February 2024. The experiment was conducted at the Pharmacy Laboratory and Laboratory Animal Management Unit (UPHL), School of Veterinary Medicine and Biomedical Sciences (SKHB), IPB University, as well as at the Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA), Pakuan University, Bogor.

### Experimental Animals

This study was approved by the Animal Ethics Committee of SKHB IPB University (approval number 138/KEH/SKE/XI/2023). Thirty male Deutschland Denken Yoken (DDY) mice, weighing 25–30 g and aged 2–2.5 months, were used. The mice were acclimatized for one week in a room maintained at 25–28°C. They were housed in cages measuring 40 ×30 ×18 cm, equipped with wire-mesh covers and rice husk bedding, and arranged in stacks. Food and water were provided *ad libitum*. The diet was formulated according to nutritional standards set by the Indonesian National Agency for Drug and Food Control (BPOM RI).

### Preparation of crude simplicia and infusion

Tamarind leaves were washed with clean water and oven-dried at 60°C for 6 h. The dried leaves were then ground using a blender to obtain powdered simplicia, which were sieved and stored in a dry container.

Tamarind leaf simplicia (10 g) was placed in an infusion pan and mixed with 100 mL distilled water. The mixture was then heated at 90°C for 15 min (Purohita *et al*., 2022). The resulting infusion was poured into a graduated cylinder at a volume of 100 mL. The volume was adjusted by adding distilled water if the volume was < 100 mL. The infusion was filtered using a muslin cloth and stored in a bottle.

### Preparation of 1% Tween 80 and loperamide hydrochloride suspension

One milliliter of Tween 80 was added to a graduated cylinder and diluted with distilled water to a final volume of 100 mL. The mixture was stirred using a glass rod until it became homogeneous. For the loperamide hydrochloride suspension, tablets (2 mg/tablet) were crushed, and 0.086 g of the resulting powder was diluted in 1 mL of 1% Tween 80 solution and mixed thoroughly. The mixture was then brought to 100 mL using distilled water.

### Phytochemical screening

Phytochemical screening included tests for alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins based on a modified method from Harborne (1987). Alkaloid testing involved mixing 2 mL of tamarind leaf extract with 2 mL of $H_2SO_3$, heating for 30 min, and filtering. The filtrate was divided into three portions, each reacting with Wagner's, Mayer's, or Dragendorff's reagent. A positive result was indicated by a white precipitate (Mayer), brown color (Wagner), or orange color (Dragendorff).

For flavonoids, 2 mL of the extract was dissolved in 5 mL of hot water and mixed with magnesium powder and 1 mL of concentrated HCl, followed by vigorous shaking. A positive result was indicated by red, yellow, or orange.

Steroid and terpenoid testing involved mixing 2 mL of extract with 2 mL of chloroform and adding the Liebermann-Burchard reagent. Green or blue indicates a positive result.

Saponin testing was performed by mixing 2 mL of the extract with 5 mL of hot water, cooling the mixture, and vigorously shaking it for 1 min. The formation of a stable froth indicated a positive result.

Tannin testing was performed by adding five drops of 1% $FeCl_3$ to 2 mL of the extract. A positive reaction is indicated by a blue to greenish-black precipitate.

### Experimental design

This study employed an intestinal protection method in five experimental groups, each consisting of six mice. The groups included a negative control group (1% Tween 80), a positive control group (Loperamide HCl suspension),

and three treatment groups receiving tamarind leaf infusion at graded concentrations (25%, 50%, and 100%).

### In vivo antidiarrheal test using the intestinal protection method

Mice were fasted for 2 h before treatment. After fasting, the mice were administered tamarind leaf infusions at different concentrations (25%, 50%, and 100%). Thirty minutes post-treatment, castor oil (0.5 mL) was orally administered to each mouse. Observations were carried out every 30 min for 9 h to monitor research parameters, including defecation frequency, fecal consistency, diarrhea onset, diarrhea duration, and recovery time (Purohita *et al*., 2022).

Defecation frequency was assessed by counting the number of defecation events, which ranged from soft to watery stools during the observation period. Fecal consistency was scored on a scale of 1 to 5: solid (score 1), semi-solid (score 2), soft (score 3), soft-watery (score 4), and watery (score 5) (Inderiyani & Sulastri, 2021). The scoring scheme is illustrated in **Figure 1**. Diarrhea onset was defined as the time from the first appearance of diarrhea, while duration was defined as the time from diarrhea onset to its resolution, indicated by the return of solid feces. The recovery time was defined as the total duration until the mouse fully recovered from diarrhea and remained healthy until the end of the observation period.

### Data analysis

Data from defecation frequency and fecal consistency variables were analyzed using one-way analysis ANOVA at a 95% confidence level ($\alpha = 0.05$). Significant differences among the treatment groups ($P<0.05$) were determined using Tukey's post-hoc test. All analyses were performed using Minitab version 18. Observations of diarrhea onset, duration, and recovery time were presented quantitatively using Microsoft Excel 2021.

## Results

### Phytochemical screening

The results of the phytochemical screening of tamarind leaves are presented in **Table 1**. Tamarind leaves yielded negative results for alkaloid tests, as indicated by the absence of white precipitate with Mayer's reagent, brown precipitate with Wagner's reagent, and formation of an orange solution with Dragendorff's reagent. The lack of precipitate formation with the first two reagents indicated the absence of alkaloid compounds in the tamarind leaves.

The flavonoid test yielded a positive result, as indicated by the formation of a yellow solution. Steroid and terpenoid tests were also positive, producing a green solution. Phytochemical screening showed a negative result for saponins, as indicated by the absence of a stable foam for more than 30 s, and a negative result for tannins due to the absence of a dark greenish-black precipitate. These findings suggest that tamarind leaves contain secondary metabolites such as flavonoids, steroids, and terpenoids, which potentially contribute to their antidiarrheal activity.

### In vivo antidiarrheal activity

The anti-diarrheal effectiveness of tamarind leaf infusion was indicated by a decrease in defecation frequency, improved stool consistency, delayed onset of diarrhea, shorter diarrhea duration, and prolonged recovery time. Observational data on the mean defecation frequency and stool consistency are shown in **Table 2**.

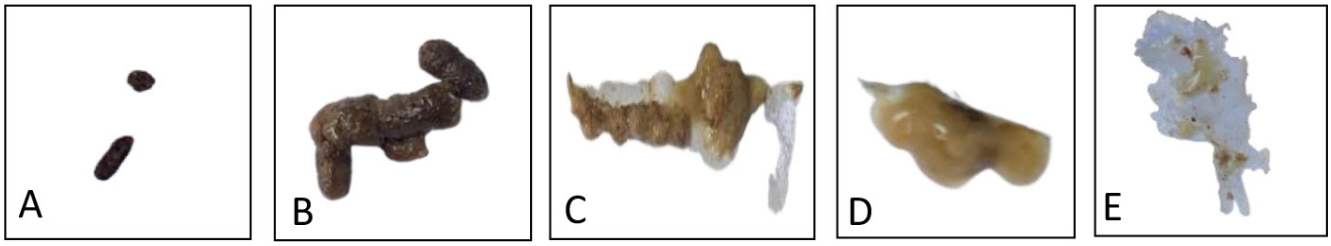


**Figure 1** Scoring system for fecal consistency in mice. (A) Score 1 (solid); (B) Score 2 (semi-solid); (C) Score 3 (soft); (D) Score 4 (soft-watery); and (E) Score 5 (watery).

**Table 1** Phytochemical screening of tamarind (*Tamarindus indica*) leaves

| Parameter | Test result | Observation |
|---|---|---|
| Alkaloids: | | |
| Mayer's reagent | Negative | No white precipitate formed |
| Wagner's reagent | Negative | No brown precipitate formed |
| Dragendorff's reagent | Positive | Orange solution formed |
| Flavonoids | Positive | Yellow-colored solution formed |
| Steroids dan Terpenoids | Positive | Green-colored solution formed |
| Saponins | Negative | No stable foam formed for more than 30 seconds |
| Tannins | Negative | No dark greenish-black precipitate observed |

**Table 2** Defecation frequency in mice treated with tamarind (*Tamarindus indica*) leaf infusion using the intestinal protection method

| Treatment Group | Defecation Frequency (times) |
|---|---|
| Negative control | $8.33 \pm 1.51^{a}$ |
| Positive control | $3.33 \pm 1.37^{b}$ |
| 25% Infusion | $5.17 \pm 1.72^{b}$ |
| 50% Infusion | $3.50 \pm 1.87^{b}$ |
| 100% Infusion | $4.00 \pm 1.79^{b}$ |

Positive control: Loperamide HCl suspension; Negative control: 1% Tween 80 solution; 25%, 50%, and 100%: Tamarind leaf infusions at corresponding concentrations. Different superscript letters in the same column indicate statistically significant differences ($P<0.05$).

The data revealed significant differences ($P<0.05$) between the negative control group and the treatment groups that received 25%, 50%, and 100% tamarind leaf infusion. The lower mean values of defecation frequency and improved stool consistency compared to the negative control indicated the antidiarrheal efficacy of the tested formulations.

The mean defecation frequency was 8.3 times in the negative control, 3.3 in the positive control, 5.2 in the 25% group, 3.5 in the 50% group, and 4.0 in the 100% group. Statistical analysis confirmed that all three infusion groups differed significantly from the negative control and showed lower defecation frequencies, suggesting the antidiarrheal activity of the tamarind leaf infusion.

The antidiarrheal activity was further supported by improvements in stool consistency, as shown in **Figure 2**. The stool consistency scores were 4.17, 1.5, 1.58, 2.13, and 2.13, in the negative control, positive control, 1.58 in the 25% group, 2.13 in the 50%, and 2.66 in the 100% groups, respectively. Stool consistency reflects the ability of a formulation to stabilize intestinal function. All treatment groups showed significant improvements in stool consistency.

The observed onset, duration, and recovery time from diarrhea are shown in the bar chart in **Figure 3**. The onset time of diarrhea was recorded every 30 min over a 9-hour period. The mean onset times were 90 min in the negative control group, 205 min in the positive control group, 130 min in the 25% group, 155 min in the 50% group, and 140 min in the 100% group. The 50% infusion group showed the most delayed onset of diarrhea compared to the negative control group, indicating its effectiveness.

Antidiarrheal activity was also assessed based on the duration of diarrhea and recovery. The mean duration of diarrhea was 300 min in the negative control group, 40 min in the positive control group, 105 min in the 25% group, 75 min in the 50% group, and 95 min in the 100% group. The corresponding recovery times were 150, 295, 305, 310, and 305 min. These results suggest that the 50% tamarind leaf infusion not only reduced the duration of diarrhea but also provided longer recovery times compared to the other treatment groups.

## Discussion

Diarrhea can be induced using laxatives such as castor oil (Oleum ricini), a triglyceride composed of ricinoleic acid that exhibits laxative effects owing to its irritant properties in the gastrointestinal tract. The triglyceride content of castor oil is hydrolyzed by lipase enzymes in the small intestine into glycerin and ricinoleic acid (EFSA Panel on Food Additives and Nutrient Sources added to Food, 2017). Ricinoleic acid stimulates prostaglandin release, which in turn enhances the secretion of

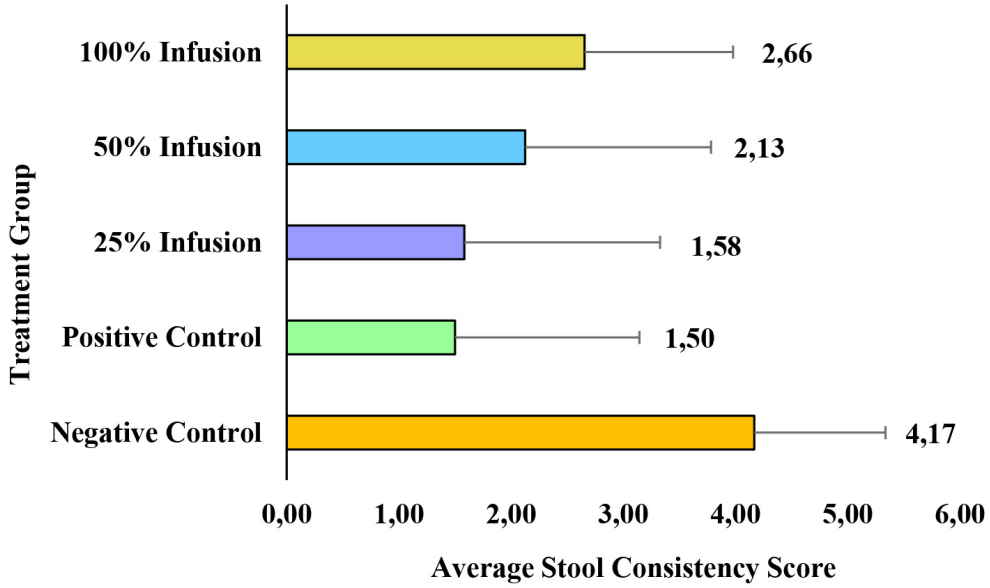


**Figure 2** Mean stool consistency scores in mice treated with tamarind (*Tamarindus indica*) leaf infusion using the intestinal protection method. Positive control: Loperamide HCl suspension; Negative control: 1% Tween 80 solution; 25%, 50%, and 100%: Tamarind leaf infusions at corresponding concentrations.

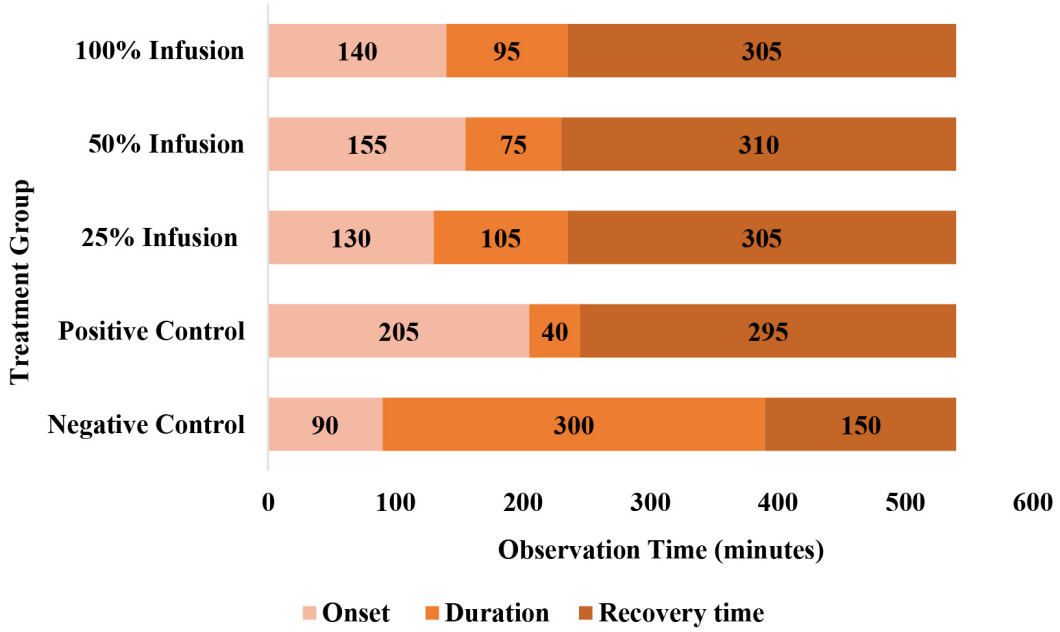


**Figure 3** Onset, duration, and recovery time of diarrhea in mice treated with tamarind (*Tamarindus indica*) leaf infusion using the intestinal protection method. Positive control: Loperamide HCl suspension; Negative control: 1% Tween 80 solution; 25%, 50%, and 100%: Tamarind leaf infusions at corresponding concentrations.

electrolytes and fluids, accelerates intestinal transit, and increases peristaltic movement (Ayalew *et al*., 2022). These effects cause the intestinal contents to be rapidly expelled, leading to diarrhea.

Diarrhea can be managed using antidiarrheal agents, including adsorbents, anti-motility agents, antisecretory drugs, probiotics, and herbal remedies (Anbhuselvam *et al*., 2019). One pharmacological approach involves the use of loperamide hydrochloride (HCl), the active compound in imodium. Loperamide HCl acts directly on intestinal smooth muscle to inhibit acetylcholine release of acetylcholine (Lina & Rahmawaty, 2021). By suppressing acetylcholine release, loperamide reduced intestinal motility and promoted muscle relaxation. Consequently, loperamide delays the onset of diarrhea, shortens its duration, and enhances recovery by suppressing intestinal peristalsis (Sadraei *et al*., 2014; Riddle *et al*., 2016). Inhibition of peristalsis also increases the time for water and electrolyte reabsorption by intestinal mucosal cells (Tjay & Rahardja, 2015).

Medicinal plants are promising sources of anti-diarrheal agents owing to their content of secondary metabolites, which act as active compounds with specific mechanisms of action. These metabolites include flavonoids, saponins, alkaloids, steroids, terpenoids, and tannins (Khafid *et al*., 2023). Their presence can be detected through phytochemical screening (Vigbedor *et al*., 2022), and each class of metabolite exerts different therapeutic effects. Phytochemical testing of tamarind leaves confirmed the presence of flavonoids, steroids, and terpenoids, which are believed to contribute to their antidiarrheal activity.

Flavonoids, for instance, exhibit antimicrobial properties by disrupting microbial membranes. This disruption impairs nutrient transport and causes nutrient deficiency in microbes, ultimately inhibiting growth (Chatri *et al*., 2022). Additionally, flavonoids may suppress acetylcholine release in the digestive tract (Pramitaningastuti & Advistasari, 2019), leading to reduced acetylcholine levels. This suppression results in decreased intestinal motility and fluid secretion (Rizal *et al*., 2016; Bawamenewi *et al*., 2023).

Steroids contribute to anti-diarrheal effects by enhancing water and electrolyte absorption in the intestine. This is partly due to the inhibition of prostaglandin release by the steroids present in the tamarind leaves (Ilmi *et al*., 2023). The activity of steroids in infusion leads to reduced defecation frequency and increased stool consistency (Toemon *et al*., 2019).

Terpenoids, another class of secondary metabolites found in tamarind leaves, are highly lipophilic and known to inhibit the release of autacoids and prostaglandins (Derebe *et al*., 2018). Their antidiarrheal activity includes antisecretory and intestinal transit inhibition properties. Terpenoids exhibit antimicrobial effects by disrupting porin proteins in bacterial outer membranes and forming strong polymeric bonds. These bonds damage the porin structure, allowing the entry of lipophilic compounds, reducing membrane permeability, and impairing bacterial nutrient uptake and growth (Nurulita *et al*., 2022).

Secondary metabolites in tamarind leaves can be extracted via infusion, a method that affects the concentration of extracted compounds. Water (aqua destillata) is commonly used as a solvent because of its

affordability, availability, low volatility, non-flammability, and non-toxicity (Risfianty & Indrawati, 2020). As a highly polar solvent, water can extract polar compounds, such as alkaloids, tannins, flavonoids, phenols, and saponins. Nonpolar compounds such as steroids and terpenoids can also be partially extracted with water (Ergina *et al*., 2014).

The intestinal protection method demonstrated that tamarind leaf infusion significantly reduced defecation frequency, improved stool consistency, delayed onset of diarrhea, and prolonged recovery in castor oil-induced mice. All tested concentrations of tamarind leaf infusions (25%, 50%, and 100%) showed effective anti-diarrheal activity. Among them, 50% concentration provided the most optimal effects compared to the other doses.

# Conclusion

Tamarind (*Tamarindus indica* L.) leaf infusion administered via the intestinal protection method at concentrations of 25, 50, and 100% exhibited significant antidiarrheal activity. Among these, 50% concentration was identified as the most effective formulation. The anti-diarrheal effects were demonstrated by reduced defecation frequency, improved stool consistency, delayed onset of diarrhea, shortened diarrhea duration, and accelerated recovery. The efficacy of the infusion is attributed to the presence of secondary metabolites (flavonoids, steroids, and terpenoids) in the tamarind leaves.

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**Conflict of interest** The authors declare no conflicts of interest associated with this study.

**Author contribution** RKN: Data curation, formal analysis, methodology, and writing – original draft. LNS: Conceptualization, project administration, validation, and writing, review, and editing. AR: Investigation and writing, review, and editing. AAM: Conceptualization and project administration.

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