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Research

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Antidiarrheal potential of *Nigella sativa* L. infusion in mice: a phytochemical and efficacy evaluation

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Abstract

Background *Nigella sativa* L., commonly known as black cumin, is a medicinal plant widely recognized for its therapeutic properties. However, limited pharmacological evidence supports its antidiarrheal potential when prepared as an infusion.

Objective This study aimed to evaluate the phytochemical composition and antidiarrheal efficacy of *Nigella sativa* infusion in mice.

Methods A total of 30 mice were divided into five groups: negative control (Tween 80 [1%]), positive control (Loperamide HCl), and treatment groups receiving *Nigella sativa* infusion at concentrations of 25%, 50%, and 100%. Antidiarrheal activity was assessed using the intestinal protection method, with parameters including defecation frequency, stool consistency, diarrheal onset time, and diarrheal duration.

Results Phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, and saponins in *Nigella sativa* infusion. The infusion demonstrated significant antidiarrheal activity across all tested concentrations (25%–100%), with the 50% concentration showing the highest efficacy, comparable to Loperamide in reducing defecation frequency, improving stool consistency, delaying diarrheal onset, and shortening diarrheal duration.

Conclusion Nigella sativa infusion at 50% concentration exhibits promising antidiarrheal potential and warrants further development as a herbal remedy.

Keywords antidiarrheal | herbal medicine | infusion | intestinal protection | *Nigella sativa* | phytochemicals

Introduction

Diarrhea is a common digestive disorder with the potential to cause outbreaks and fatalities (Kemenkes, 2019). According to the World Health Organization (WHO), diarrhea is the second leading cause of death among children under five, contributing to approximately 525,000 deaths annually (WHO, 2017). In Indonesia, diarrhea remains a significant health concern. The 2019 Indonesia Health Profile reported a total of 2,549 diarrhea cases with a case fatality rate (CFR) of 1.14% (Apriani *et al.*, 2022).

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The onset of diarrhea is typically caused by the increased intestinal peristalsis, leading to the altered stool consistency and increased defecation frequency (Farhani *et al.*, 2020). Various pathogens, including bacteria, viruses, and parasites, can trigger diarrhea, primarily through cross-contamination from food and environmental sources. Poor sanitation practices, especially in food and beverage handling, are major contributors to this contamination. Enhancing hygiene and improving food sanitation can significantly reduce the incidence of diarrhea (Tuang, 2021).

Diarrhea can result from osmotic, secretory, and motility disorders within the intestines. Osmotic disturbances occur due to an increase in osmotic pressure, which in turn affects secretion processes. Unabsorbed nutrients in the intestines lead to a shift of water and electrolytes into the intestinal lumen. This results in the increased intestinal motility, causing more frequent defecation and softer stool consistency (Kelly *et al*, 2018).

Conventional treatments for diarrhea include chemical agents such as loperamide, oral rehydration solutions, bismuth subsalicylate, and attapulgite. However, these drugs may cause side effects like nausea, vomiting, dizziness, and abdominal pain (Lina & Rahmawaty, 2021). In contrast, herbal medicine offers an alternative treatment approach. Herbal remedies, widely used in Indonesia for their affordability and accessibility, are composed of medicinal plants with empirically proven efficacy against various diseases, including diarrhea (Adiyasa & Meiyanti, 2021). Several plants with secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, steroids, glycosides, essential oils, and triterpenoids, exhibit antidiarrheal properties through synergistic activity (Astuti *et al.*, 2019; Simanjuntak, 2021).

One notable herbal plant is black cumin (*Nigella sativa* L.), which contains tannins, saponins, arachidic acid, alkaloids, and essential oils, all of which are potential antidiarrheal agents (Astuti *et al.*, 2019). Despite its widespread use in traditional medicine, no pharmacological studies have specifically evaluated the antidiarrheal properties of black cumin infusion. Therefore, this study aims to investigate the phytochemical composition of *Nigella sativa* infusion and its antidiarrheal efficacy using the intestinal protection method in mice. Additionally, this study seeks to determine the most effective concentration of *Nigella sativa* infusion for antidiarrheal use.

Methods

Time and location of study

The research was conducted from November to December 2023 at three locations: the Laboratory of Pharmacy at the School of Veterinary Medicine and Biomedical Sciences, IPB University (SKHB IPB); the Laboratory Animal Management Unit (UPHL) of SKHB IPB; and the Laboratory of FMIPA, Universitas Pakuan, Bogor.

Preparation of black cumin infusion, tween 80, and loperamide HCl suspension

The *Nigella sativa* (black cumin) infusion was prepared by mixing 10 g of powdered black cumin simplicia with 100 mL of distilled water. The mixture was boiled at 90°C for 15 minutes (BPOM R1 2012). After boiling, the solution was filtered and adjusted to 100 mL with distilled water. The prepared infusion was stored separately. Dilutions of the infusion were made with distilled water to obtain concentrations of 50% and 25%.

A 1% solution of Tween 80 (Merck, Germany) was prepared by measuring 1 mL of Tween 80 into a graduated cylinder, and then distilled water was added to make up the volume to 100 mL. The solution was homogenized by stirring.

For the preparation of 1.56% loperamide HCl suspension, loperamide HCl tablets were crushed into a fine powder. A total of 0.086 g of the loperamide powder was mixed with 1 mL of 1% Tween 80 and distilled water was added to achieve a final volume of 100 mL. The mixture was stirred until homogenous.

Phytochemical screening of black cumin (*Nigella sativa* L.) infusion

Phytochemical screening was performed on the *Nigella sativa* simplicia using qualitative methods by adding specific reagents to detect various compounds (Erwan & Parbuntari, 2023). The compounds tested in this study include tannins, flavonoids, saponins, alkaloids, steroids, and terpenoids.

Tannins were detected by adding 5 drops of 1% $FeCl_3$ solution to 2 mL of *Nigella sativa* infusion. The presence of tannins was indicated by the formation of a blue-black or greenish-black precipitate.

Flavonoids were detected by adding 2 mL of *Nigella sativa* infusion to 5 mL of hot water, followed by the addition of 0.1 g of magnesium powder, 1 mL of concentrated HCl, and 1 mL of amyl alcohol, then shaking vigorously. The formation of red, yellow, or orange coloration in the amyl alcohol layer indicated the presence of flavonoids.

Saponins were detected by vigorously shaking 2 mL of *Nigella sativa* infusion with 5 mL of water in a test tube for 30 seconds. The presence of stable foam indicated the presence of saponins.

Alkaloids were detected by adding 2 mL of sulfuric acid (H2SO4) to the *Nigella sativa* infusion and heating for 30 minutes. The acidic layer was then divided into three portions, and each portion was treated with Wagner's reagent (brown precipitate), Mayer's reagent (white precipitate), and Dragendorff's reagent (orange precipitate), respectively, to indicate the presence of alkaloids.

Steroids and terpenoids were detected by mixing 2 mL of chloroform with 2 mL of *Nigella sativa* infusion, shaking, and adding Liebermann-Burchard reagent. The formation of green or blue color indicated the presence of steroids or terpenoids.

Experimental design

The study received approval from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, under protocol number 139/KEH/SKE/XI/2023. Thirty DDY strain albino mice (*Mus musculus*), weighing 20–30 g, were obtained from BPOM RI Jakarta. The study followed a completely randomized design. Based on Federer's formula, 30 mice were divided into five groups with six mice per group (Ilmi *et al.*, 2023). The antidiarrheal effectiveness of the black cumin infusion was evaluated using the intestinal protection method with treatments as shown in **Table 1**.

Animal maintenance and acclimatization

The mice were housed in plastic cages measuring 40 cm×30 cm×18 cm, equipped with wire mesh covers and wood shavings for bedding. The room was maintained at 25–30°C with a 12-hour light-dark cycle. Standard laboratory animal feed and water were provided ad libitum. The experimental mice were acclimatized for seven days prior to the experimental treatments.

In vivo antidiarrheal test using the intestinal protection method

The antidiarrheal efficacy was evaluated using the intestinal protection method. The mice were fasted for two hours prior to the administration of treatments. Each group received black cumin infusion at concentrations of 100%, 50%, or 25%, administered orally. Thirty minutes after the infusion administration, all groups were orally given 0.5 mL of castor oil (0.75 mL/20 g body weight) to induce diarrhea. Defecation frequency, stool consistency, onset of diarrhea, and duration of diarrhea were observed every 30 minutes for nine hours (Sukmawati *et al.*, 2017). Stool consistency was scored on a scale from 1 to 5, where 1 represents hard stool and 5 represents watery stool (**Figure 1**). Each mouse was observed individually at 30-minute intervals for the nine-hour period. The duration of diarrhea was calculated as the average time from the onset to the cessation of diarrhea in each group.

Data analysis

All data were compiled in Microsoft Excel for frequency and average value calculations. The stool consistency and defecation frequency data were analyzed

Table 1 Treatment groups for testing the antidiarrheal effect of black cumin in mice

Treatment group	Description
Negative control	Mice given 1% Tween 80 solution
Positive control	Mice given 1.56% loperamide HCl suspension and 1% Tween 80
100% concentration	Mice given 100% black cumin infusion and 1% Tween 80
50% concentration	Mice given 50% black cumin infusion and 1% Tween 80
25% concentration	Mice given 25% black cumin infusion and 1% Tween 80

Table 2 Qualitative phytochemical screening results of *Nigella sativa* infusion

Parameter	Test Result	Observation
Alkaloids		
Mayer	Positive (+)	Formation of white precipitate
Wagner	Positive (+)	Formation of brown precipitate
Dragendorf	Positive (+)	Formation of orange solution
Flavonoids	Positive (+)	Yellow-colored solution
Steroids/terpenoids	Negative (-)	No green color formed
Saponins	Positive (+)	Stable foam formed, lasting more than 30 sec
Tannins	Positive (+)	Formation of greenish-black precipitate

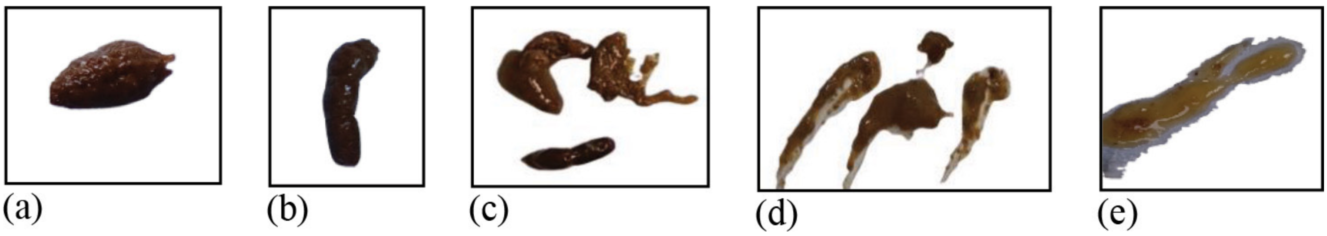

Figure 1 Stool consistency scoring system: (a) Score 1: hard stool, (b) Score 2: semi-hard, watery stool, (c) Score 3: soft stool, (d) Score 4: semi-soft, watery stool, (e) Score 5: watery stool

quantitatively using Minitab 18 software. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc test with a 95% confidence interval and a significance level of α=0.05.

Results

Phytochemical screening

The phytochemical screening results are presented in **Table 2**. Four compounds tested were positive: alkaloids (Mayer, Wagner, Dragendorff), flavonoids, saponins, and tannins. Positive results for alkaloids were indicated by the formation of a white precipitate (Mayer's reagent), a brown precipitate (Wagner's reagent), and an orange solution (Dragendorff's reagent). Flavonoids produced a yellow solution, while saponins formed stable foam lasting more than 30 seconds, and tannins formed a greenish-black precipitate. Alkaloids were considered present when at least two reagents yielded positive results (Meigaria *et al.*, 2016). Conversely, the tests for steroids and terpenoids were negative, indicated by the absence of a green color in the tested solution. These findings confirmed the presence of the aforementioned compounds in *Nigella sativa* infusion, which were subsequently tested for their antidiarrheal potentials.

Antidiarrheal efficacy via intestinal protection method

The efficacy of *Nigella sativa* infusion as an antidiarrheal was evaluated by its ability to reduce defecation frequency, improve stool consistency, delay the onset of diarrhea, and shorten the duration of diarrhea. The average defecation frequency results are presented in **Table 3**. Castor oil induction increased defecation frequency in the negative control group (1% Tween 80) to 7.83 defecations, while the positive control group (loperamide HCl) showed a significant reduction in defecation frequency to 4.17 ($p<0.05$). Treatment with *Nigella sativa* infusion at concentrations of 25%, 50%, and 100% also resulted in a significant reduction ($p<0.05$) in defecation frequency.

The stool consistency results, shown in **Figure 2**, indicated that diarrhea could be identified by a stool consistency score >3. The negative control group had an average stool consistency score of 3.18, confirming the diarrheal effect of castor oil. Among the treatment groups, the 50% *Nigella sativa* infusion had the best result, with an average stool consistency score of 1.81, although this was not significantly different from the 100% and 25% concentrations. These results suggest that the 50% concentration provided the most optimal improvement in stool consistency.

The average times for diarrhea onset, duration, and recovery for each treatment group are presented in **Figure 3**. The negative control group (1% Tween 80) exhibited the fastest onset of diarrhea (70 minutes) and the shortest recovery time (128 minutes). The duration of diarrhea in the negative control group was the longest, at 342 minutes. In contrast, the positive control group (loperamide) had an average diarrhea onset time of 115 minutes, a duration of 110 minutes, and a recovery time of 128 minutes. The 50% *Nigella sativa* infusion exhibited the best antidiarrheal effect, with diarrhea onset at 170 minutes, a duration of 90 minutes, and a recovery time of 285 minutes. The 25% and 100% concentrations of *Nigella sativa* also delayed diarrhea onset, shortened the duration, and extended recovery time compared to the negative control. Statistical analysis of these parameters confirmed that all tested concentrations of *Nigella sativa* infusion exhibited antidiarrheal activity.

Discussion

Plants can be used in various herbal treatments by utilizing the secondary metabolites they contain. Medicinal plants used in treatments can include seeds, leaves, stems, fruits, and roots (Yulianto, 2017). The part

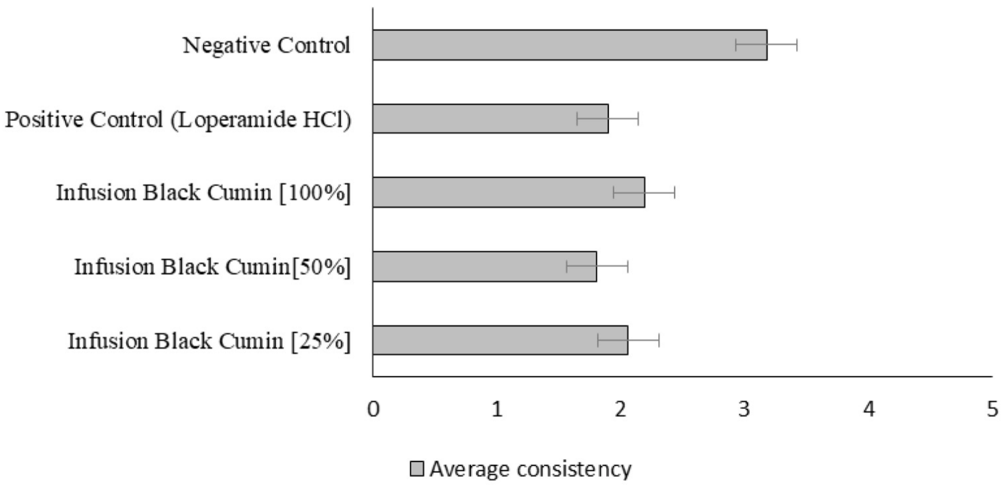

Figure 2 Average stool consistency scores of mice treated with *Nigella sativa* infusion

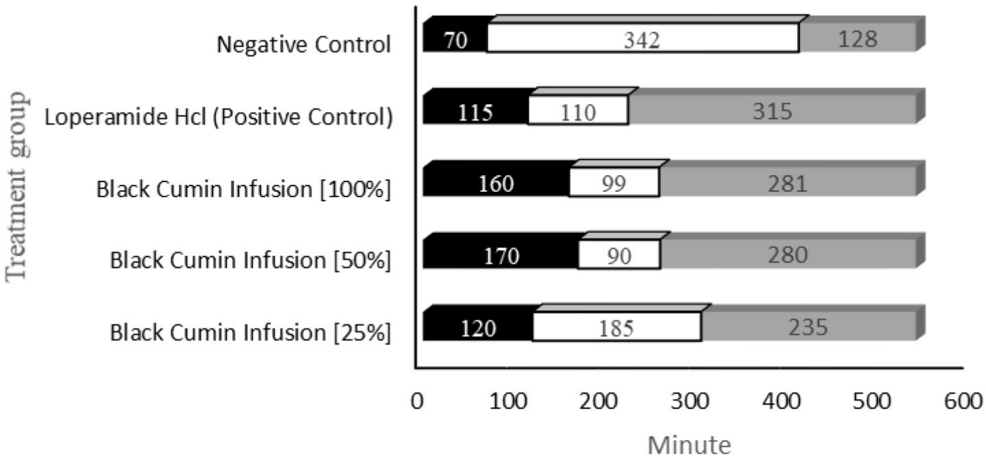

Figure 3 Time of diarrhea onset, duration, and recovery in mice treated with *Nigella sativa* infusion at 25%, 50%, 100%, Tween 80 (negative control), and loperamide (positive control). The time of diarrhea onset () is indicated by a stool consistency score of 1–2 and a defecation frequency of 1–2 times. The duration of diarrhea () is characterized by a stool consistency score >3 and defecation frequency >3. The recovery time () is indicated by a stool consistency score of 1–2 and a defecation frequency of 1–2 times.

of the black cumin plant used in this study was its seeds. Secondary metabolites consist of three main groups: terpenes, phenolics, and nitrogen compounds. Terpenes include volatiles, cardiac glycosides, carotenoids, and sterols. Phenolic compounds include phenolic acids, coumarins, stilbenes, flavonoids, tannins, and lignin. The nitrogen-containing group includes alkaloids and glucosinolates (Sila *et al.*, 2022). In general, plants with antidiarrheal potential contain five compounds: alkaloids, saponins, flavonoids, steroids, and tannins (Anas *et al.*, 2012).

The extraction method used in this study was infusion. Infusion involves extracting the plant material with water at 90°C for 15 minutes (Khafidhoh *et al.*, 2015). Water is used as the solvent because it is polar, and compounds with similar polarity dissolve easily. Water was chosen because it is easily accessible and can dissolve secondary metabolite compounds. Infusion can be an effective method for isolating active components like saponins, tannins, alkaloids, and flavonoids, as these compounds dissolve in water (Khafidhoh *et al.*, 2015). This method is economically advantageous as it uses water and is easy to apply by the general public (Fatimatuzzahra & Lestari, 2022).

The intestinal protection method used to test the antidiarrheal potential of black cumin infusion showed that all the test groups had antidiarrheal activity. The secondary metabolites in the black cumin infusion delayed the onset of diarrhea after castor oil induction, shortened the duration of diarrhea, extended the recovery period (**Figure 3**), reduced the frequency of defecation (**Table 3**), and improved stool consistency (**Figure 2**).

This study demonstrates that black cumin possesses antidiarrheal activity, containing secondary metabolites like tannins, flavonoids, alkaloids, saponins, and steroids, which have been previously reported to exhibit antidiarrheal effects (Suwendar *et al.*, 2021). The antidiarrheal effect of black cumin infusion can be attributed to the positive test results of these secondary metabolites, specifically tannins, alkaloids, saponins, and flavonoids.

Tannins act as astringents by denaturing proteins on the intestinal mucosa. As an astringent, tannins shrink the pores and mucous membranes of the intestine, reducing water absorption and peristaltic movement. The reduction in intestinal peristalsis is also due to the spasmolytic effect of tannins as chelating agents (Birdi *et al*, 2010; Fauzi *et al.*, 2020). According to Yu *et al.* (2020), tannins work on the intestinal mucosa by promoting cell regeneration, reducing the severity of intestinal wounds through tissue reconstruction, increasing villus height, deepening intestinal crypts, and restoring morphological integrity. This crypt deepening is beneficial in the post-diarrheal period because the crypts function as secretory tissues.

Sunani and Hendriani (2023) found that hydrolysable tannins exhibit antidiarrheal activity. Hydrolyzed tannins bind to protein tannate, pass through the intestines, and reduce secretion from the small intestine, thereby improving stool consistency. Tannins are also responsible for antibacterial action. The spasmolytic effect of these compounds shrinks bacterial cell walls (Mufti *et al.*, 2017).

The secondary metabolite flavonoid in black cumin works by inhibiting intestinal motility and secretion. The flavonoid content antagonizes castor oil's mechanism of action by preventing excessive fluid secretion. Ricinoleic acid in castor oil acts on receptors in the small intestine, increasing smooth muscle contraction and excessive fluid secretion, whereas flavonoids reduce smooth muscle contraction and inhibit fluid secretion in the intestine. Flavonoids also lower the extracellular calcium concentration in intestinal smooth muscle, leading to relaxation and reduced intestinal motility (Ningsih *et al.*, 2023).

Black cumin infusion contains alkaloid compounds that exhibit antidiarrheal effects by inhibiting prostaglandins, reducing intestinal motility and fluid secretion (Ilmi *et al.*, 2023). Alkaloids also serve as antibacterial agents (Anggraini *et al.*, 2019). Saponins in black cumin infusion work by dissolving castor oil to prevent diarrhea (Ilmi *et al.*, 2023). Saponins also exhibit antibacterial effects by forming complexes that damage bacterial cell membranes (Ilmi *et al.*, 2023). The interaction of these secondary metabolites in black cumin provides a synergistic antidiarrheal effect by inhibiting mucosal secretion, increasing water absorption, lysing bacterial cell walls, and reducing peristalsis and intestinal motility.

Conclusion

Black cumin infusion contains secondary metabolites such as tannins, flavonoids, saponins, and alkaloids. The black cumin infusion at concentrations of 25%, 50%, and 100% has proven antidiarrheal efficacy. All tested concentrations of black cumin infusion delayed the onset of diarrhea after castor oil induction, extended the recovery period, and shortened the duration of diarrhea. The best antidiarrheal effect was observed at a 50% concentration, comparable to loperamide.

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Conflicts of interest All authors declare no conflicts of interest regarding this study.

Author contributions AAM, LNS, DNP, and ASP were responsible for the study design and data processing. NS analyzed the research results and wrote the manuscript.

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