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In Vitro Antibacterial and In Vivo Antidiarrhoeal Activities of a 96% Ethanol Extract of *Eriobotrya japonica* Stem Bark

Friska Dewi Sari Hutauruk[1], Yesiska Kristina Hartanti[2], Meilani Roma Ito Sihaloho[2], Anastasia Setyopuspito Pramitaningastuti[3*]

[1] *Diploma in Pharmacy Program, Faculty of Health Sciences, Universitas Pelita Harapan, Tangerang, Banten 15811, Indonesia*
[2] *Pharmacy Undergraduate Program, Faculty of Health Sciences, Universitas Pelita Harapan, Tangerang, Banten 15811, Indonesia*
[3] *Pharmacist Professional Program, Faculty of Health Sciences, Universitas Pelita Harapan, Tangerang, Banten 15811, Indonesia*
[*] *Email: anastasia.pramitaningastuti@uph.edu*

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Corresponding Author:
Anastasia Setyopuspito Pramitaningastuti
Pharmacist Professional Program,
Faculty of Health Sciences,
Universitas Pelita Harapan,
Tangerang,
Banten,
Indonesia
Email:
anastasia.pramitaningastuti@uph.edu

ABSTRACT

Eriobotrya japonica (Thunb.) Lindl. (loquat) has been reported to possess various health benefits. Phytochemical studies have identified flavonoids and triterpene acids in its stem bark; however, its biological potential remains underexplored. This study aimed to evaluate the *in vitro* antibacterial and *in vivo* antidiarrhoeal activities of the 96% ethanol extract of *Eriobotrya japonica* stem bark as a candidate natural antibacterial and antidiarrhoeal agent. The extract was prepared by maceration using 96% ethanol and subjected to phytochemical screening. Acute oral toxicity was assessed in Wistar rats according to OECD Guideline 423 using three animals per step. Antibacterial activity was evaluated using the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli* at concentrations ranging from 25 to 500 mg/mL (n = 3). Antidiarrhoeal activity was assessed in male BALB/c mice at doses of 200–600 mg/kg BW (n = 4) using a castor oil-induced diarrhoea model. The extract was found to be practically non-toxic at doses up to 5,000 mg/kg BW. Concentration-dependent antibacterial activity was observed against both bacterial strains, with the highest concentration producing the largest inhibition zone against *E. coli* (12.17 ± 0.61 mm; $p < 0.05$). In the antidiarrhoeal assay, the extract at 200 mg/kg BW showed the most pronounced inhibitory effect on wet faeces and total faecal output, with an inhibition percentage of 83.14%, exceeding that of loperamide. Higher extract doses did not enhance the antidiarrhoeal effect. These findings suggest that *Eriobotrya japonica* stem bark extract has potential antibacterial and antidiarrhoeal activities with a favourable

acute safety profile, although further phytochemical standardisation and mechanistic studies are required.

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ABSTRAK

Eriobotrya japonica (Thunb.) Lindl. atau loquat dilaporkan memiliki berbagai manfaat kesehatan. Studi fitokimia menunjukkan bahwa kulit batang tanaman ini mengandung flavonoid dan asam triterpen, tetapi potensi biologisnya masih belum banyak dieksplorasi. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri secara *in vitro* dan aktivitas antidiare secara *in vivo* dari ekstrak etanol 96% kulit batang *Eriobotrya japonica* sebagai kandidat agen antibakteri dan antidiare alami. Ekstrak dibuat melalui metode maserasi menggunakan etanol 96% dan dilakukan skrining fitokimia. Uji toksisitas akut oral dilakukan pada tikus Wistar berdasarkan OECD Guideline 423 dengan tiga hewan uji pada setiap tahap pengujian. Aktivitas antibakteri dievaluasi menggunakan metode difusi cakram terhadap *Staphylococcus aureus* dan *Escherichia coli* pada konsentrasi 25–500 mg/mL (n = 3). Aktivitas antidiare dievaluasi pada mencit BALB/c jantan dengan dosis 200–600 mg/kg BB (n = 4) menggunakan model diare induksi minyak jarak. Ekstrak menunjukkan profil praktis tidak toksik hingga dosis 5.000 mg/kg BB. Aktivitas antibakteri yang bergantung pada konsentrasi diamati terhadap kedua strain bakteri, dengan konsentrasi tertinggi menghasilkan zona hambat terbesar terhadap *E. coli* (12,17 ± 0,61 mm; $p < 0,05$). Pada uji antidiare, ekstrak dosis 200 mg/kg BB menunjukkan efek inhibisi paling besar terhadap feses cair dan total keluaran feses, dengan persentase inhibisi sebesar 83,14%, melebihi loperamide. Peningkatan dosis ekstrak tidak meningkatkan efek antidiare. Temuan ini menunjukkan bahwa ekstrak kulit batang *Eriobotrya japonica* memiliki potensi aktivitas antibakteri dan antidiare dengan profil keamanan akut yang baik, meskipun standardisasi fitokimia dan studi mekanistik lebih lanjut masih diperlukan.

Kata Kunci: *Eriobotrya japonica*; Ekstrak kulit batang; Antibakteri; Antidiare; Toksisitas akut

1. Introduction

Diarrhoea is a disorder of the gastrointestinal system characterised by an increased frequency of bowel movements, a change in stool consistency to a more liquid state, and a daily stool volume exceeding 200 mL [1] . To date, diarrhoea remains a significant public health problem globally as it contributes substantially to morbidity and mortality rates, particularly among children under five years of age. The WHO reports that diarrhoeal diseases cause approximately 444,000 child deaths annually, with the highest incidence occurring in developing countries. In addition to causing direct clinical effects, diarrhoea is also associated with malnutrition and growth failure in vulnerable groups. Therefore, the development of effective and sustainable therapeutic strategies remains essential to address this health issue [2] .

The majority of diarrhoea cases are caused by infections with pathogenic microorganisms, such as bacteria, viruses, and protozoa. Among the bacteria causing diarrhoea, *Escherichia coli* is one of the most commonly found pathogens and is generally transmitted via contaminated food or drink. Some strains *of E. coli*, particularly *enterotoxigenic Escherichia coli* (ETEC), are capable of producing enterotoxins in the form

of heat-stable toxins (ST) and heat-labile toxins (LT). Both toxins can stimulate increased secretion of fluid and electrolytes in the intestinal mucosa, thereby disrupting the regulatory mechanisms of ion transport. This disruption leads to fluid accumulation in the intestinal lumen, ultimately triggering secretory diarrhoea [3]. The management of diarrhoea generally involves the use of antimotility and antisecretory drugs, whilst antibiotics are administered in specific cases caused by bacterial infection. However, the irrational or prolonged use of antibiotics can increase the risk of antimicrobial resistance and reduce the effectiveness of therapy, particularly in diarrhoea mediated by bacterial toxins [4],[5]. This situation highlights the need to explore alternative sources of therapeutic compounds with broader pharmacological activity, including antibacterial activity and the ability to regulate intestinal motility.

One plant with potential for development as a source of natural medicine is the loquat (*Eriobotrya japonica* (Thunb.) Lindl.), which belongs to the Rosaceae family. This plant originates from China and has been widely cultivated in various Asian countries, including Indonesia, where it is known as the loquat or Berastagi grape. Various parts of the loquat plant have long been utilised in traditional medicine due to their content of diverse secondary metabolites with biological activity. Medicinal plants are known to contain various bioactive compounds capable of producing pharmacological effects such as antibacterial, anti-inflammatory, and antioxidant actions. Previous research has reported that loquat fruit contains a number of important components, such as organic acids, carotenoids, vitamins, and minerals, which contribute to anti-inflammatory and hepatoprotective effects [6]. Furthermore, the leaves and seeds of this plant are also known to contain various secondary metabolites, such as triterpenoids, flavonoids, tannins, and amygdalin, which exhibit diverse pharmacological activities, including antibacterial, antioxidant, anticancer, and anti-inflammatory effects [7]–[9] .

Traditionally, loquat extract has also been used to treat various digestive disorders. Several studies report that this plant extract is capable of reducing the frequency of bowel movements and inhibiting intestinal motility in a castor oil-induced diarrhoea model [10]. However, scientific studies specifically evaluating the pharmacological potential of the loquat stem bark remain limited. Several reports suggest that the bark tissue possesses a different phytochemical composition compared to other parts of the plant, thereby potentially yielding distinctive biological activities. To the best of our knowledge, no previous study has simultaneously investigated the antibacterial, antidiarrhoeal, and acute toxicity profiles of *Eriobotrya japonica* stem bark extract in a single integrated study. This combinatorial approach represents the novelty of the present work, as it addresses a critical gap by providing a comprehensive pharmacological and safety assessment of this underexplored plant part.

In addition to efficacy, safety is also a crucial factor in the development of natural medicinal compounds. Therefore, toxicity testing is required to ensure the safety of plant extract use at specific doses. Acute toxicity testing is one of the common initial approaches used to evaluate the toxic potential of a substance. The OECD 423 method (*Acute Oral Toxicity – Acute Toxic Class Method*) is widely used to determine the safety level of a compound based on the biological response of test animals to the administration of specific doses.

Against this background, this study aims to evaluate the *in vitro* antibacterial activity and the *in vivo* antidiarrhoeal effects of a 96% ethanol extract of *Eriobotrya japonica* stem bark. Additionally, acute toxicity testing was conducted using the OECD 423 method to assess the safety of the extract in test animals. Antibacterial activity was tested using the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*,

whilst antidiarrhoeal activity was evaluated in a castor oil-induced diarrhoea model in mice. This study is expected to provide an integrated scientific basis for the potential of loquat stem bark extract development as a natural therapeutic agent for diarrhoea.

2. Methods

Materials

Loquat stem bark (*Eriobotrya japonica* (Thunb.) Lindl.) (Kabanjahe, Indonesia), 96% ethanol (Pasifik Kimia, Jakarta, Indonesia), distilled water (Pasifik Kimia, Jakarta, Indonesia), 0.9% sodium chloride (Otsuka Indonesia, Jakarta, Indonesia), Mueller-Hinton agar (Himedia, Maharashtra, India), gentamicin sulphate injection (Sanbe Farma, Bandung, Indonesia), sterile paper discs (6 mm), test bacterial strains *Escherichia coli* and *Staphylococcus aureus*, loperamide HCl (Kimia Farma, Jakarta, Indonesia), male mice (BALB/c) (Bogor, Indonesia), and male rats (Wistar) (Bogor, Indonesia).

Plant Identification

The loquat bark was identified as *Eriobotrya japonica* (Thunb.) Lindl. (No. 2867/MEDA/2024) at the Plant Systematics Laboratory, Herbarium Medanense (MEDA), University of North Sumatra, Indonesia.

Extraction

Eriobotrya japonica bark extract was obtained through several stages, including the selection of fresh material, wet sorting, washing, drying, dry sorting, and grinding into a crude powder. The extraction process was carried out using the maceration method with 96% ethanol as the solvent at a ratio of 1:5 (w/v) for 4 × 24 hours at room temperature. The resulting macerate is then filtered and evaporated using a rotary evaporator at 40°C at a speed of 45 rpm until a concentrated extract is obtained. The concentrated extract is subsequently dried in an oven at 40°C until a dry extract is obtained.

Phytochemical Screening

Qualitative identification of secondary metabolites was carried out via phytochemical screening using standard colour reaction techniques. Alkaloid testing was carried out using Mayer's and Dragendorff's reagents. Flavonoids were identified using magnesium powder and concentrated hydrochloric acid. Terpenoids and steroids were tested using the Liebermann-Burchard reaction. Polyphenolic compounds and tannins were identified using iron(III) chloride solution, whilst saponins were tested using the foam formation method [11] .

Antibacterial Activity Test

The antibacterial activity of *Eriobotrya japonica* stem bark extract was tested using the disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*. The extract was dissolved in sterile distilled water to obtain concentration variations of 25, 50, 100, 250, 500 mg/mL. Gentamicin (10 µg/20 µL) was used as the positive control, whilst sterile distilled water was used as the negative control. Bacterial suspensions were prepared in 0.9% NaCl solution and their turbidity was adjusted to McFarland standard 0.5. These suspensions were then evenly inoculated onto the surface of *Mueller-Hinton Agar* medium. Sterile 6 mm diameter discs, each containing 20 µL of the respective test solutions, were placed on the surface of the medium and then incubated at 37°C for 18–24 hours. The inhibition zone was measured as the clear halo surrounding the disc, excluding the 6 mm disc diameter, using a digital caliper. All tests were performed in triplicate.

Antidiarrhoeal Activity Test

Antidiarrhoeal activity was evaluated following approval from the Animal Research Ethics Committee of the Integrated Research and Testing Laboratory, Preclinical Research Ethics Commission, UGM (No. 00011/VI/UN1/LPPT/EC/2024). The test animals used were male BALB/c mice weighing 20–30 g and aged approximately 8 weeks. The mice were acclimatised for five days under standard laboratory conditions, fed a standard diet of 10 g/mouse/day and provided with water *ad libitum*. Prior to treatment, the animals were fasted for 18 hours whilst still having access to water.

The mice were then randomly divided into five groups. The negative control group was administered a 0.5% CMC-Na suspension orally, whilst the positive control group was administered loperamide at a dose of 0.0146 mg/20 g body weight, one hour prior to diarrhoea induction. The treatment groups were administered *Eriobotrya japonica* extract at doses of 200, 400, and 600 mg/Kg BW. Diarrhoea was induced by oral administration of castor oil (0.5 mL/mouse). Thirty minutes after induction, each group received the treatment according to allocation.

Each mouse was placed in an individual cage for observation of diarrhoea parameters, including the time of onset of watery stools, defecation frequency, and total stool weight over a 3-hour period. Following the observation period, the animals were returned to their housing cages and monitored for up to three days to observe potential mortality.

The percentage inhibition of diarrhoea was calculated using the formula:

$$\%\, inhibition = [(WNC - WT) / WNC] \times 100$$

where WNC represents the liquid faecal weight in the negative control group, and WT represents the liquid faecal weight in each treatment group, including loperamide and EBB at doses of 200, 400, and 600 mg/kg BW.

Acute Toxicity Test Based on OECD Guideline 423

This acute toxicity study was conducted under separate ethical approval granted by The Research Ethics Committee, Faculty of Health Sciences, Universitas Pelita Harapan (Approval No. 0027/PE.KEPK-FIKes-UPH/XII/2024). The acute oral toxicity test was conducted using the Acute Toxicity Class method. The test animals were male Wistar strain rats (*Rattus norvegicus*) weighing at least 120 g and classified as BCS 3. The rats were acclimatised for 5–7 days prior to testing, fed a standard rat diet and given water ad libitum. During the testing phase, the animals were fasted for 14–18 hours whilst still having access to drinking water. The first dose was administered orally at a concentration of 2000 mg/Kg BW of EBB in a 0.5% CMC-Na solvent to 3 test animals. Following administration of the single dose, observations for signs of toxicity were carried out over a 14-day period. After 3–4 hours following sample administration, food was reintroduced. If 2–3 test animals died, the sample was classified as GHS Category 4. Conversely, if 0–1 death occurred, the 2000 mg/Kg BW dose was repeated on 3 test animals. In this repeat test, if 2–3 deaths occur, the sample is classified as GHS Category 4. If death occurs in only 1 test animal or there are no deaths, the sample is classified as GHS Category 5. Limit tests are conducted up to a dose of 5000 mg/Kg BW.

Data Analysis

Data were analysed using SPSS software. For the antibacterial assay (n = 3 per group), inhibition zone diameters were expressed as mean ± standard deviation (SD)

and analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Post hoc comparisons were expressed using superscript letters (a–f), where values with different superscript letters within the same column indicate statistically significant differences between groups at $p < 0.05$. For the antidiarrhoeal assay ($n = 4$ per group), data on onset of diarrhoea, number of stools, liquid faecal weight, and percentage inhibition were presented as mean ± SD and evaluated descriptively, as the primary outcome measure was the percentage inhibition of diarrhoea relative to the negative control. No inferential statistical test was applied to the antidiarrhoeal data. For the acute toxicity test conducted according to OECD Guideline 423, results were reported descriptively based on mortality, body weight changes, and clinical signs of toxicity observed during the 14-day observation period. No formal statistical analysis was performed in accordance with the OECD 423 guideline.

3. Results and Discussion

Extract and Phytochemical Screening

As shown in **Table 1**, the yield of *Eriobotrya japonica* stem bark extract was 10.14% with a moisture content of 8.6%. This yield indicates good extraction efficiency and suggests that 96% ethanol is effective in dissolving semi-polar to polar secondary metabolites [12] . The use of a solvent with appropriate polarity plays a crucial role in enhancing the diffusion and solubility of bioactive compounds from the plant matrix. Furthermore, a moisture content within the 5–10% range indicates that the extract meets the required stability parameters. Controlled moisture content is essential for maintaining extract quality as it minimises the risk of microbial growth and degradation of active compounds during storage [13] .

Table 1. Yield and moisture content of the extract

Raw Material	Raw material weight (g)	Concentrated extract weight (g)	Yield (%)	Moisture content (%)
Fine powder of dried loquat bark	600	60.83	10.14	8.6

The phytochemical profile presented in **Table 2** shows that *Eriobotrya japonica* extract contains phenolic compounds, tannins, saponins, flavonoids, alkaloids, quinones, and triterpenoids/steroids. The intensity of the colour formed indicates a predominance of phenolic compounds, saponins, and tannins. Phenolic compounds and tannins are known to play a role in enhancing the reabsorption of water and electrolytes in the intestine and reducing intestinal fluid secretion, thereby supporting an antidiarrhoeal effect via a mucosal protection mechanism [14],[15]. Meanwhile, saponins possess an antibacterial mechanism through disruption of bacterial cell membrane permeability, which can inhibit the growth of pathogenic microorganisms [16]. The presence of flavonoids and alkaloids supports the pharmacological potential of the extract, given that both compounds play a role in modulating intestinal motility through their influence on smooth muscle contraction, as well as contributing to the control of the inflammatory response [17]. However, quantitative phytochemical standardisation, such as Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), was not performed in the present study. Therefore, the correlation between phytochemical concentration and biological activity could not be quantitatively established.

Table 2. Phytochemical Screening of Ethanol Extracts of Loquat Bark.

Metabolite Compounds	Reagent	Result
Alkaloids	Dragendorff, Mayer	(+)
Flavonoids	Magnesium, HCl	(+)
Phenol	$FeCl_3$	(+++)
Saponin	-	(++)
Tannin	$FeCl_3$ 3%	(+++)
Quinone	NaOH 1 N	(++)
Triterpenes/Steroids	Acetic anhydride, concentrated H_2SO_4	(++)

Notes: (+) = The number of '+' indicates the relative intensity of the qualitative reaction and does not represent quantitative concentration.

Antibacterial Activity

As shown in **Table 3**, the extract of *Eriobotrya japonica* stem bark exhibits antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at all tested concentrations. The increase in the diameter of the inhibition zone with increasing extract concentration indicates a dose-response relationship, suggesting that the antibacterial efficacy of the *Eriobotrya japonica* stem bark extract is influenced by the amount of active compounds present. The highest antibacterial activity was observed at a concentration (500 mg/mL), with an inhibition zone diameter of 11.09 ± 0.46 mm against *S. aureus* and 12.17 ± 0.61 mm against *E. coli*.

Table 3. Inhibition zone of *Eriobotrya japonica* stem bark extract

Sample	*Staphylococcus aureus* (mm)	*Escherichia coli* (mm)
Negative control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
EBB 25 mg/mL	2.89 ± 0.75^{b}	2.87 ± 0.75^{b}
EBB 50 mg/mL	5.09 ± 0.40^{c}	3.96 ± 0.59^{b}
EBB 100 mg/mL	8.13 ± 0.58^{d}	6.25 ± 0.25^{c}
EBB 250 mg/mL	9.61 ± 0.64^{cd}	8.79 ± 0.69^{d}
EBB 500 mg/mL	11.09 ± 0.46^{e}	12.17 ± 0.61^{e}
Positive control (gentamicin)	14.94 ± 0.86^{f}	12.70 ± 0.95^{e}

Note: Data are presented as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Different superscript letters within the same column indicate statistically significant differences between groups at $p < 0.05$. The inhibition zone was measured as the clear inhibition halo surrounding the disc, excluding the 6 mm disc diameter. The negative control showed no antibacterial activity. EBB: Eriobotrya japonica stem bark extract.

The difference in sensitivity levels between the two bacteria indicates that the response to the extract is influenced by the structural characteristics of each bacterium's cell wall. As shown in **Figure 1**, the sensitivity pattern differed between *S. aureus* and *E. coli* and varied across extract concentrations, indicating that the antibacterial response may be influenced by both bacterial cell-wall structure and concentration-dependent phytochemical diffusion. Structurally, Gram-positive bacteria have a thick peptidoglycan layer without an outer membrane, making them easily penetrated by antibacterial compounds. Conversely, Gram-negative bacteria have an outer membrane

containing lipopolysaccharides, which act as an additional barrier against the penetration of antibacterial compounds [16].

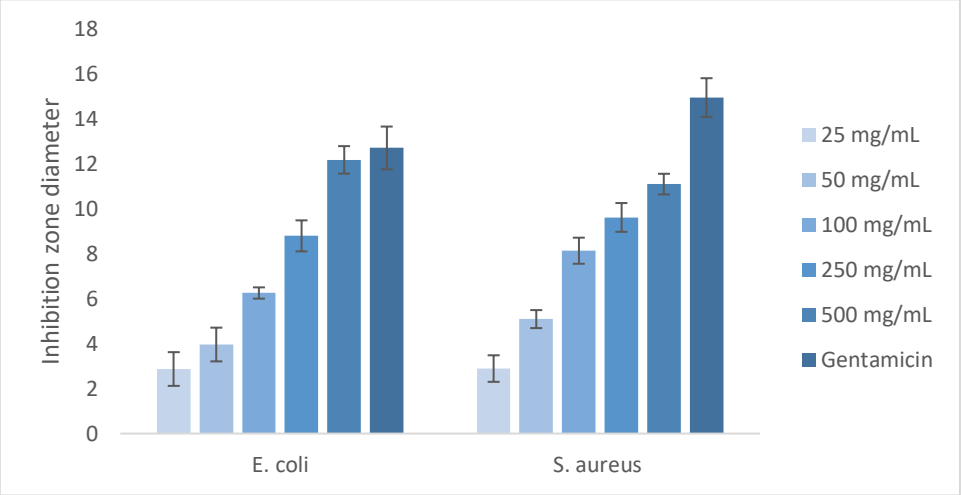

Figure 1. Effect of varying extract concentrations on the inhibition zone diameters of *E. coli* and *S. aureus*

These findings indicate that *Eriobotrya japonica* stem bark extract exhibits antibacterial activity against both Gram-positive and Gram-negative bacteria, although the response pattern differs between strains. This activity is thought to be related to the presence of secondary metabolites capable of disrupting membrane permeability and inhibiting bacterial cell activity [15],[16].

Antidiarrhoeal Activity

The antidiarrhoeal activity of *Eriobotrya japonica* stem bark extract in mice was assessed using a castor oil-induced diarrhoea model. Administration of loperamide as a positive control delayed the onset of diarrhoea compared with the negative control, demonstrating the expected pharmacological response in the test model. As shown in **Table 4**, administration of *Eriobotrya japonica* stem bark extract at concentrations of 200 and 400 mg/Kg BW resulted in a delay in the onset of diarrhoea of 72.50 ± 9.76 minutes and 87.00 ± 6.12 minutes, respectively. Conversely, the 600 mg/Kg BW dose resulted in a shorter onset of diarrhoea (44.5 ± 8.20 minutes), indicating that increasing the dose did not produce a delaying effect on diarrhoea. From the evaluation of liquid faecal weight, mice in the negative control group showed a value of 676.85 mg, whereas loperamide reduced this value to 206.00 mg, with a diarrhoea inhibition rate of 69.56%. The effect of *Eriobotrya japonica* stem bark extract at a dose of 200 mg/Kg BW provided greater inhibition than loperamide, namely 83.14%. Conversely, *Eriobotrya japonica* stem bark extract at 400 mg/kg BW exhibited lower activity than loperamide. At the highest dose, *Eriobotrya japonica* stem bark extract did not demonstrate diarrhoea-inhibiting activity, as indicated by a higher weight of liquid faeces compared to the negative control. At a dose of 600 mg/kg BW, the percentage inhibition was −52.99%, indicating increased liquid faecal output compared to the negative control group. This result suggests that the extract did not exhibit antidiarrhoeal activity at higher doses. The reduced activity observed at 600 mg/kg BW may reflect dose-dependent differences in the biological effects of the extract.

Table 4. Table evaluating the antidiarrhoeal activity of *Eriobotrya japonica* stem bark extract in *castor oil-induced* mice.

Group	Onset of diarrhoea (minutes)	Amount of liquid faeces	Total number of stools	Total faecal weight (mg)	Proportion of liquid stool weight (mg)	% inhibition
CMC-Na	50.75 ± 1.48	4.75 ± 1.64	7.50 ± 1.80	1145	676.84	-
Loperamide	102.5 ± 10.87	2.25 ± 1.09	7.00 ± 3.08	614	206.00	69.56
EBB 200 mg/Kg BW	72.5 ± 9.76	2.00 ± 1.09	11.75 ± 3.34	746	114.12	83.14
EBB 400 mg/Kg BW	87.00 ± 6.12	4.50 ± 1.12	12.00 ± 1.22	1250	455.37	32.72
EBB 600 mg/Kg BW	44.5 ± 8.20	5.25 ± 1.64	6.25 ± 1.48	1277	1035.46	-52.99

Note: Data are presented as mean ± SD where applicable (n = 4). The antidiarrhoeal assay was evaluated descriptively, and no inferential statistical analysis was applied. Percentage inhibition was calculated relative to the negative control group.

Acute Toxicity Test Based on OECD Guideline 423

An acute toxicity test of *Eriobotrya japonica* stem bark extract was conducted using the OECD Guideline 423 method. Administration of the extract at an initial dose of 2000 mg/Kg BW did not cause mortality or clinical toxicity symptoms in the test animals. Some rats showed a decrease in locomotor activity and *grooming* behaviour at the start of observation; however, these conditions were temporary and returned to normal after a few hours. Repeated doses of 2000 mg/Kg BW and a limit test at a dose of 5000 mg/Kg BW also showed no deaths during the 14-day observation period. Furthermore, the body weight of test animals in the treatment group did not show a significant decrease compared to the control group.

Based on these results, the LD_{50} value of the extract is estimated to be greater than 5000 mg/Kg BW and falls within Category 5 of *the Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), indicating a low level of acute toxicity. Compounds with an LD_{50} value of more than 5000 mg/Kg BW are generally considered to have a high safety margin in acute toxicity testing on test animals.

Limitation of the Study

Several limitations of this study should be acknowledged. First, quantitative phytochemical standardisation, specifically Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), was not performed. The absence of these measurements limits the ability to directly correlate the phytochemical composition of the extract with the observed antibacterial and antidiarrhoeal activities. Future studies should therefore include quantitative phytochemical analysis.

Second, the antidiarrhoeal assay was evaluated descriptively without inferential statistical analysis, which limits the strength of comparisons between treatment groups. Third, the antibacterial activity was evaluated only against *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion method. Further studies involving a broader range of clinically relevant microorganisms as well as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays are recommended to better characterise the antibacterial potential of the extract.

Fourth, the in vivo antidiarrhoeal study was limited to a castor oil-induced diarrhoea model in male BALB/c mice, and other pharmacological models to evaluate intestinal motility and secretion were not employed. Fifth, the study used a crude ethanol extract without fractionation or isolation of active compounds; therefore, the specific constituents responsible for the observed biological activities remain unclear.

Despite these limitations, the findings of this study provide preliminary scientific evidence supporting the potential development of *Eriobotrya japonica* stem bark extract as a natural antibacterial and antidiarrhoeal agent.

4. Conclusion

The results of this study indicate that the 96% ethanol extract of *Eriobotrya japonica* stem bark exhibits concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in the disc diffusion assay, as well as antidiarrhoeal activity in a castor oil-induced diarrhoea model in mice, with the most effective response observed at 200 mg/kg BW. Acute toxicity testing according to OECD Guideline 423 showed no mortality or marked signs of toxicity up to 5000 mg/kg BW, suggesting low acute toxicity with an estimated LD_{50} > 5000 mg/kg BW. These findings provide preliminary evidence that *Eriobotrya japonica* stem bark extract may serve as a potential natural antibacterial and antidiarrhoeal agent; however, further studies are required to determine its active compounds, clarify its pharmacological mechanisms, evaluate long-term safety, and establish quantitative phytochemical standardisation. The reduced antidiarrhoeal effect observed at 600 mg/kg BW also warrants further dose–response evaluation to clarify whether this pattern reflects reduced efficacy, dose-dependent pharmacological variation, or other extract-related effects.

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Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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