BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 1, April 2026 | Pages: 823-831 | DOI: 10.14421/biomedich.2026.151.823-831 ISSN 2540-9328 (online)

LC–MS/QTOF-Based Metabolite Profiling and Antidiarrheal Activity of Ethyl Acetate Fraction of *Jernang* Fruit

Siti Samaniyah[1]*, Ulfa Husna Dhirah[2], Refa Alaydrus[3], Aida Apriani[4]
[1]Department of Pharmacy, Faculty of Health Sciences, Ubudiyah University of Indonesia, Banda Aceh, Indonesia;
[2]Department of Midwifery, Faculty of Health Sciences, Ubudiyah University of Indonesia, Banda Aceh, Indonesia;
[3]National Population and Family Planning Agency, Aceh, Indonesia.
[4]Assyifa Aceh College of Health Sciences, Banda Aceh, Indonesia.

Corresponding author*
sitisamaniyah@uui.ac.id

Manuscript received: 21 January 2026. Revision accepted: 22 April 2026, Published: 03 June 2026.

Abstract

Diarrhea remains a major public health problem in Indonesia, particularly in rural areas with limited access to healthcare. In Aceh Province, it is a leading cause of medical visits among children under five. Reliance on traditional herbal medicine persists due to limited access to conventional drugs and concerns over the side effects of synthetic antidiarrheals. The aim of this study was to evaluate the secondary metabolite profile and antidiarrheal activity of the ethyl acetate fraction of *Daemonorops draco* (Willd.) Blume (*jernang* fruit) as a potential Indonesian herbal medicine. Dried fruits of *D. draco* were macerated using 70% ethanol, followed by liquid-liquid fractionation with n-hexane and ethyl acetate. The ethyl acetate fraction was subjected to liquid chromatography-mass spectrometry quadrupole time-of-flight (LCMS-QTOF) analysis for secondary metabolite identification. Antidiarrheal activity was evaluated in vivo using male mice induced with castor oil. Parameters assessed included diarrhea onset time, fecal frequency, fecal weight, and fecal consistency. Pharmacognostic evaluation of the simplicia was conducted in accordance with the Indonesian Herbal Pharmacopoeia standards. The simplicia met pharmacopeial standards with a high ethanol-soluble extract content. LC-MS/QTOF analysis identified flavonoids, terpenoids, lignans, alkaloids, and sphingolipids. The ethyl acetate fraction significantly delayed diarrhea onset and reduced fecal frequency and weight in a dose-dependent manner, with the highest dose (100 mg/kg body weight) showing the strongest effect, although lower than loperamide. The ethyl acetate fraction of *D. draco* fruit exhibits significant antidiarrheal activity and contains diverse bioactive secondary metabolites, supporting its potential development as a safe, locally sourced herbal antidiarrheal agent.

Keywords: Antidiarrheal agents; diarrhea; flavonoids; herbal medicine; liquid chromatography-mass spectrometry.

INTRODUCTION

Diarrhea remains a major public health problem worldwide, particularly in regions with poor sanitation and limited access to healthcare services.(Lin et al., 2016) The condition can lead to severe dehydration, electrolyte imbalance, and death if not treated appropriately.(Soleimani et al., 2016) In developing countries, diarrhea continues to be a leading cause of morbidity and mortality, especially among children under five years of age.(Guerrant et al., 1990) In Indonesia, diarrhea is consistently reported as one of the most common causes of visits to healthcare facilities, with a particularly high incidence in Aceh Province.(At Thobari et al., 2022; Simaremare et al., 2024) The predominantly rural geography and socio-economic conditions of Aceh limit access to conventional medicines and healthcare infrastructure, compelling many communities to rely on traditional medical practices.(Simaremare et al., 2024)

The global use of medicinal plants as alternative and complementary therapies has increased markedly in recent decades.(Møller et al., 2024) This trend is supported by a growing body of scientific evidence demonstrating the therapeutic potential of plant-derived compounds.(Zeppa et al., 2025) Increased public awareness of health and safety has further encouraged the use of herbal medicines, which are often perceived as more affordable and associated with fewer side effects than synthetic drugs.(Ekor, 2014) In Indonesia, the use of medicinal plants has been deeply rooted in cultural traditions for centuries and has served as a means to maintain health, prevent disease, and treat various ailments.(2021) To date, approximately 5,000 plant species in Indonesia have been identified as having medicinal potential.(Arozal et al., 2020)

Conventional antidiarrheal therapy commonly involves synthetic drugs such as loperamide.(Lee, 2015) Although effective, these agents may cause adverse effects and raise concerns related to long-term use and drug resistance.(Lääveri et al., 2016) These limitations highlight the need for alternative antidiarrheal therapies that are safe, effective, and derived from locally available

natural resources. Research into herbal medicines is therefore essential to support the development of accessible and culturally acceptable treatments, particularly for communities with limited access to modern healthcare.

Jernang fruit (*Daemonorops draco* (Willd.) Blume) is a plant traditionally used in Indonesia for the treatment of various ailments, including digestive disorders and diarrhea.(Yusnelti & Muhaimin, 2019) In Aceh Province, the fruit is widely distributed in lowland forests and has been utilized for generations in traditional medicine.(Andini et al., 2020) Previous studies have reported that jernang fruit contains secondary metabolites such as flavonoids, tannins, and phenolic compounds, which exhibit pharmacological activities including anti-inflammatory and antibacterial effects. (Samaniyah et al., 2022) However, comprehensive profiling of its secondary metabolites using advanced analytical techniques remains limited.

In particular, studies employing liquid chromatography–mass spectrometry/quadrupole time-of-flight mass spectrometry (LC-MS/QTOF) to characterize the secondary metabolite profile of the ethyl acetate fraction of jernang fruit, as well as systematic in vivo evaluation of its antidiarrheal activity, are scarce. The present study addresses this gap by identifying secondary metabolites in the ethyl acetate fraction of D. draco fruit using LC-MS/QTOF and evaluating its antidiarrheal activity in a castor oil–induced mouse model. This study aims to provide scientific evidence supporting the potential development of *jernang* fruit as a safe and effective herbal antidiarrheal agent based on local Indonesian resources.

MATERIALS AND METHODS

Study design

This experimental laboratory study investigated the secondary metabolite profile and antidiarrheal activity of the ethyl acetate fraction of *jernang* fruit (*Daemonorops draco* (Willd.) Blume).

Plant Material Collection and Identification

Fresh *jernang* fruits were collected purposively from Sabet Village, Jaya District, Aceh Jaya Regency, Aceh Province, Indonesia. Botanical identification was performed by the Biology Research Center, Indonesian Institute of Sciences (LIPI), Cibinong, Bogor, Indonesia. A voucher specimen was authenticated and archived for reference.

Preparation and Characterization of Plant Material

The fruits were washed, air-drained, and dried in a cabinet dryer at 40–50 °C until brittle. The dried material was weighed, pulverized into powder, and stored in airtight containers protected from light. Pharmacognostic evaluation included macroscopic examination (shape, color, size, and odor) and microscopic analysis of powdered simplicia using chloral hydrate as a clearing agent.

Extraction and Fractionation

A total of 20 g of the crude ethanol extract of *D. draco* fruit was dissolved in 96% ethanol until completely solubilized, followed by the addition of 60 mL of distilled water to obtain a hydroalcoholic solution. The solution was transferred into a separatory funnel, and liquid–liquid partitioning was performed by adding 100 mL of n-hexane. The mixture was vigorously shaken and allowed to stand for approximately 30 min to ensure complete phase separation. The n-hexane layer (upper phase) was collected, and the partitioning process was repeated until the n-hexane fraction gave a negative result with the Liebermann–Burchard reagent, indicating the absence of nonpolar compounds. The combined n-hexane fractions were concentrated under reduced pressure using a rotary evaporator to yield the n-hexane fraction.

The remaining hydroalcoholic residue was subsequently partitioned with 100 mL of ethyl acetate using the same procedure. After vigorous shaking and standing for approximately 30 min, the ethyl acetate layer (upper phase) was collected. Fractionation was repeated until the ethyl acetate layer showed a negative reaction with ferric chloride ($FeCl_3$) reagent. The combined ethyl acetate fractions were then concentrated under reduced pressure using a rotary evaporator to obtain the ethyl acetate fraction, which was used for subsequent LC–MS/QTOF analysis and in vivo antidiarrheal activity testing.

LC-MS/QTOF Analysis

The ethyl acetate fraction of *jernang* fruit (*D. draco* (Willd.) Blume) was analyzed using LC–MS/QTOF. The sample was prepared by dissolving the fraction in HPLC-grade methanol at a concentration of 1 mg/mL and filtering through a 0.22 µm membrane filter prior to injection. Chromatographic separation was performed on a reversed-phase C18 column (2.1 × 100 mm, 1.7 µm particle size) maintained at 40 °C. The mobile phase consisted of solvent A (water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid). Elution was carried out using a gradient program of 5% B (0–2 min), increased to 95% B (2–20 min), held at 95% B (20–25 min), and returned to 5% B (25–30 min) for column re-equilibration. The flow rate was set at 0.3 mL/min with an injection volume of 5 µL.

Mass spectrometric detection was conducted in both positive and negative electrospray ionization (ESI) modes over an m/z range of 50–1200. Instrument parameters included a capillary voltage of 3.5 kV, an ion source temperature of 120 °C, and nitrogen as the nebulizing gas at a flow rate of 10 L/min. Mass

calibration was performed using a standard formate cluster solution to ensure mass accuracy. Data acquisition and processing were performed using MassHunter software (Agilent Technologies), and secondary metabolites were tentatively identified by comparing accurate mass measurements and fragmentation patterns with established secondary metabolite databases.

Experimental Animals and Sampling

Twenty-five healthy adult male mice (*Mus musculus*; 20–30 g) were used and randomly divided into five groups (n= 5): negative control (1% CMC), positive control (lodia), and three treatment groups receiving ethyl acetate fraction at doses of 25, 50, and 100 mg/kg body weight.

Antidiarrheal Activity Assay

Antidiarrheal activity was evaluated using an oleum ricini–induced diarrhea model in male mice. Following an overnight fasting period with free access to water, mice were randomly assigned to experimental groups and administered the respective treatments orally. Diarrhea was induced by oral administration of oleum ricini at a dose of 1 mL per 20 g body weight.

Animals were observed continuously for 6 h. The onset of diarrhea was recorded as the time elapsed between oleum ricini administration and the appearance of the first unformed or watery stool. Fecal output was collected at 30-min intervals, and fecal frequency was determined by counting the number of diarrheal stools during the observation period. Fecal weight was measured gravimetrically using an analytical balance. Stool consistency was visually assessed and classified as normal (N), soft (L), or slimy/watery (B).

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's honestly significant difference (HSD) post hoc test was applied to identify pairwise differences between groups. A p-value <0.05 was considered statistically significant.

Ethical Clearance

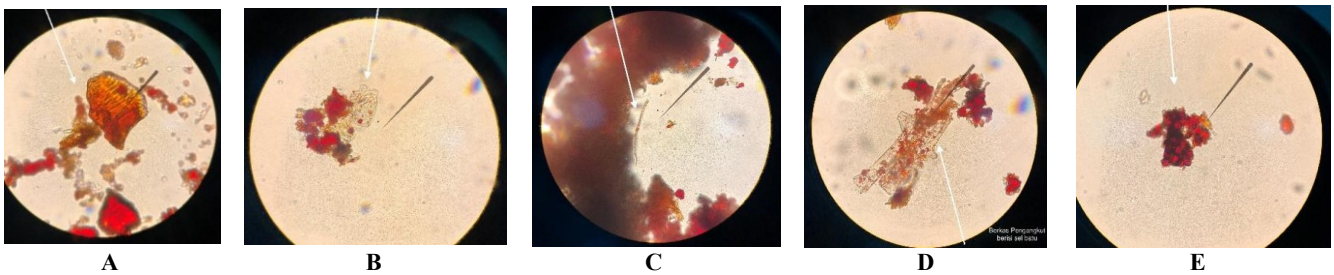
All experimental procedures involving animals were reviewed and approved by the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Indonesia (Ethical Clearance Certificate No. 436/KEPH/IX/2025).

RESULTS AND DISCUSSION

Characterization of *D. draco* and Ethyl Acetate Fraction

Macroscopic examination showed that the jernang fruit was reddish brown, hard, and scaly, with a pointed apex, a bitter taste, and an average length of approximately 1–2 cm (Figure 1). Microscopic examination of the powdered simplicia at magnifications of 40× and 100× revealed the presence of sclerenchyma tissue, epidermal cells from the flower base, unicellular hairs, and vascular bundles containing stone cells and essential oil components (Figure 2). These diagnostic features were consistent with the standards established by the Indonesian Ministry of Health, confirming the identity and quality of the simplicia for use as traditional medicinal raw material.

Figure 1. Macroscopic appearance of jernang fruit.



A **B** **C** **D** **E**

Figure 2. Microscopic appearance of the powdered simplicia at magnifications of 40× and 100×. (A) sclerenchyma tissue, (B) epidermal cells from the flower base, (C) unicellular hairs, and vascular bundles containing (D) stone cells and (E) essential oil components.

Physicochemical evaluation demonstrated that the simplicia possessed a relatively high ethanol-soluble extract content, supporting the selection of 70% ethanol as the maceration solvent and indicating effective extraction of semi-polar constituents. Examination of the ethyl acetate fraction showed a moisture content of 6.67%, which remained within acceptable limits and suggests good stability and extended shelf life. The total ash content of the ethyl acetate fraction was 5.17%, while the acid-insoluble ash content was low (0.67%), indicating minimal contamination by silicates or soil-derived impurities during processing.

Physicochemical screening results showed that triterpenoid–steroid compounds were detected in the powdered simplicia but were not observed in the ethyl acetate fraction (Table 1). In contrast, flavonoids, alkaloids, saponins, and tannins were detected in the ethyl acetate fraction of jernang fruit. These findings indicate differences in compound group presence between the powdered crude drug and the ethyl acetate fraction.

Table 1. Phytochemical Screening Results of Simplicia Powder and Ethyl Acetate Fraction.

Compound	Simple Powder	Ethyl Acetate Fraction
Alkaloid	+	+
Flavonoid	+	+
Glycosides	+	+
Saponin	+	+
Tannin	+	+
Triterpenoid Steroids	+	-

Note: + : detected; - : not detected

LC–MS/QTOF-Based Secondary Metabolite Profile of the Ethyl Acetate Fraction

LC–MS/QTOF analysis of the ethyl acetate fraction of *jernang* fruit (*Daemonorops draco* (Willd.) Blume) revealed a complex chromatographic profile with multiple peaks distributed across a retention time range of approximately 4–28 min. Several dominant peaks were observed, particularly at retention times of 8.86, 11.91, 13.37, and 14.46 min, indicating the presence of major secondary metabolite constituents in the fraction. The full chromatographic profile and detected compounds are presented in Figure 3.

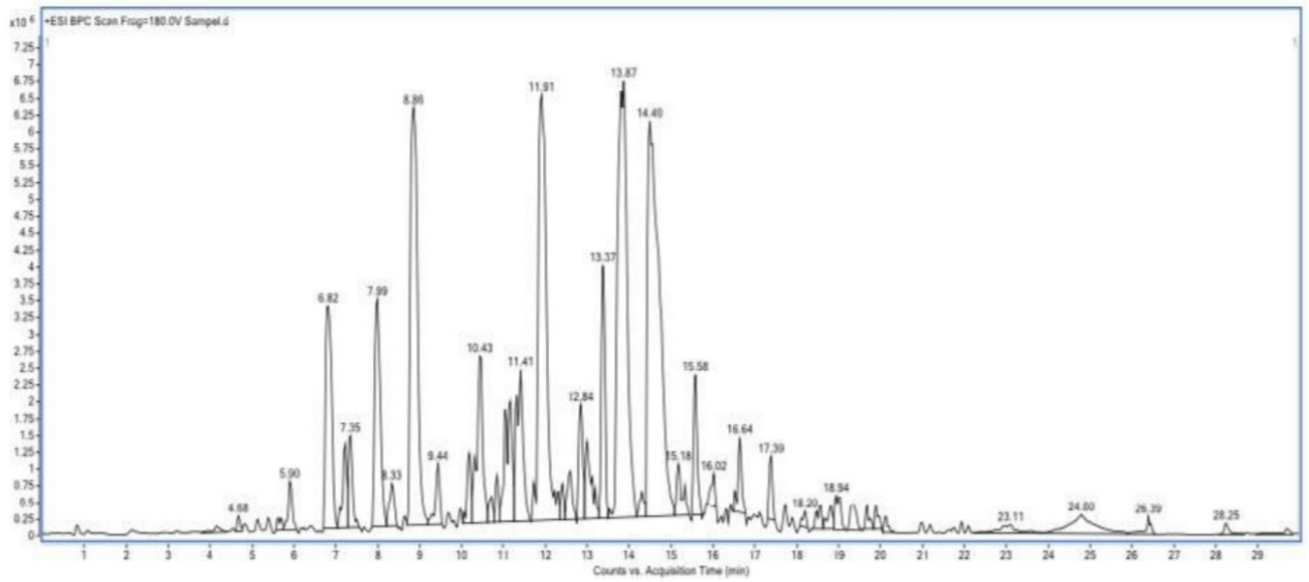


Figure 3. LC–MS/QTOF chromatogram of the ethyl acetate fraction of jernang fruit.

Accurate mass measurement and fragmentation pattern analysis enabled tentative identification of secondary metabolites belonging to multiple chemical classes (Table 2). Detected compounds included flavonoids and isoflavonoids, such as formononetin, isoliquiritigenin, ipriflavone, and 4′-O-methylisoflavone. Lignan-type compounds, including lariciresinol, were also identified. In addition, terpenoid-related compounds such as glaucarubin and gibberellin derivatives were detected in the ethyl acetate fraction.

Table 2. Secondary metabolites tentatively identified in the ethyl acetate fraction of jernang fruit by LC–MS/QTOF analysis.

Compound	Molecular Formula	Molecular Weight (m/z)	Compound Groups
Valine	C5H11NO2	117.08	Amino acids
Formononetin	C16H12O4	268.07	Flavonoids (isoflavones)
(-)-cis-Deguelin	C23H22O6	394.14	Flavonoids (rotenoids)
O-Methylodoratol	C18H20O5	316.13	Flavonoid
Isoliquiritigenin	C15H12O4	256.07	Flavonoids (chalcones)
5,6,7,4′- Tetramethoxyflavanone	C19H20O6	344.12	Flavonoid
4′-O-Methylisoflavone	C16H12O3	252.08	Flavonoid
Asticolorin B	C33H28O7	536.18	Flavonoid
Lariciresinol	C20H24O6	360.16	Lignan
Glaucoma	C25H36O10	496.23	Terpenoids (quassinoids)
Gibberellin A34-catabolite	C19H22O6	346.14	Terpenoids (gibberellins)
Gibberellin A66	C20H26O7	378.17	Terpenoids (gibberellins)
Ophiopogonin B	C39H62O12	722.43	Saponins (steroidglycosides)
3α,12α-Dihydroxy-5β- pregnan-20-one diacetate	C25H38O5	418.27	Steroid
Raucaffricine	C27H32N2O8	512.22	Alkaloids (indoles)
Catheduline E2	C38H40N2O11	700.26	Alkaloidpeptides
Valerianine	C11H15NO	177.12	Alkaloid
Creatine A	C34H40N4O4	568.31	Alkaloidpeptides
Sphinganine	C18H39NO2	301.30	Sphingolipids
Tylosin	C46H77NO17	915.53	Macrolides (antibiotics)
Melleolide D	C24H31ClO8	482.17	Polyketide
Melleolide M	C23H29ClO7	452.16	Polyketide

Alkaloid compounds identified included coniine, tamsulosin, and N-demethylnarwedine. Furthermore, sphingolipid-related metabolites were detected, represented by sphinganine. Overall, the LC–MS/QTOF analysis demonstrated that the ethyl acetate fraction of jernang fruit contains a diverse range of secondary metabolites spanning flavonoid, isoflavonoid, lignan, terpenoid, alkaloid, and sphingolipid classes.

Antidiarrheal Activity

Antidiarrheal activity was evaluated in oleum ricini–induced male mice by assessing diarrhea onset time, fecal weight, diarrhea frequency, and stool consistency. The negative control group exhibited an average diarrhea onset of 63 min following induction, whereas the positive control group showed a shorter onset time, as presented in Table 3. Administration of the ethyl acetate fraction of *jernang* fruit resulted in diarrhea onset times comparable to the negative control across all tested doses. Among the treatment groups, the longest mean onset time was observed at a dose of 100 mg/kg body weight, followed by 25 and 50 mg/kg body weight.

Table 3. Effect of the ethyl acetate fraction of jernang fruit on diarrhea onset time.

Treatment	Diarrhea starts (min)					Average (min)
	Replication					
	I	II	III	IV	V	
Na-CMC 1% (Negative Control)	70	67	54	65	59	63
Lodia (Positive control)	57	56	44	53	55	53
Ethyl Acetate Fraction Dose of Jernang Fruit 25 mg/kgBW	69	53	57	65	58	60.4
Ethyl Acetate Fraction Dose of Jernang Fruit 50% mg/kgBW	62	56	58	52	60	57.6
Ethyl Acetate Fraction Dose of Jernang Fruit 100% mg/kgBW	74	70	40	73	56	61.4

The effect of the ethyl acetate fraction on fecal weight is summarized in Table 4. The negative control group exhibited the highest cumulative fecal weight during the 6-h observation period. In contrast, the positive control group showed a markedly lower cumulative fecal weight. Administration of the ethyl acetate fraction resulted in a reduction in fecal weight compared with the negative control across all tested doses.

A dose-dependent trend was observed, with progressively lower mean fecal weights recorded at increasing doses of the ethyl acetate fraction. The group treated with 25 mg/kg body weight showed a reduction in cumulative fecal weight compared with the negative control, while further decreases were observed at doses of 50 and 100 mg/kg body weight. The lowest mean fecal weight among treatment groups was recorded at 100 mg/kg body weight and approached that of the positive control group.

Table 4. Cumulative fecal weight (g) over 6 h in oleum ricini–induced mice.

Replication	Treatment	Total Fecal Weight over 6 h (g)	Average (g)
1	Na-CMC 1% (Negative Control)	1.17	1.274
2		1.15	
3		1.29	
4		1.26	
5		1.5	
1	Lodia (Positive control)	0.562	0.628
2		0.74	
3		0.64	
4		0.65	
5		0.55	
1	Ethyl Acetate Fraction Dose of Jernang Fruit 25 mg/kgBW	0.64	0.842
2		0.97	
3		0.95	
4		0.81	
5		0.84	
1	Ethyl Acetate Fraction Dose of Jernang Fruit 50% mg/kgBW	0.77	0.788
2		0.86	
3		0.92	
4		0.77	
5		0.62	
1	Ethyl Acetate Fraction Dose of Jernang Fruit 100% mg/kgBW	0.56	0.692
2		0.54	
3		0.91	
4		0.72	
5		0.73	

The frequency of diarrhea observed over the 6-h period is summarized in Table 5. The negative control group (1% Na-CMC) exhibited the highest total number of diarrhea episodes, with 48 occurrences recorded across all animals. The positive control group showed a lower total frequency, with 32 episodes.

Administration of the ethyl acetate fraction of jernang fruit reduced diarrhea frequency compared with the negative control at all tested doses. Total diarrhea frequencies of 43, 41, and 36 episodes were recorded for doses of 25, 50, and 100 mg/kg body weight, respectively. A decreasing trend in diarrhea frequency was observed with increasing dose of the ethyl acetate fraction. The lowest total frequency among treatment groups was observed at a dose of 100 mg/kg body weight, although this value remained higher than that of the positive control group.

Table 5. Diarrhea frequency during a 6-h observation period.

Treatment	Frequency of diarrhea during 6 h					Total
	Replication					
	I	II	III	IV	V	
Na-CMC 1% (Negative Control)	9	9	10	9	11	48
Lodia (Positive control)	7	7	6	6	6	32
Ethyl Acetate Fraction Dose of Jernang Fruit 25 mg/kgBW	8	9	9	8	9	43
Ethyl Acetate Fraction Dose of Jernang Fruit 50% mg/kgBW	8	9	9	8	7	41
Ethyl Acetate Fraction Dose of Jernang Fruit 100% mg/kgBW	6	6	9	7	8	36

Stool consistency results are presented in Table 6. The negative control group (1% Na-CMC) predominantly produced slimy (B) and soft (L) stools, with no normal stools observed. In contrast, the positive control group showed improved stool consistency, with stools classified as soft (L) or normal (N). Treatment with the ethyl acetate fraction resulted in dose-dependent improvement in stool consistency. At doses of 25 and 50 mg/kg body weight, stools were mainly soft (L), whereas the 100 mg/kg body weight group exhibited the presence of normal (N) stool consistency.

Table 6. Stool consistency in oleum ricini–induced mice during the observation period.

Treatment	Replication				
	I	II	III	IV	V
Na-CMC 1% (Negative Control)	B	B	B	L	L
Lodia (Positive control)	B	L	L	L	N
Ethyl Acetate Fraction Dose of Jernang Fruit 25 mg/kgBW	B	L	L	L	L
Ethyl Acetate Fraction Dose of Jernang Fruit 50% mg/kgBW	B	L	L	L	l
Ethyl Acetate Fraction Dose of Jernang Fruit 100% mg/kgBW	B	B	L	L	N

Notes: B : slimy; L : soft; N : normal

Discussion

This study was designed to evaluate the secondary metabolite profile and antidiarrheal activity of the ethyl acetate fraction of *Daemonorops draco* fruit, addressing the lack of comprehensive chemical and biological validation of this traditionally used medicinal plant. The findings demonstrate that the ethyl acetate fraction contains diverse secondary metabolites and exhibits measurable antidiarrheal activity in an oleum ricini–induced mouse model.

Quality characterization confirmed that the simplicia and ethyl acetate fraction met pharmacopeial standards, supporting the reliability of the subsequent chemical and biological analyses. The physicochemical parameters, including moisture content, total ash, and acid-insoluble ash, indicated acceptable stability and low inorganic contamination, which are critical prerequisites for phytopharmaceutical development. These results align with standard quality requirements for herbal raw materials and extracts reported in previous pharmacognostic studies. (Wang et al., 2023)

LC–MS/QTOF analysis revealed that the ethyl acetate fraction contains a wide range of secondary metabolites, including flavonoids, isoflavonoids, lignans, terpenoids, alkaloids, and sphingolipid-related compounds. The detection of these metabolite classes is consistent with earlier reports describing the phytochemical richness of D. draco and related species, although previous studies have largely relied on conventional qualitative screening methods.(Fan et al., 2025; Sari et al., 2022; Wang et al., 2020) The use of LC–MS/QTOF in this study provides a more sensitive and comprehensive metabolite profile, highlighting the advantage of instrumental analysis in identifying compounds that may not be detected by classical phytochemical tests.

The antidiarrheal evaluation showed that the ethyl acetate fraction influenced multiple diarrhea-related parameters, including diarrhea onset, fecal weight, diarrhea frequency, and stool consistency. Although the delay in diarrhea onset was modest and comparable to the negative control, reductions in fecal weight and diarrhea frequency, as well as improvements in stool consistency, were observed in a dose-dependent manner. These findings indicate that the antidiarrheal effect of the ethyl acetate fraction is more pronounced in reducing diarrhea severity rather than completely preventing diarrhea initiation. Similar patterns have been reported in other plant-based antidiarrheal studies using oleum ricini–induced models. (Ananda et al., 2024; Astuti et al., 2019)

Comparison with the positive control revealed that the ethyl acetate fraction exhibited weaker effects than loperamide; however, this outcome is expected, as loperamide is a synthetic antidiarrheal agent with a targeted pharmacological mechanism.(Pannemans & Corsetti, 2018) The observation that the highest dose (100 mg/kg body weight) consistently produced the most favorable outcomes suggests a dose–response relationship, supporting the presence of bioactive constituents contributing to antidiarrheal activity. These results are in agreement with studies reporting that flavonoid- and tannin-rich plant extracts reduce fecal output and defecation frequency in experimental diarrhea models. (Damtie & Smaoui, 2023; Muhammad et al., 2021)

Despite the promising findings, several gaps and limitations should be acknowledged. First, compound identification was based on tentative LC–MS/QTOF analysis without confirmation using authentic standards, which limits structural certainty. Second, the antidiarrheal activity was evaluated using a single experimental model and a relatively small sample size, which may reduce statistical power. Third, this study did not assess toxicity, pharmacokinetics, or long-term safety, which are essential considerations for further development.

Future studies should focus on isolating and quantifying key bioactive compounds, confirming their structures using complementary analytical techniques, and evaluating their individual and synergistic effects. Additional investigations into toxicity, formulation stability, and alternative diarrhea models, as well as clinical evaluation, are necessary to further validate the therapeutic potential of *D. draco* fruit.

CONCLUSIONS

The ethyl acetate fraction of *jernang* fruit (*D. draco*) contains diverse secondary metabolites and exhibits antidiarrheal activity in an oleum ricini–induced mouse model. LC–MS/QTOF analysis confirms the presence of bioactive compounds, while in vivo evaluation demonstrates dose-dependent effects on fecal weight, diarrhea frequency, and stool consistency. These findings support the potential of *jernang* fruit as a locally sourced

herbal antidiarrheal agent and provide a scientific basis for further pharmacological and formulation studies.

Acknowledgements: The authors would like to thank the Faculty of Health Sciences, Ubudiyah University of Indonesia, for providing research facilities and technical support.

Authors' Contributions: Siti Samaniyah designed the study, supervised the research, and contributed to manuscript preparation. Ulfa Husna Dhirah conducted the laboratory experiments and collected the data. Refa Alaydrus performed data analysis and assisted in interpretation of the results. Aida Apriani contributed to data interpretation and manuscript revision. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: The authors gratefully acknowledge BIMA–Kemdiktisaintek for financial support of this research.

REFERENCES

Ananda, R. A. D. S., Kusumawardani, N., Fauzi, R., Estiningsih, D., Emelda, E., Solikah, W. Y., Nurinda, E., Gunawan, A., Ramandani, W., & Stiawati, R. (2024). Evaluation of analgesic and antidiarrheal activity of Clitoria ternatea Linn. ethanol flower extract. *Journal of Pharmaceutical and Sciences*, 358–364. https://doi.org/10.36490/journal-jps.com.v7i3.558

Andini, R., Ismullah, F., Bakri, S., Sulaiman, M. I., & Anhar, A. (2020). Current status of Aceh jernang (Daemonorops sp.) and its traditional conservation efforts. *IOP Conference Series: Earth and Environmental Science*, *482*(1). https://doi.org/10.1088/1755-1315/482/1/012035

Arozal, W., Louisa, M., & Soetikno, V. (2020). Selected Indonesian Medicinal Plants for the Management of Metabolic Syndrome: Molecular Basis and Recent Studies. *Frontiers in Cardiovascular Medicine*, *7*. https://doi.org/10.3389/fcvm.2020.00082

Astuti, K. I., Wulandari, L. D., & Susiani, E. F. (2019). Antidiarrheal Activity of Ethanolic Extract of Vernonia amygdalina Del Leaves againts Male Mice Induced by Oleum Ricini. *Borneo Journal of Pharmacy*, *2*(1), 10–14. https://doi.org/10.33084/bjop.v2i1.707

At Thobari, J., Sutarman, Mulyadi, A. W. E., Watts, E., Carvalho, N., Debellut, F., Clark, A., Soenarto, Y., & Bines, J. E. (2022). Direct and indirect costs of acute diarrhea in children under five years of age in Indonesia: Health facilities and community survey. *The Lancet Regional Health - Western Pacific*, *19*. https://doi.org/10.1016/j.lanwpc.2021.100333

Damtie, D., & Smaoui, S. (2023). Review of Medicinal Plants Traditionally Used to Treat Diarrhea by the People in the Amhara Region of Ethiopia. *Evidence-Based Complementary and Alternative Medicine*, *2023*(1). https://doi.org/10.1155/2023/8173543

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, *4*. https://doi.org/10.3389/fphar.2013.00177

Fan, W., Li, Z., Liu, L., Wang, Y., Chen, K., Li, L., Wang, Z., & Yang, L. (2025). An integrated 3-M workflow for accelerated annotation of natural products: Flavonoids in Daemonorops draco as a case study. *Talanta*, *282*. https://doi.org/10.1016/j.talanta.2024.126921

Guerrant, R. L., Hughes, J. M., Lima, N. L., & Crane, J. (1990). Diarrhea in Developed and Developing Countries: Magnitude, Special Settings, and Etiologies. *Clinical Infectious Diseases*, *12*(Supplement_1), S41–S50. https://doi.org/10.1093/clinids/12.Supplement_1.S41

Lääveri, T., Sterne, J., Rombo, L., & Kantele, A. (2016). Systematic review of loperamide: No proof of antibiotics being superior to loperamide in treatment of mild/moderate travellers' diarrhoea. *Travel Medicine and Infectious Disease*, *14*(4), 299–312. https://doi.org/10.1016/j.tmaid.2016.06.006

Lee, K. J. (2015). Pharmacologic Agents for Chronic Diarrhea. *Intestinal Research*, *13*(4). https://doi.org/10.5217/ir.2015.13.4.306

Lin, H. Y., Lee, Y. T., Chan, Y. W., & Tse, G. (2016). Animal models for the study of primary and secondary hypertension in humans. *Biomedical Reports*, *5*(6), 653–659. https://doi.org/10.3892/br.2016.784

Møller, S. R., Ekholm, O., & Christensen, A. I. (2024). Trends in the use of complementary and alternative medicine between 1987 and 2021 in Denmark. *BMC Complementary Medicine and Therapies*, *24*(1). https://doi.org/10.1186/s12906-023-04327-8

Muhaimin, M., Tarigan, I. L., Devy, D. M., & Gusti, D. R. (2021). Antibacterial Activities of Ethanol Extract of Jernang Resin (Daemonorops draco Blume). *Pharmacology and Clinical Pharmacy Research*, *6*(3). https://doi.org/10.15416/pcpr.v6i3.31166

Muhammad, N., Ullah, S., Rauf, A., Atif, M., Patel, S., Israr, M., Akbar, S., Shehzad, O., Saeed, M., Bawazeer, S., Uddin, M. S., Derkho, M., Shariati, M. A., & Mubarak, M. S. (2021). Evaluation of the anti-diarrheal effects of the whole plant extracts of Cuscuta reflexa Roxb in pigeons. *Toxicology Reports*, *8*, 395–404. https://doi.org/10.1016/j.toxrep.2021.02.013

Pannemans, J., & Corsetti, M. (2018). Opioid receptors in the GI tract: targets for treatment of both diarrhea and constipation in functional bowel disorders? *Current Opinion in Pharmacology*, *43*, 53–58. https://doi.org/10.1016/j.coph.2018.08.008

Sari, R. K., Prayogo, Y. H., Rozan, S. A., Rafi, M., & Wientarsih, I. (2022). Antioxidant Activity, Sun Protection Activity, and Phytochemical Profile of Ethanolic Extracts of Daemonorops acehensis Resin and Its Phytosomes. *Scientia Pharmaceutica*, *90*(1). https://doi.org/10.3390/scipharm90010010

Samaniyah, S., Masfria, & Sitorus, P. (2022). Antibacterial activity of ethanol extract and fraction of *Daemonorops draco* (Willd.) Blume fruit. *International Journal of Science, Technology & Management*, 3(4), 875–879. https://doi.org/10.46729/ijstm.v3i4.544

Simaremare, S. R. S., Rachmat, B., Nugraheni, W. P., Faisal, D. R., Nirwan, M., Ipa, M., Puspita, T., Kurniasih, D. A. A., & Senewe, F. P. (2024). How Sociodemographic, Water, and Sanitation Factors Influence Diarrhea in Children Under Five: Insights from Indonesia's Underdeveloped Regions. Journal of Research in Health Sciences, 25. https://doi.org/10.34172/jrhs.2025.171

Soleimani, A., Foroozanfard, F., & Tamadon, M. R. (2016). Evaluation of water and electrolytes disorders in severe acute diarrhea patients treated by WHO protocol in eight large hospitals in Tehran; a nephrology viewpoint. Journal of Renal Injury Prevention, 6(2), 109–112. https://doi.org/10.15171/jrip.2017.21

Wang, H., Chen, Y., Wang, L., Liu, Q., Yang, S., & Wang, C. (2023). Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Frontiers in Pharmacology, 14. https://doi.org/10.3389/fphar.2023.1265178

Wang, Y.-Y., Dai, Y.-W., Cao, J., Chen, Y.-T., Zhao, C.-X., & Jiang, L. (2020). Eight new flavonoids from the fruits of Daemonorops draco. Fitoterapia, 143. https://doi.org/10.1016/j.fitote.2020.104549

Y, Y., A, A., & M, W. (2021). Phytochemical Screening and Emulgel Formulation of Jernang Resin Extract (Daemonorops Draco (Willd) Blume). Asian Journal of Pharmaceutical Research and Development, 9(1), 33–37. https://doi.org/10.22270/ajprd.v9i1.918

Yusnelti, & Muhaimin. (2019). Utilization of Jernang Resin (Daemonorops draco) as the Basic Material for Making Liquid Wound Medicine. Journal of Physics: Conference Series, 1338(1). https://doi.org/10.1088/1742-6596/1338/1/012011

Zeppa, L., Aguzzi, C., & Morelli, M. B. (2025). Exploring the Therapeutic Potential of Natural Compounds and Plant Extracts in Human Health. Biomolecules, 15(6). https://doi.org/10.3390/biom15060774

THIS PAGE INTENTIONALLY LEFT BLANK