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GC-MS Profiles, Anti-inflammatory and Antibacterial Activities of *Mucuna pruriens* Seed Against Gastrointestinal Pathogens

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Abstract. *Escherichia coli* and *Bacillus cereus* are pathogenic bacteria commonly implicated in gastrointestinal infection (GI). Severe GI can lead to some gastrointestinal diseases, such as diarrhea, gastroenteritis, and chronic inflammation. While antibiotic therapy remains the standard treatment, the rise in antimicrobial resistance (AMR) cases necessitates alternative therapeutic approaches. This study aimed to investigate the antibacterial and anti-inflammatory potential of Velvet bean (*Mucuna pruriens* L.) and to identify its bioactive compounds using gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was assessed against *E. coli* and *B. cereus* using the disc diffusion method. Meanwhile, anti-inflammatory activity was measured by the red blood cell membrane stabilization method at extract concentrations of 15, 60, 250, and 1000 ppm. Antibacterial effectiveness was determined by measuring the diameter of inhibition zones, while anti-inflammatory potential was quantified through stability percentages. The results showed that the velvet bean's ethanolic extract had antibacterial activity against both bacteria, with the strongest inhibition observed at 100% extract concentration, producing inhibition zones of 9.2 mm on *E. coli* and 7.15 mm on *B. cereus*. For the anti-inflammatory assay, the 1000 ppm extract demonstrated the highest stability value (76.36%), nearly equivalent to the standard non-steroidal anti-inflammatory drug (NSAID), ibuprofen (76.35% at 120 ppm), as a positive control. GC-MS analysis revealed the presence of 18 bioactive compounds in the velvet bean extract, classified into nine chemical groups, which are mostly known for their antimicrobial and anti-inflammatory potential. These findings highlight the potential of *M. pruriens* to address gastrointestinal infections, particularly those caused by *E. coli* and *B. cereus*, as well as its anti-inflammatory effect. This also contributes to achieving sustainable development goals (SDGs), especially to encourage good health in the community through utilizing local resources for herbal-based treatment.

Keywords: *B. cereus*; bioactive compounds; *E. coli*; *Mucuna pruriens*; red blood cell membrane

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INTRODUCTION

Gastrointestinal infection (GI) is a global health problem that affects millions of people each year, which causes morbidity and mortality worldwide, particularly in developing countries (Troeger et al., 2018). These infections can be caused by pathogens such as viruses and fungi, but are primarily caused by bacteria, including *Escherichia coli* and *Bacillus cereus. These bacteria* are commonly associated with severe gastrointestinal diseases such as diarrhea, food poisoning, and gastroenteritis (Burd & Hinrichs, 2016; Enosi Tuipulotu et al., 2021; Messelhäußer & Ehling-Schulz, 2018). In the gastrointestinal tract, these pathogens disrupt normal digestive functions and can lead to acute or chronic health complications. Numerous antibiotics have been developed and used for bacterial infection treatment, including chloramphenicol, penicillin, cephalosporins, new β-lactam, aminoglycosides, macrolides, lincosamides, and quinolones (Yan et al. 2021; Ishak et al. 2025). However, inappropriate or excessive use of these drugs has significantly contributed to the emergence of antimicrobial resistance (AMR), making certain bacterial strains less responsive to existing treatments. This condition undermines the effectiveness of some existing antibiotics and poses a serious threat to global health, potentially leading to more severe infections, prolonged illness, and in some cases, even death (Zaman et

al., 2017).

Bacterial infection often induces inflammation, a natural biological response of the immune system that serves as an early protection when the body is exposed to harmful stimuli, including infections of pathogens, injury, toxins, or irradiation (Ayertey et al., 2021; Karrat et al., 2022) serving as a crucial defense mechanism for maintaining health. While inflammation plays a vital role in protecting the body, uncontrolled inflammation can lead to necrosis or further tissue damage, and also contribute to the development of chronic inflammation or systemic inflammatory diseases and even cancer (Ayertey et al., 2021; Chen et al., 2018). Consequently, controlling inflammation is essential for infectious disease treatment. Although synthetic anti-inflammatory drugs such as NSAIDs (Nonsteroidal anti-inflammatory drugs) and corticosteroids are widely used, their prolonged or improper use often results in adverse side effects (Kustiawan et al., 2023; Rousdy et al., 2022). Therefore, it is necessary to explore safer alternative therapies that combine both antibacterial and anti-inflammatory to address the infection as well as to prevent chronic inflammation, which offer effective treatment that is safe and less risks, such as plant-based medication.

Velvet bean (*Mucuna pruriens* L.) is a legume commonly found in tropical and subtropical regions, including Indonesia. This bean has high nutrient content, especially protein and carbohydrates (Boniface et al., 2024), but its utilization is still limited. This species has been reported to exhibit a broad range of pharmacological properties, including antibacterial, anti-inflammatory, antianxiety, antioxidant, anti-aging, and anticancer activities, which are attributed to its bioactive compounds (Fadilaturahmah et al., 2023; Jitpimai et al., 2023; Theansungnoen et al., 2022). Furthermore, metabolites such as alkaloids, terpenoids, steroids, glycosides, various phenolic compounds including flavonoids (notably isoflavones), tannins, aromatic amino acids such as L-Dopa and several fatty acids (ascorbic acid, gallic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid) have reported antibacterial effects (Jitpimai et al., 2023; More et al., 2022; Yu et al., 2016). Studies have demonstrated the antibacterial potential of *M. pruriens* extracts. For instance, an alcoholic seed extract of *M. pruriens* from India inhibited the growth of *Vibrio harveyi*, *V. cholerae*, *E. coli*, and *Staphylococcus aureus* through disc diffusion and MIC assays (Govindan et al., 2015). Similarly, ethanolic extracts from related *Mucuna* sp. have shown effectiveness against common skin pathogens (*S. aureus*, *S. epidermidis,* and *C. albicans*), while *M. interrupta* ethanolic extract showed antimicrobial effect against *C. albicans* according to the MIC and MBC assay (Theansungnoen et al., 2022). However, despite these promising findings, there is currently a lack of research on the *M. pruriens seeds* cultivated in Indonesia, particularly regarding their potential against gastrointestinal pathogens. In addition, no studies have yet evaluated their comparative effectiveness against both Gram-positive and Gram-negative gastrointestinal bacteria, as well as their anti-inflammatory potential, which has not yet been investigated.

Based on these concerns, the present study was conducted to evaluate the antibacterial activity of ethanolic extracts from Indonesian *M. pruriens* seeds against *Escherichia coli* and *Bacillus cereus*, which are representative Gram-negative and Gram-positive bacteria commonly associated with gastrointestinal infections (GI) and foodborne illnesses. Additionally, the study aimed to assess the extract's anti-inflammatory potential. This research represents one of the earliest GC-MS-guided bioprospecting studies on *M. pruriens* in Indonesia, focused on gastrointestinal pathogens and inflammation.

METHODS

Sample Preparation and Extraction of *M. pruriens* Seed

Velvet bean (*M. pruriens* var. utilis) was obtained from the Dinas Pertanian Kulon Progo Regency, Yogyakarta. Plant identification was carried out at the Faculty of Biology, Gadjah Mada University, to verify the samples. The extract was obtained using the maceration method, with 70% ethanol as solvent. The 3 months post-harvest, grayish-brown beans were randomly selected, sorted, and cleaned from contaminants, then soaked in hot water (1 kg in 2 L) for 24 hours with water replacement every 6 hours. The seeds were peeled, dried, and ground into simplicia powder. Subsequently, 200 g of powder was dissolved in 2 L of 70% ethanol, sealed, and left for 24 hours with occasional stirring. The solution was filtered, and the residue was re-macerated with fresh solvent in the same way as before. The combined filtrates were concentrated using a rotary evaporator at 50°C and 120 rpm to obtain a thick or concentrated extract (CE) (100% conc.).

Yield Extract Calculation and Bioactive Compound Analysis Using GC-MS

The CE of Velvet bean was then analyzed using gas chromatography-mass spectrometry (GC-MS) to identify its bioactive compounds and to confirm the absence of the solvent used (ethanol). This analysis was conducted at the Central Laboratory, Universitas Padjadjaran, Bandung, Indonesia. One µl aliquots of the organic phase were injected into a GC–MS system (Agilent Technologies 7890A GC coupled with 5977B MSD) equipped with a DB-5 capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.02 mL/min. The injection was performed in split mode with a split ratio of 100:1. The injector temperature was maintained at 220 °C, and the transfer line temperature was set to 240 °C. The oven temperature program was set as follows: initial temperature at 50 °C, held for 2 minutes, ramped at 5 °C/min to 280 °C, held for 2 minutes. The total run time was 50 minutes. Mass spectra were obtained using electron ionization (EI) at 70 eV, with a scan range of m/z 15–800 in 895,1 V (Electron Multiplier Voltage/EMV). The temperatures were maintained at 230 °C and 150 °C, respectively. A solvent delay of 3 minutes was applied prior to data acquisition. The extract, which shows no ethanol, was then quantified using the formula (Nofita et al., 2022):

$$\% \text{ Yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of koro benguk bean powder (g)}} \times 100\%$$

While the relative quantity of each compound was calculated by formula as follows (More et al., 2022):

$$\text{The relative quantity of compounds} = \frac{\text{chromatographic peak area of each compound}}{\text{Total peak area of all chromatographic peaks}} \times 100\%$$

Preparation of Bacterial Growth Medium

Nutrient Agar (NA) was used for bacteria subcultures and Mueller-Hinton Agar (MHA) for antibacterial tests. The media were prepared and sterilized according to standard procedures by dissolving the appropriate amount of NA or MHA powder in distilled water, homogenizing the mixture, and sterilizing it in an autoclave at 121ºC for 30 minutes.

Bacterial Culture and Preparation of Bacterial Suspension

The bacterial isolates (*E. coli* and *B. cereus*) were obtained from the Pharmacy Laboratory of Bandung Institute of Technology. These isolates were then cultured on nutrient agar (NA) medium at 37°C for 24 hours before antibacterial activity testing. The bacterial suspension was prepared by diluting the isolates in 0.9% NaCl solution. A loopful of each isolate was aseptically transferred into different tubes, each containing approximately 10 mL of 0.9% NaCl solution, and then vortexed until homogenous. The suspension absorbance was measured at 600 nm using a UV-VIS spectrophotometer, aiming for a range of 0.08 to 0.1, corresponding to a 0.5 McFarland standard or approximately $1{,}5\times10^{8}$CFU/mL (Eni et al., 2017).

Antibacterial Activity Test

Antibacterial activity of Velvet bean extract against *E. coli* (Gram-negative) and *B. cereus* (Gram-positive) was tested using the disc diffusion method (Kirby-Bauer). Each bacterial suspension was spread over MHA plates using a sterile swab. The paper discs soaked in the respective treatments namely negative control (aquadest), positive control (5 µg/50 µL of Chloramphenicol antibiotics), velvet bean extracts at concentrations 25% (P25), 50% (P50), 75% (P75), and 100% (P100) then each were placed on the inoculated plates then incubated at 37°C for 24 hours and each test was conducted in three replicates. Antibacterial activity was evaluated by measuring the inhibition zones formed around each disc using a caliper, as shown in Figure 1, and calculated with the formula:

$$\text{Inhibition zone} = \frac{(AB - ab) + (CD - cd) + (EF - ef) + (GH - gh)}{4}$$

Note:

AB=Diameter of horizontal zone, ab=Diameter of paper disc (horizontal), CD=Diameter of vertical zone, cd=Diameter of paper disc (vertical), EF=Diameter of diagonal zone 1, ef=Diameter of paper disc (diagonal 1), GH=Diameter of diagonal zone 2, gh=Diameter of paper disc (diagonal 2)

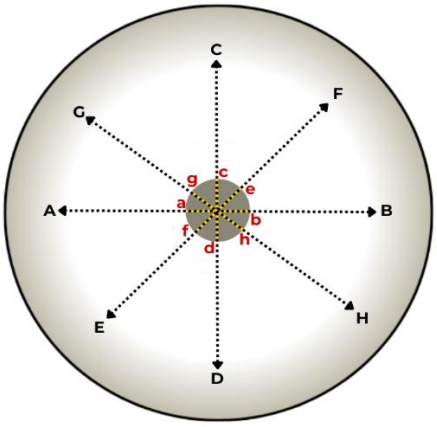

Figure 1. Inhibition zone calculation

The effectiveness of bacteria inhibition was classified into very strong (inhibition zone of ≥20 mm), strong (11-20 mm), moderate (5-10 mm), and weak inhibition (≤5 mm).

Anti-inflammatory Test using Red Blood Cell Membrane Stabilization

Anti-inflammatory activity was assessed using the red blood cell (RBC) membrane stabilization method. Due to the structural similarity between RBC and lysosomal membranes (Fujiati et al., 2022). Blood samples were obtained from white male Wistar rats (ethical approval No: 985/UN6.KEP/EC/2024). Wistar rats were chosen as experimental models due to their genetic uniformity, ease of handling, and extensive use in pharmacological studies. Male rats specifically chosen for their more stable physiological condition compared to females (Karrat et al., 2022; Yesmin et al., 2020). A total of 10 ml of blood was mixed with 3 ml of EDTA, centrifuged at 3000 rpm for 10 minutes at a temperature of 27°C, and washed repeatedly with isosaline until clear. The RBC were resuspended in isosaline (10% v/v) and stored at 4°C (Oyedapo et al., 2010). Anti-inflammatory tests were performed using velvet bean extract at concentrations of 15, 60, 250, and 1000 ppm, alongside ibuprofen (15, 30, 60, and 120 ppm) as a positive control and isosaline as a negative control, with each conducted in triplicate. 1 mL of each sample solution was mixed with 1 mL of phosphate buffer (pH 7.4, 0.15 M), 0.5 mL of RBC suspension, and 2 mL of hyposaline. The mixtures were then incubated at 37°C for 30 minutes and centrifuged at 5000 rpm for 10 minutes. The absorbance of the supernatant was measured at 577,5 nm using a UV/Vis spectrophotometer. The percentage of RBC membrane stabilization was then calculated using the following formula:

$$\text{Percentage of RBC membrane Stabilization (\%)} = \frac{100 - (\text{A2})}{(\text{A1})} \times 100\%$$

Note:
A1= Absorbance of control; A2 = Absorbance of test solution

Data Analysis

GC-MS data were analyzed qualitatively, while the antibacterial and anti-inflammatory activity data were analyzed quantitatively using SPSS version 25. The inhibition zone data were analyzed using a non-parametric test (Kruskal-Wallis), and post hoc analysis was performed using the Mann-Whitney test. Meanwhile, the data on red blood cell membrane stabilization percentage, used to assess anti-inflammatory activity, were analyzed using one-way ANOVA, as the data were normally distributed and homogeneous. To identify significant differences among treatments, post hoc analysis was conducted using LSD and Duncan's tests.

RESULTS AND DISCUSSION

Bioactive Compounds of Ethanolic *M. Pruriens* Seed Extract using GC-MS

The identification results confirmed that the seeds were *M. pruriens* var. utilis from the Fabaceae family (certificate number: 00567/S.Tb//II/2024), and the extraction with 70% ethanol of these seeds, yielded 10% (20.1g) of concentrated extract (CE). A high yield reflects the efficiency of the extraction method used and the high content of bioactive components in the samples (Benmeziane et al., 2023). GC-MS analysis was performed to identify bioactive

contents, specifically volatile compounds present in the extract and to confirm that the extract was free from any ethanol as an extraction solvent, since it is known to possess antibacterial properties; the presence of ethanol in the extract could potentially affect the results. Therefore, it is crucial to ensure that the extracts used in both antibacterial and anti-inflammatory tests contain no ethanol. GC-MS results showed that no ethanol was present in the extract, as indicated by "n.d." (non-detection) in Figure 2A-C, confirming that the evaporation process effectively removed ethanol.

On the other hand, the GC-MS result also shows that the Ethanolic extract of velvet bean contains various compounds, which are presented by peaks on the chromatogram in Figure 2D. It has been known that velvet bean contains a wide range of bioactive compounds that have antibacterial, antioxidant, anti-inflammatory, anticancer, and neuroprotective properties. These compounds include tannins, alkaloids, triterpenoids, steroids, polyphenols, flavonoids, saponins, glycosides, carotenoids, serotonin, L-Dopa, and several fatty acids such as ascorbic acid, gallic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid (Jitpimai et al., 2023; More et al., 2022; Yu et al., 2016). GC-MS analysis in this study identified 18 volatile-bioactive compounds that were categorized into nine chemical groups, including esters, ethers, alcohols, aliphatic amines, aromatic rings, weak acids, fatty acids, silanes, and siloxanes (Table 2). It is higher than the number of bioactive compounds identified in the ethanolic extract of velvet bean by More et al., (2022), which reported only eight compounds. Variations in velvet bean phytochemical profiles may result from ecological and geographical differences. More et al., (2022) used samples originating from India that have different ecological and geographical conditions compared with Kulon Progo, Yogyakarta, Indonesia, as a sample location of this study. Geographical location may lead to genotypic diversity among plant populations, contributing to differences in phytochemical profiles. In addition, variations in climate, soil type, altitude, and exposure to biotic and abiotic stressors can significantly impact secondary metabolite production (Boniface et al., 2024).

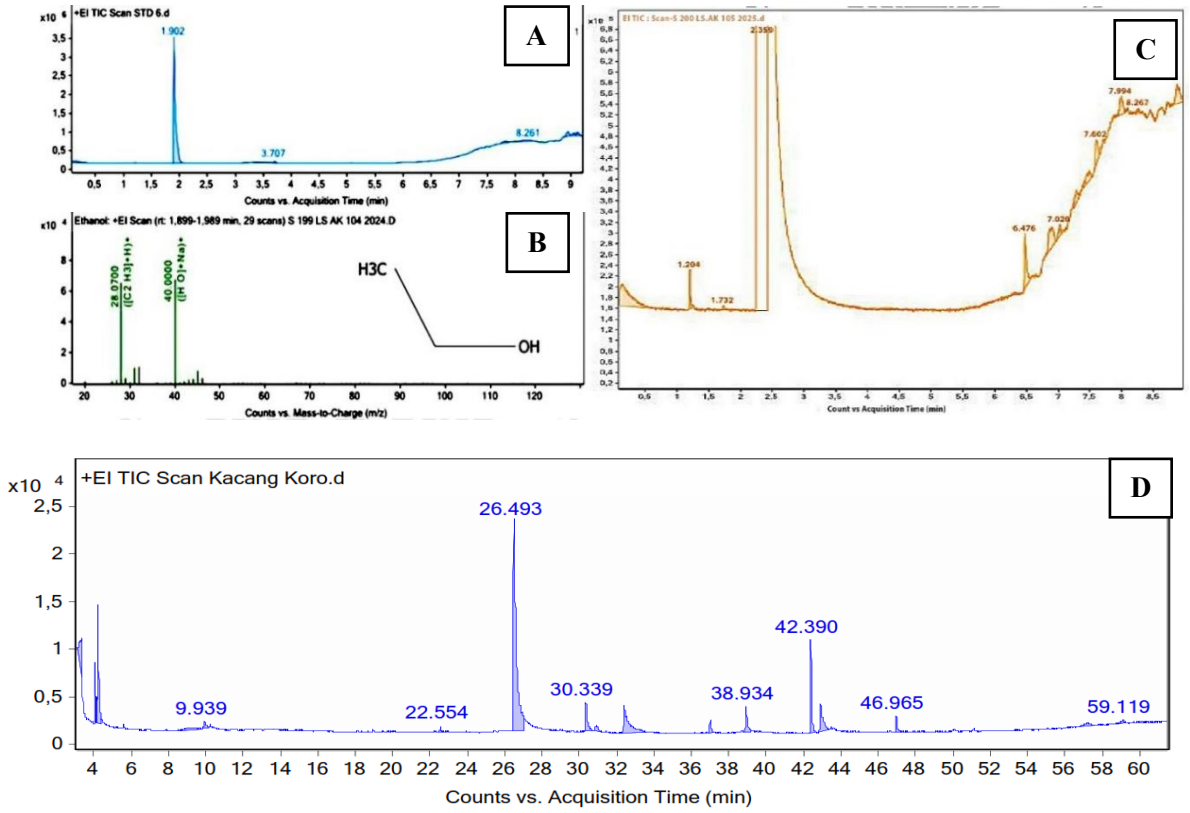

Figure 2. (A) Chromatogram of etanol standart, (B) Chromatogram of etanol/alkohol conc. from *Mucuna pruriens* seed extract and (C) MS Spectrum of *Mucuna pruriens* seed extract, (D) Chromatogram of *Mucuna pruriens* seed Extract

Table 2. Compounds identified in of *Mucuna pruriens* seed's extract by GC-MS

No	Compounds Name	RT(min)	Area%	Chemical groups	Biological activity
1	*1-dodecanamine, N, N-dimethyl-*	26.462	40.19*	Aliphatic amine	Antioxidant, antibacterial, antiseptic (Al-Tameme et al., 2015)
2	*2,3-butanediol*	4.164	13.34	Secondary Alcohol	Antimicrobial, anti-inflammatory, anti-nematode (Yi et al., 2016)
3	*N,1-Dimethylhexylamine*	32.339	9.89	Aliphatic amine	-
4	*N.methyl-N-benzylglycine ethyl ester*	42.349	8.16	Amino ester	-
5	*13-Tetradice-11-yn-1-ol*	42.841	5.63	Alcohol	Antibacterial, antifungal and antioxidant (Jahan et al., 2020)
6	*boric acid*	3.159	5.39	Weak acid	Antimicrobial (antifungal, antibacterial, antiviral), anti-inflammatory (Abdel-Fatah et al., 2025; Ceviz İnce, 2022)
7	*Silane,[3,(2,3-epoxypropoxy)propyl] ethoxydimetyhl-]*	30.288	4.30	Organosilane	-
8	*n-Hexadecanoic acid*	38.882	2.85	Fatty acid	Antioxidant, antimicrobial, anti-inflammatory (Ganesan et al., 2024)
9	*2-Methyl-beta-alanine, N-benzyl-N-methyl, Methyl ester*	46.913	1.53	Amino ester	-
10	*1,1,1,3,5,5,5-Heptamethyltrisiloxane*	56.821	1.53	Organosiloxane	-
11	*1,2-propanediol diformate*	4.113	1.33	Formate diester	-
12	*Benzemethanol, alpha.-[1-(ethylmethylamino)ethyl]-*	36.954	1.33	Alcohol and amine	-
13	*2-propanol,1-(1-methylethoxy)-*	8.728	1.13	Hydroxyalkyl ether	-
14	*Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl-*	30.831	1.00	Allyl ether silane	-
15	*benzyl chlorides*	9.846	0.96	Aromatic rings	-
16	*1,4-Bis(trimethylsilyl)benzene*	58.811	0.60	Organosilane/Aryl silane	-
17	*amphetamine, N-pentafluoropropionyl*	22.503	0.44	Amine derivatives	-
18	*Arsenous acid, tris(trimethylsilyl) ester*	59.929	0.41	Arsenic ester	-

Note: RT= Retention Time, * The largest percentage area

The compounds of velvet bean extract in this study have varying peak percentages that represent their abundance in the sample. The higher the peak percentage of a compound in a GC-MS chromatogram, the higher its abundance or presence in the sample (Suharti et al., 2023). Based on a GC-MS analysis, three dominant compounds were identified in velvet bean, which showed the highest peak percentages include *1-Dodecanamine*, *N, N-dimethyl-* (40,19%), *2,3-Butanediol* (13.34%), and *N,1-Dimethylhexylamine* (9.89%).

Based on Table 2, the majority of the identified compounds possess antibacterial and anti-inflammatory activities. This observation aligns with the results of the antibacterial and anti-inflammatory activity assays, which revealed significant effects in this study (Table 3 and Figure 5). Moreover, it shows that *1-Dodecanamine, N, N-dimethyl-* and *N,1-Dimethylhexylamine* (Figures 3A and 3C) are the highest compounds found in the velvet bean ethanolic extract and belong to the aliphatic amine groups. This group can interact with the phospholipids of bacterial cell membranes, causing damage to the structure and leakage of cellular contents. Some aliphatic amines can also bind to bacterial DNA and RNA, disrupting replication and transcription processes (Fayeulle et al., 2021; Long et al., 2023). Furthermore, *2,3-Butanediol* (Figure 3b) belongs to the glycol and secondary alcohol group (PubChem) that has anti-inflammatory and antimicrobial functions, including antifungal, antibacterial, and anti-nematode properties. According to a previous report, it is often applied to prevent plant diseases (Li et al., 2023). This compound is known to be produced by various plants, including *Arabidopsis thaliana*, *Solanum incanum*, and *Pinus armandii* leachates, as well as by the yeast *S. cerevisiae* and several bacterial species from the genus Bacillus (Ravichandran & Yetayih, 2022; Yi et al., 2016).

Several other compounds, including acids (boric acid), fatty acids (*n-Hexadecanoic acid*) (Figure 3E), esters (*N-methyl-N-benzylglycine ethyl ester*) (Figure 3F), and aromatic alcohols, are also known to have antibacterial or antifungal activity (Ganesan et al., 2024; Zumreoglu-Karan & Kose, 2015). *n-Hexadecanoic acid* can cause bacterial lysis. It alters the permeability of the cytoplasmic membrane by changing the structure of lipopolysaccharide (LPS) membrane asymmetrical due to its interaction with the hydroxyl group of LPS, and that leads to the leakage of nutrients from the cell and membrane damage, ultimately inhibiting bacterial growth and causing cell death (Idris et al., n.d.). It also reported that this compound can inhibit biofilm synthesis in *P. aeruginosa* (Sajayan et al., 2023). *Boric acid* (Figure 3D) is commonly used industrially as an ingredient in various pharmaceutical products, cosmetics, lotions, soaps, mouthwashes, toothpaste, astringents, and eye rinses. It is recognized for antibacterial properties, particularly in treating infections, including vaginosis and candidiasis (PubChem). Methyl ester and fatty acid demonstrate antibacterial activity by some mechanisms, such as inhibiting DNA/RNA replication, inhibiting protein synthesis, or disrupting cell wall and cytoplasmic membrane structures through synergistic mechanisms (Casillas-Vargas et al., 2021).

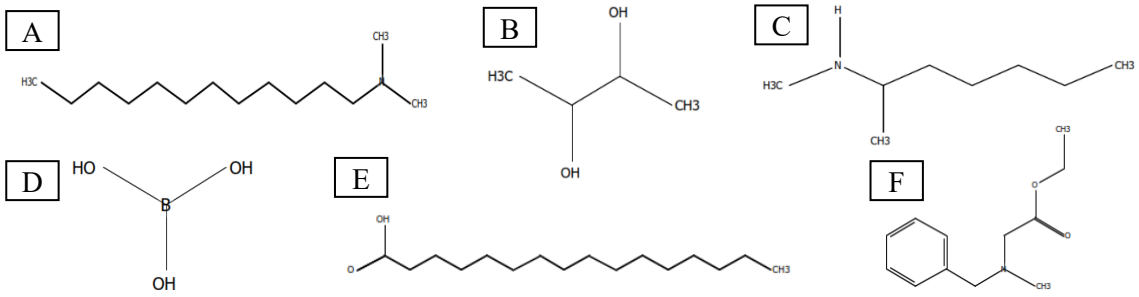

Figure 3. Some chemical structures of bioactive compounds as antibacterial or anti-inflammatory in Velvet bean (A) *1-Dodecanamine*, *N, N-dimethyl-*; (B) *2,3-Butanediol*; (C*) N,1-Dimethylhexylamine*; (D) *boric acid*; (E) *n-Hexadecanoic acid* and (F) *N-methyl-N-benzylglycine ethyl ester*

Table 3. Antibacterial activity of Velvet beans extract in *E. coli* and *B. cereus*

Tested Bacteria	Extract Conc.	Inhibition zone diameter Average	Antibacterial activities Category
E. coli	P25	6.33±2.23[b]	Moderate
	P50	7.5±3.75[b]	Moderate
	P75	8.88±1.36[b]	Moderate
	P100	9.2±0.62[b]	Moderate
	K+	17.49±1.16[a]	Strong
	K-	-	-
B. cereus	P25	2.22±2.12[c]	Weak
	P50	4.98±1.45[c]	Weak
	P75	6.38±3.24[bc]	Moderate
	P100	7.15±0.35[b]	Moderate
	K+	21.93±0.85[a]	Very strong
	K-	-	-

Note: (-) means no inhibition or clear Zone formed, K- (Aquadest), K+ (Chloramphenicol); P25 (25% of velvet bean extract); P50 (50% of velvet bean extract); P75 (75% of velvet bean extract), and P100 (100% of velvet bean extract).

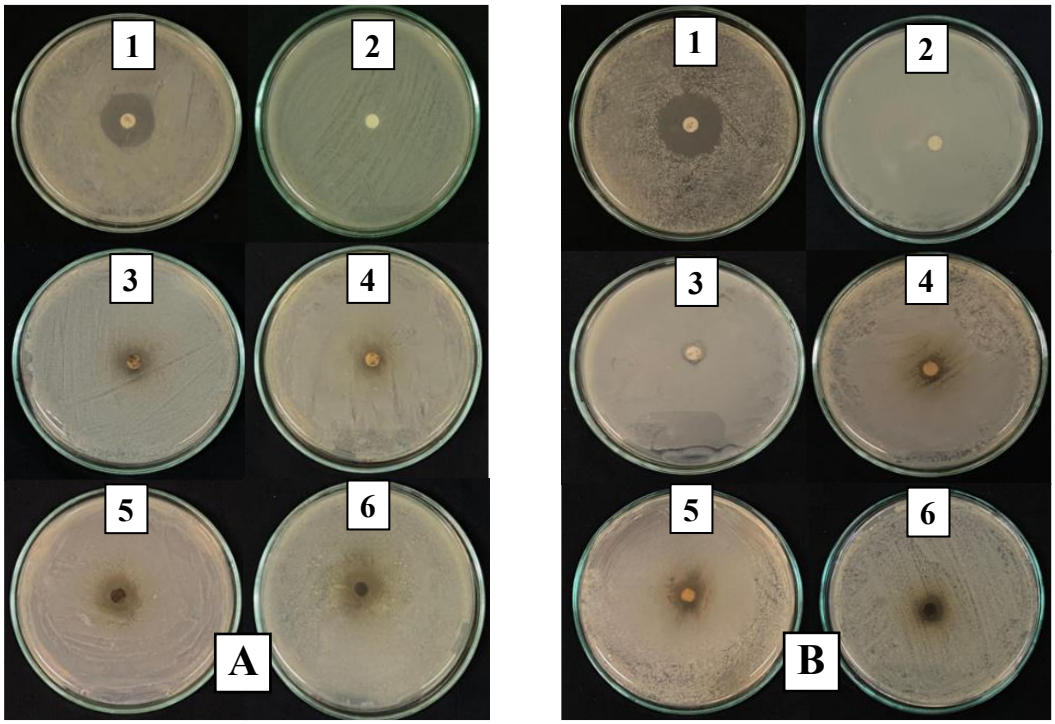

Figure 4. An inhibition zone of velvet bean extract formed in bacteria. (A) *E. coli*, (B) *B. cereus*, (1) (+) control, (2) (–) control, (3) 25% of velvet bean extract, (4) 50% of velvet bean extract, (5) 75% of velvet bean extract, (6) 100% of velvet bean extract

Antibacterial Activity of Velvet Bean (*Mucuna pruriens* L) Seed Extract

This study tested the antimicrobial activity of velvet bean seed extract against *E. coli* (Gram-negative bacteria) and *B. cereus* (Gram-positive bacteria), to determine its spectrum of activity against gastrointestinal pathogens. The extract concentrations used were 100%, 75%, 50%, and 25%, with chloramphenicol as a positive control and sterile distilled water as a negative control. The results showed the presence of inhibition zones for both bacteria, as presented in Table 3.

The results demonstrated that velvet bean extract exhibited antibacterial activity against both test bacteria. Statistical analysis using the Kruskal-Wallis test revealed a significant effect of treatment on the inhibition zone, with a p-value of 0.004 ($p < 0.05$), indicating significant differences among the treatment groups. Further post-hoc analysis using the Mann-Whitney test showed no

statistically significant differences in antibacterial activity against *E. coli* among all extract concentrations (25%, 50%, 75%, and 100%), with all treatments categorized as having moderate activity. The inhibition zones observed were 6.33 mm, 7.5 mm, 8.88 mm, and 9.2 mm, respectively. In contrast, for *B. cereus*, significant differences were observed in the antibacterial activity of the extract at different concentrations. Against *B. cereus*, the extract showed weak to moderate antibacterial activity: 25% and 50% concentrations resulted in weak inhibition zones (2.22 mm and 4.98 mm), while the 75% and 100% concentrations produced moderate inhibition zones (6.38 mm and 7.15 mm). Compared to the positive control (chloramphenicol), all extract treatments still produced smaller inhibition zones. The positive control showed strong antibacterial activity against *E. coli* (17.49 mm) and very strong inhibition against *B. cereus* (21.93 mm). Nevertheless, the inhibition zones observed around the discs in all treatment groups (Figure 4) indicate that velvet bean extract still possesses antibacterial activity.

Antibacterial activity of velvet bean extract was shown by the formation of inhibition zones (clear zones) around the discs, denoting no bacterial growth at that area, indicating bacterial sensitivity to bioactive compounds in the plant extract. The size of inhibition zone reflects the level of bacterial sensitivity, where a larger zone indicates stronger antibacterial activity, while a small or no zone indicate low sensitivity or resistance of the bacteria to the tested substance. Velvet bean contains a variety of bioactive compounds, including volatiles identified in Table 2. These compounds have the potential to inhibit the growth or kill pathogenic bacteria. Other compounds contributing to the antibacterial activity of this plant include alkaloids, terpenoids, steroids, phenolics such as flavonoids (including isoflavones), and tannins (Jitpimai et al., 2023; More et al., 2022; Yu et al., 2016). Each group of these compounds operates through distinct mechanisms for inhibiting pathogenic bacteria, including the inhibition of cell wall synthesis, disruption of cell membrane function, and interference with protein and nucleic acid synthesis (Yu et al., 2016).

In this study, the ethanol extract of velvet bean demonstrated a greater inhibitory effect against *E. coli* compared to *B. cereus*, although the difference was not statistically significant. These result suggest that velvet bean extract is more effective against Gram-negative bacteria than Gram-positive bacteria, which is contrary to several previous studies. Typically, Gram-negative bacteria are more resistant to antibacterial agents due to their more complex cell wall structure. These bacteria have an outer membrane containing lipopolysaccharides (LPS) and a thin peptidoglycan layer, which contribute to higher resistance to antibacterial compounds. While Gram-positive bacteria have thick peptidoglycan but lack an outer membrane (Choi & Lee, 2019). This research shows a different result, where the antibacterial activity of the tested extract was more effective against *E. coli* than *B. cereus*. The disparity in this results may be attributed to the types of bioactive compounds present in the velvet bean extract, which may possess distinct mechanisms of action that are more effective in inhibiting or killing Gram-negative bacteria than Gram-positive ones. The effectiveness of phytochemical compounds often varies depending on the type of compound and the bacterial strain tested (Jitpimai et al., 2023). For example, chalcones synthesized from aldehydes and ketones have been reported to show greater antibacterial activity against Gram-negative bacteria such as *E. coli* than against Gram-positive bacteria, including *S. aureus*, *S. epidermidis*, and *Bacillus* sp. (Sepvianti & Kusumaningrum, 2022).

This finding is further supported by the study conducted by Widiyastuti et al. (2022), which reported differential antibacterial activities against *E. coli* and *S. aureus* in in vitro assays of *Melaleuca alternifolia* extracts obtained through two different extraction methods (maceration and soxhlet). The macerated extract exhibited stronger antibacterial activity against *E. coli*, whereas the Soxhlet extract demonstrated greater activity against *S. aureus*. These differences are likely attributed to variations in the phytochemical constituents present in the respective extracts based on thin-layer chromatography results. Similar results were also reported by Eni et al. (2017), showing that fermented "nira" had stronger antimicrobial effects against *E. coli* than *S. aureus*. Additionally, the differences in resistance capabilities of each species or bacterial strains often contribute to the effectiveness of antibacterial compounds. Each bacterial strain may show varying degrees of resistance to specific antibacterial compounds (Kowalska-Krochmal et al., 2022; Tajer et al., 2024). In this study, *B. cereus* possesses a greater tolerance to the active compounds contained in the extract compared to *E. coli*, resulting in lower inhibition activity.

Anti-Inflammatory Activity of *Mucuna pruriens* Seed Extract

The red blood cell (erythrocyte) membrane stabilization method was used to assess anti-inflammatory activity. This method can be used to evaluate anti-inflammatory activity in vitro, as erythrocyte membranes bear structural similarities to lysosomal membranes (Karrat et al., 2022). Thus, stabilization of erythrocyte membranes can serve as indicator of lysosomal membranes stabilization that is crucial to reduce inflammatory responses by preventing the release of lysosomal contents from neutrophil activity, such as protease enzymes that contribute to inflammation in tissues and extracellular fluid.

Hypotonic solution as a hemolysis inducer, a 10% red blood cell suspension, and the compound in the form of ethanol extract from velvet bean at concentrations of 15, 60, 250, and 1000 ppm were used for this test. Membrane stabilization can be observed when red blood cells are exposed to a hypotonic solutions, causing oxidative stress and disrupting membrane stability. This oxidative stress results in lipid and protein oxidation, leading to membrane damage charaterized by hemolysis. The extent or level of hemolysis can be used as an indicator of anti-inflammatory activity, which is indicated by absorbance values, where the value is inversely proportional to anti-inflammatory effects. Lower absorbance indicates less red blood cell lysis and high anti-inflammatory activity. The precentage of membrane stability that approached or exceeded the positive control indicates significant strength, as it showed comparable or greater anti-inflammatory activity than positive control. In this study, ibuprofen with concentration of 15, 30, 60, and 120 ppm was used as positive control. Ibuprofen is a commonly used NSAID or standart drug, and several of these drugs are known to possess membrane-stabilizing properties that contribute to anti-inflammatory effects (Jahnavi et al., 2019). Meanwhile, the negative control solution was used as an indicator of 100% hemolysis, where the test compound was replaced with isotonic saline. From the observations, the percentage of stabilization of red blood cell membranes was obtained, as shown in Figure 5.

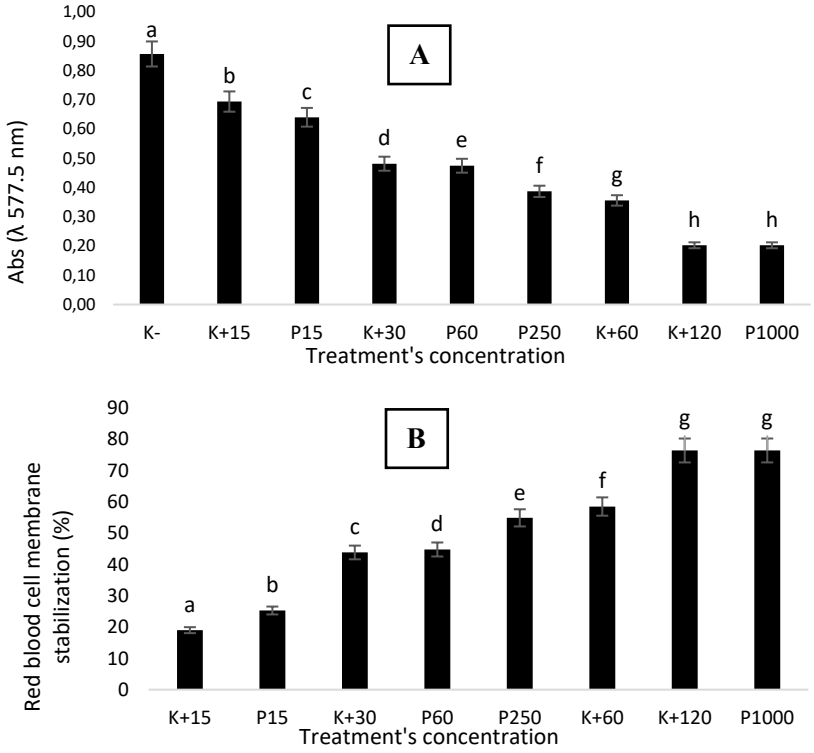

Figure 5. (A) Absorbance value of treatment and control on λ 577.5 nm, (B) Percentage of red blood cell membrane stabilization (%) for each treatment and control. K-: Negatif control (Isosalin); K+15 (ibuprofen conc. of 15 ppm); K+30 (ibuprofen conc. of 30 ppm); K+60 (ibuprofen conc. of 60 ppm); K+120 (ibuprofen conc. of 120 ppm); P15 (15 ppm velvet bean extract; P60 (60 ppm velvet bean extract); P250 (250 ppm velvet bean extract); P1000 (1000 ppm velvet bean extract). Different letter notations indicate statistically significant differences

Statistical analysis using ANOVA shows a significant difference in all concetration treatments ($P < 0.05$). Figures 5a and 5b show that the 1000 ppm velvet bean extract (P1000) has the lowest average absorbance (0.2024) and the highest membrane stability (76.36%) compared to other concentrations including the positive control. Membrane stability is measured by comparing the absorbance of the test solution with the negative control. The percentage of stabilization is a measure that indicates the ability of a sample to stabilize the erythrocyte membrane, calculated from the comparison between the absorbance of the test solution and the negative control (Oyedapo et al., 2010). Anti-inflammatory activity of the test sample can be observed from the decrease in hemoglobin absorbance in the test solution. The lower hemoglobin absorption detected, the less hemoglobin is released, indicating that the red blood cell membrane is more stable and does not lyse. The hemolysis value of red blood cell membrane is inversely proportional to the percentage of stabilization, implying that the lower the absorbance value, t the hemolysis less occur, meaning the higher the percentage of stabilization produced (Khairinisa et al., 2022). Based on the results, velvet bean extract in all concentrations had higher stability than the 15 ppm positive control (K+15). Meanwhile, when compared to the 120 ppm control (K+120), only the P1000 concentration showed higher anti-inflammatory activity. Furthermore, in the ethanolic extract of other plants such as *Piper chaba* produced inflammatory activity of 52.67% at 500 ppm concentration reported by Yesmin et al., (2020). This shows that the P1000 velvet bean extract 1000ppm demonstrated significantly higher anti-inflammatory activity (76.36%), while the P250 (250 ppm) concentration was slightly lower (54.84%). These findings indicate strong anti-inflammatory activity of velvet bean extract, with even the lowest concentration showing notable membrane stabilization.

The anti-inflammatory effect can be attributed to the activity of secondary metabolites found in the extract of velvet bean through GC-MS analysis, such as *2,3-butanediol, boric acid, and n-Hexadecanoic acid.* Additionally, other compounds such as phenolics and alkaloids in the velvet bean also play crucial roles in anti-inflammatory activity. The mechanism of action of phenolic compounds as anti-inflammatories comprises several pathways, including inhibition of cyclooxygenase (COX), histamine, lipoxygenase activity, leukocyte accumulation, and neutrophil degranulation (Amri et al., 2018; Kustiawan et al., 2023; Nijveldt et al., 2001). Phenolic compounds inhibit inflammation in two ways, namely by preventing arachidonic acid and the secretion of lysosomal as well as endothelial enzymes, thereby preventing proliferation and exudation during the inflammatory process. Inhibition of arachidonic acid released from inflammatory cells will result in reduced arachidonic substrates for the cyclooxygenase and the lipoxygenase pathway. In addition, alkaloids are bioactive compounds containing heterocyclic nitrogen, widely distributed in medicinal plants. The anti-inflammatory activities of this compound is associated to their ability to modulate various molecular signaling pathways involved in inflammatory process, such as inhibiting the production of proinflammatory mediators. Alkaloids are known to suppress histamine secretion from mast cells and reduce the secretion of pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) by monocytes and macrophages (Tang et al., 2022).

Antibacterial and anti-inflammatory activity assays of natural compounds have been widely conducted, including those derived from *Mucuna pruriens* seed extracts. The novelty of this study lies in the integration of antibacterial assays specifically targeting pathogenic bacteria that cause gastrointestinal infections, anti-inflammatory assays, and GC-MS–based phytochemical analysis of *Mucuna pruriens* seed extracts simultaneously, thereby providing more comprehensive information on the bioactive compounds potentially responsible for these activities. This comprehensive approach has rarely been reported in previous studies, which generally focused on only one activity in isolation. In addition, this study employed two bacterial models representing Gram-positive and Gram-negative groups to evaluate the antibacterial spectrum. The findings provide scientific insights into the potential of *Mucuna pruriens* seeds as a source of bioactive compounds with dual effects, both inhibiting the growth of pathogenic bacteria and alleviating inflammation, which are relevant for infection therapy.

Furthermore, the result of this study also contributes significantly to science and becomes a base knowledge for further research to develop alternative herbal medicines that are eco-friendly, cost-effective, and safer for public health, particularly in addressing the prevalent gastrointestinal infections in society. So, this study

will directly support the achievement of sustainable development goals (SDGs) point 3 (Good Health and Well-being), and indirectly to the SDGs 8 (Decent Work and Economic Growth) and SDGs 12 (Responsible Consumption and Production).

CONCLUSION

In conclusion, the velvet bean ethanolic extract exhibited weak to moderate antibacterial activity against *E. coli* and *B. cereus*, with inhibition zones of 9.2 ± 0.62 mm and 7.15 ± 0.35 mm, respectively, at a 100% concentration. In addition, the extract demonstrated anti-inflammatory potential based on membrane stabilization assays, showing the highest stability of 76.36% at a concentration of 1000 ppm. GC-MS analysis revealed the presence of various bioactive phytochemical compounds, belonging to 9 chemical groups (esters, ethers, alcohols, aliphatic amines, aromatic rings, weak acids, fatty acids, silanes, and siloxanes). Although these findings are promising for the treatment of gastrointestinal infections, this study is limited by the absence of MIC/MBC determinations, in vivo evaluations, and isolation of individual active compounds. Further studies are required to assess the toxicity, identify specific bioactive constituents, and confirm the efficacy and pharmacological potential of this extract.

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AUTHOR CONTRIBUTION STATEMENT

All authors have an equal contribution to the research and publication. MDH, YS and AVD designed the study and collected the samples. While MWB and MM conducted the research, including data collection and analysis. MDH analyzed the data and also wrote the manuscript. AZE reviews and carries out proofreading of the manuscript.

INFORMED CONSENT STATEMENT

This study involved the use of white male Wistar rats as experimental subjects. All procedures involving animals were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals. Ethical approval for this research was obtained from the Health Research Ethics Committee, Universitas Padjadjaran, with approval number: 985/UN6.KEP/EC/2024.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper. This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.

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