Jurnal Jamu Indonesia (2026) 11(1): 39–47
https://jamu-journal.ipb.ac.id/index.php/JJI/index
ISSN 2407-7178
eISSN 2407-7763

Research Article
Doi: https://doi.org/10.29244/jji.v11i1.422

LC-MS/MS Profiling and Anti-inflammatory Activity of Red Onion (*Allium cepa* L. var. Bima Brebes) Extract-Based Liniment in *Mus musculus*

Profil LC-MS/MS dan Aktivitas Antiinflamasi Linimen Berbasis Ekstrak Bawang Merah (*Allium cepa* L. var. Bima Brebes) pada *Mus musculus*

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ARTICLE INFO

Article history
Received on: 2025-07-07
Revised on: 2025-10-31
Accepted on: 2026-01-05

Keyword:
Allium cepa L.;
Anti-inflammatory;
Liniment;
Mus musculus

Kata kunci:
Allium cepa L.;
Antiinflamasi;
Liniment;
Mus musculus

ABSTRACT

Allium cepa L. (Bima Brebes variety) is a prolific source of quercetin-type flavonols and organosulfur compounds, recognized for their potent anti-inflammatory and antioxidant activities. Topical herbal liniments offer a practical approach to manage localized inflammation while minimizing systemic exposure. This study aimed to evaluate the anti-inflammatory activity of a topical liniment formulated with a 70% ethanolic extract of *A. cepa*. LC–MS/MS (UHPLC Vanquish Tandem Q) had identified ten secondary metabolites putatively linked to anti-inflammatory effects. Anti-inflammatory efficacy was evaluated in vivo using a carrageenan-induced paw oedema model in male mice. Oedema inhibition was recorded at 18% in the negative control, 47% in the positive control, and 38%, 42%, and 44% for the 2 g, 3 g, and 5 g formulations, respectively. One-way ANOVA revealed significant differences among groups ($p = 0.001$). These findings suggested that *A. cepa*-based liniment formulations may serve as promising candidates as natural topical anti-inflammatory agents.

ABSTRAK

Allium cepa L. (varietas Bima Brebes) merupakan sumber yang melimpah akan flavonol tipe kuersetin dan senyawa organosulfur dengan aktivitas antiinflamasi serta antioksidan yang poten. Penggunaan linimen herbal topikal menawarkan strategi praktis dalam manajemen inflamasi lokal guna meminimalkan paparan sistemik. Penelitian ini mengevaluasi aktivitas antiinflamasi linimen yang diformulasikan dengan ekstrak etanol 70% *A. cepa*. Karakterisasi fitokimia menggunakan LC-MS/MS (UHPLC Vanquish Tandem Q) mengidentifikasi sepuluh metabolit sekunder yang berkontribusi pada aktivitas biologis tersebut. Efek antiinflamasi diuji secara in vivo melalui model edema pada kaki mencit jantan yang dinduksi karagenan. Hasil menunjukkan inhibisi edema sebesar 18% pada kontrol negatif, 47% pada kontrol positif, serta 38%, 42%, dan 44% berturut-turut untuk formulasi 2 g, 3 g, dan 5 g. Analisis One-way ANOVA mengonfirmasi perbedaan signifikan antar kelompok ($p = 0.001$). Temuan ini menegaskan potensi sediaan linimen *A. cepa* sebagai agen terapeutik topikal alami yang menjanjikan dalam mengatasi inflamasi lokal.

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Citation: Saragih, M., Harefa, S., Rahel, K., Ruhi, R. S., Rahmaningtyas, E. L., Eliasari, L., C., & Riskianto. (2026). LC-MS/MS Profiling and Anti-inflammatory Activity of Red Onion (*Allium cepa* L. var. Bima Brebes) Extract-Based Liniment in *Mus musculus*. *Jurnal Jamu Indonesia*, *11*(1), 39–47. https://doi.org/10.29244/jji.v11i1.422

1. INTRODUCTION

Medicinal plants, commonly referred to as *biofarmaka*, are plant-derived materials containing bioactive constituents that exert therapeutic effects either individually or synergistically. Among these, red onion (*Allium cepa* L.) is widely reported to contain quercetin, flavonoids, and sulphur-containing compounds with anti-inflammatory, antioxidant, immunomodulatory, and analgesic activities (Soemarie, 2016; Sarno, 2019; Marefati et al., 2021; Jose & Vinod, 2024). Mechanistically, *A. cepa* and its constituents have demonstrated modulation of inflammatory responses through multiple pathways, including inhibition of cyclooxygenase enzymes and suppression of the JAK–STAT signaling pathway in lipopolysaccharide-stimulated macrophages (Tuuk et al., 2020; Lee et al., 2023). These findings support the potential of *A. cepa* as a natural source of anti-inflammatory agents.

Topical liniments are considered an effective dosage form for self-medication due to their ease of application and localized action. Virgin Coconut Oil (VCO) has often been used as a liniment base owing to its favorable physicochemical properties and reported anti-inflammatory, analgesic, and antipyretic effects (Intahphuak et al., 2010; Indriyani et al., 2021). Several liniment-based formulations derived from natural products have shown significant anti-inflammatory efficacy. For example, Huangbai Liniment was reported to reduce skin inflammation and proinflammatory cytokine expression in atopic dermatitis models (Zheng et al., 2021), while *Polygonum minus* liniment (Lineminus™) demonstrated potent anti-inflammatory activity in both in vitro and in vivo studies (George et al., 2014). These studies indicate highlight the effectiveness of bioactive plant-extract-based liniments in topical anti-inflammatory therapy.

Despite reports on the wound-healing potential of *A. cepa* topical preparations (Yunanda & Rinanda, 2016; Puspariki & Suharti, 2019), studies examining their anti-inflammatory effects remain limited. Notably, systematic evaluation of liniments made from Bima Brebes *A. cepa* using a standardized in vivo model is lacking. Accordingly, this study aimed to test the hypothesis that liniment containing *A. cepa* extract in a VCO base exerts significant anti-inflammatory effects. The hypothesis was tested using a carrageenan-induced paw oedema model in male mice (*Mus musculus*).

2. METHODS

2.1. Materials

The equipment used included glassware (Iwaki, Jakarta, Indonesia), analytical balance (Ohaus, Parsippany, United States), hot plate (Thermo Scientific, Waltham, United States), oven (Memmert, Schwabach, Germany), rotary evaporator (Heidolph, Schwabach, Germany), grinder (Fomac, Jakarta, Indonesia), and ultrasonic cleaner (Digital Pro, Jakarta, Indonesia), plethysmometer (Orchid, Nashik, India), LC-MS UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS (Thermo Fisher Scientific, Waltham, United States), SPSS version 26 (IBM Corporation, Armonk, United States).

Materials included Bima Brebes variety of red onions (*Allium cepa* L.) (Bogor, Indonesia), Virgin Coconut Oil (VCO) (Nutrifarm, Tangerang, Indonesia), diclofenac sodium ointment (Voltaren Emulgel) (PT Glaxo Wellcome Indonesia, Jakarta, Indonesia), carrageenan (IndoGum) (PT Gumindo Perkasa Industri, Jakarta, Indonesia), 70% ethanol (Pasifik Kimia, Jakarta, Indonesia), distilled water (Pasifik Kimia, Jakarta, Indonesia), and male mice (*Mus musculus*) (Jakarta Barat, Indonesia).

2.2. Plant Determination

The Bima Brebes variety of red onions (**Figure 1**) was taxonomically verified as *Allium cepa* L. (No. 35/HB/04/2024) at the Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, Indonesia.

Figure 1. Bima Brebes variety of red onion (*Allium cepa* L.)

2.3. Herb dan Extraction

The preparation of *A. cepa* extract involved selecting fresh bulbs, performing wet sorting, washing, removing outer skins, drying, dry sorting, and pulverizing. The resulting powdered herb was extracted with 70% ethanol for 5 × 24 hours. The extract was then concentrated using a rotary evaporator at 40°C and 80 rpm, followed by oven-drying at 40°C until a dry extract was obtained (Riskianto et al., 2025).

2.4. Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

A 5 mg sample was dissolved in 1 mL methanol and filtered using a 0.2 µm nylon membrane. The analysis was conducted using a ThermoScientific LC-MS UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS with an Accucore C18 column (100 × 2.1 mm, 1.5 µm). The flow rate was set to 0.2 mL/min with eluent A (H_2O + 0.1% formic acid) and eluent B (acetonitrile + 0.1% formic acid). The gradient program was as follows: 0–1 min (5% B), 1–25 min (5–95% B), 25–28 min (95% B), and 28–33 min (5% B). The column temperature was maintained at 30°C, with an injection volume of 2 µL. The mass range was set at 100–1500 m/z in positive ionization mode (Audah et al., 2022). Data were processed using ChemSpider (www.chemspider.com) and mzCloud (https://www.mzcloud.org/).

2.5. Formulation of Liniment 70% Ethanol Extract of *A. cepa*

The liniment was prepared by mixing the *A. cepa* extract with VCO in ratios of 3:10, 5:10, and 10:10. The mixtures were heated on a hotplate for 8 hours with continuous stirring. Subsequently, each sample underwent maceration at room temperature for 24 hours with intermittent stirring. After filtration, the liniments were ready for in vivo testing on mice (Pramitha et al., 2023). The liniment formulations were evaluated organoleptically for color, odor, form, taste, and texture.

2.6. Liniment Anti-inflammatory Effect Test on Mice (*M. musculus*)

The anti-inflammatory experiment was conducted following ethical approval from the Ethics Committee of the Faculty of Health Sciences, Universitas Pelita Harapan (Approval No. 0019/PE.KEPK-FIKes-UPH/V/2024). Twenty male mice were randomly assigned into five groups (n = 4 per group). Group I (negative control) received VCO, while Group II (positive control) was treated with diclofenac sodium ointment. Groups III, IV, and V received liniment formulations I (2 g), II (3 g), and III (5 g), respectively. Baseline paw oedema volumes (T0) were measured before carrageenan induction. Inflammation was induced via intraplantar injection of carrageenan, and oedema volumes were measured 30 minutes post-induction (T1). Treatments were topically applied, and paw volumes were subsequently measured at 30 (T2), 60 (T3), and 90 minutes (T4) post-application using a plethysmometer (Juliadi et al., 2019).

2.7. Data Analysis

The results of the anti-inflammatory test were analyzed using Statistical Package for Social Sciences (SPSS) software version 26. A one-way analysis of variance (ANOVA) was conducted with a 95% confidence level. Tukey's Honestly Significant Difference (HSD) post hoc test was then conducted at a significance level of p = 0.05 to identify statistically significant differences in oedema volume reduction between the positive control group and the groups treated with liniment formulas I, II, and III.

3. RESULTS AND DISCUSSION

3.1. Extraction

The extraction process was conducted using the maceration method with 70% ethanol as the solvent. Ethanol was selected due to its polarity, which allows it to effectively penetrate plant cell walls, promote diffusion, and facilitate the extraction of bioactive compounds (Yulianti et al., 2020). Maceration was preferred for its simplicity and non-thermal nature, thereby preventing the degradation of thermolabile secondary metabolites in the Bima Brebes variety of *A. cepa*. This method facilitated the extraction of a broader spectrum of compounds.

During herb preparation, the weight of the dried material was 286 grams, with a moisture loss of 16.57% during the drying process. During extraction, the pressure differential across cell membranes disrupted the cell structure, enabling the release of cytoplasmic secondary metabolites into the 70% ethanol solvent (Handini, 2020). The process yielded 68.1 grams of extract, resulting in an extractive yield of 23.8% (**Table 1**). A higher yield indicates a greater quantity of bioactive compounds successfully extracted.

Table 1. The results of herb extraction from *A. cepa* bulbs with 70% ethanol extract

Part of Plant	Weight of Herb (g)	Weight of Extract (g)	Extract Yield (%)
A. cepa bulbs	286	68.1	23.8

3.2. Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Analysis

The extract was analyzed for its secondary metabolite content using LC-MS/MS. A total of 66 secondary metabolites were detected in the 70% ethanol extract of *A. cepa* (**Figure 2**), with ten compounds identified as having anti-inflammatory activity based on previously published literature (**Table 2**).

Table 2. Anti-Inflammatory Compounds in 70% Ethanol Extract of Bima Brebes Red Onion (*A. cepa*) Identified by LC-MS/MS

Compound Name	Formula	Annot. DeltaMass [ppm]	Calc. MW	RT [min]	ChemSpider Results	mzCloud Best Match	Reference
DL-Arginine	C6 H14 N4 O2	-0.76	174,11154	1.012	3	82.4	(Oyovwi & Atere, 2024; Yin et al., 2024)
gamma-Glutamyl-gamma-glutamyl-S-methylcysteine	C14 H23 N3 O8 S	-1.3	393,12007	2.635	1		(Thomas et al., 2015)
L-(+)-Leucine	C6 H13 N O2	0.3	131,09467	1.645	20	97.8	(Hirai et al., 2014)
DL-Phenylalanine	C9 H11 N O2	0.02	165,07898	2.426	19	95.2	(Bai et al., 2024)
L-gamma-Glutamyl-L-valine	C10 H18 N2 O5	-1	246,12132	2.122	12		(Guha et al., 2021)
DL-Glutamine	C5 H10 N2 O3	-0.54	146,06906	1.06	9	95.4	(Kim et al., 2017; Raizel et al., 2016)
L-(-)-Asparagine	C4 H8 N2 O3	-0.29	132,05345	1.052	8	70.9	(Vimal & Kumar, 2018; Chen et al., 2016)
N-Acetylcystathionine	C9 H16 N2 O5 S	-1.51	264,07759	1.543	4		(Uraz et al., 2013; Emelda 2022)
DL-Tyrosine	C9 H11 N O3	-0.38	181,07382	1.147	22	97.4	(Andriyono, 2019)
Tricine	C6 H13 N O5	-0.39	179,0793	1.01	12		(Li et al., 2021)

LC-MS/MS combines the separation capacity of liquid chromatography with the high specificity and sensitivity of mass spectrometry. In this technique, sample components are separated based on their interactions with the stationary and mobile phases and then ionized for detection as charged ions. The method enables the identification, structural elucidation, and quantification of compounds based on their molecular weights and spectral profiles (Mangurana et al., 2019). Ten compounds were identified that have been previously reported to exert anti-inflammatory activity through various molecular mechanisms. The mechanisms of action of these compounds are detailed in **Table 3.**

3.3. Evaluation of the Liniment Formulation

Three liniment formulations containing 1 g, 2 g, and 3 g of *A. cepa* extract in 50 mL of virgin coconut oil (VCO) were prepared and subjected to organoleptic evaluation. The results are summarized in **Table 4.** Anti-inflammatory activity tests revealed oedema reduction percentages of 18% (negative control), 47% (positive control), 38% (formulation 1/F1), 42% (formulation 2/F2), and 44% (formulation 3/F3). To optimize the formulation, fresh *A. cepa* was used and extracted via maceration without heating, distillation, bleaching, or deodorization steps (Pranata et al., 2020). Subsequent formulations were prepared using 2 g, 3 g, and 5 g of fresh Bima Brebes *A. cepa* extract in 50 mL of VCO. Organoleptic evaluation indicated that Formula I was the most preferred, primarily due to its clear color.

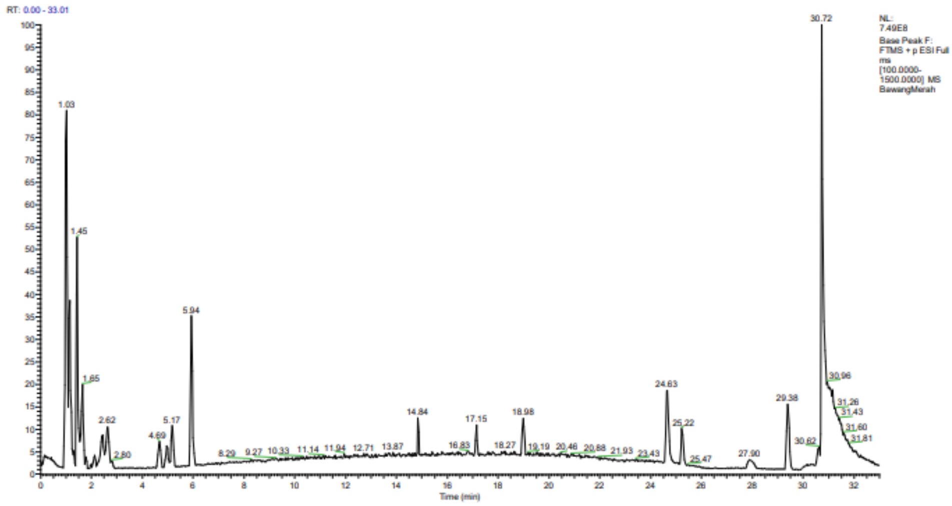

Figure 2. LC-MS/MS Analysis of 70% Ethanol Extract of *A. cepa*

Table 3. Anti-Inflammatory Mechanisms of Compounds in Bima Brebes Red Onion (*A. cepa*) Extract Based on LC-MS/MS.

Compound Name	Mechanism of Action
DL-Arginine	Involves the enhancement of nitric oxide production, modulation of immune cell activity (regulation of macrophages and increased T cell proliferation), regulation of inflammatory signalling pathways (inhibition of NF-kB transcription and activation of PPARs), and reduction of oxidative stress (Oyovwi & Atere, 2024).
gamma-Glutamyl-gamma-glutamyl-S-methylcysteine	Inhibition of NF-kB, reduction of COX-2, decrease in oxidative stress, inhibition of MAPK, activation of PPARs, enhancement of T cell proliferation, and alteration of macrophage polarity (Thomas et al., 2015).
L-(+)-Leucine	Through activation of the mTOR pathway by regulating protein synthesis and inhibiting NF-kB, reducing oxidative stress, modulating macrophages, and producing adipokines (Hirai et al., 2014).
DL-Phenylalanine	Inhibits the expression of Tph-1 and SERT, as well as suppresses the expression of MMP-2/-9, TIMP-1/-2, interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), and intercellular adhesion molecule-1 (ICAM-1) (Bai et al., 2024).
L-gamma-Glutamyl-L-valine	Inhibits the activity of cyclooxygenase (COX) or lipoxygenase (LOX), which produce inflammatory mediators such as prostaglandins and leukotrienes (Guha et al., 2021).
DL-Glutamine	Inhibits the nuclear factor-κB (NF-κB) signalling pathway (expression of pro-inflammatory genes including cytokines, chemokines, and adhesion molecules) and the signal transducers and activators of transcription (STAT) pathway (Kim et al., 2017).
L-(-)-Asparagine	Modulates T-cell mediated B-cell responses, inhibiting both humoral and cell-mediated immune responses to T-cell dependent immunogens in sheep red blood cells (SRBC) (Vimal & Kumar, 2018).
N-Acetylcystathionine	Inhibits tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), myeloperoxidase (MPO), and malondialdehyde (MDA) (Uraz et al., 2013).
DL-Tyrosine	Inhibits NFκB (Andriyono, 2019).
Tricine	It is a commonly used electrophoresis buffer and is also used for resuspending cell pellets (Li et al., 2021).

Table 4. Organoleptic Evaluation of Three Formulations of 70% Ethanol Extract of Bima Brebes Red Onion (*A. cepa*).

Formula	Weight (g)	Observation			
		Colour	Odor	Form	Result
F-I	2	Clear	Dominant VCO	No Sediment	No Sensation of Heat on the Skin
F-II	3	Slightly Cloudy	*A. cepa*	No Sediment	Slight Sensation of Heat on the Skin
F-III	5	Cloudy	Dominant *A. cepa*	No Sediment	Sensation of Heat on the Skin

3.4. Anti-Inflammatory Effect of Liniment on Mice *(Mus musculus)*

The anti-inflammatory activity was evaluated in vivo using mice as experimental subjects. Mice were chosen for their small size, rapid reproductive cycle, and genetic similarity to humans, which makes them suitable models for studying human-related diseases. They are also amenable to genetic modifications, allowing researchers to model human pathologies. Additional benefits include ease of handling, compact housing requirements, and low maintenance costs. Inflammation was induced by intraplantar injection of carrageenan, which activates phospholipase enzymes, hydrolyzing membrane phospholipids and releasing arachidonic acid. The resulting arachidonic acid triggers the release of early-phase inflammatory mediators, including histamine, serotonin, and bradykinin. Prostaglandins (PGs) further contribute by increasing vascular permeability, which promotes plasma protein extravasation and oedema formation. Carrageenan is advantageous experimentally as it causes no permanent tissue damage, leaves no residual marks, and produces a more sensitive inflammatory response than many other irritants (Zamanabadi, 2025).

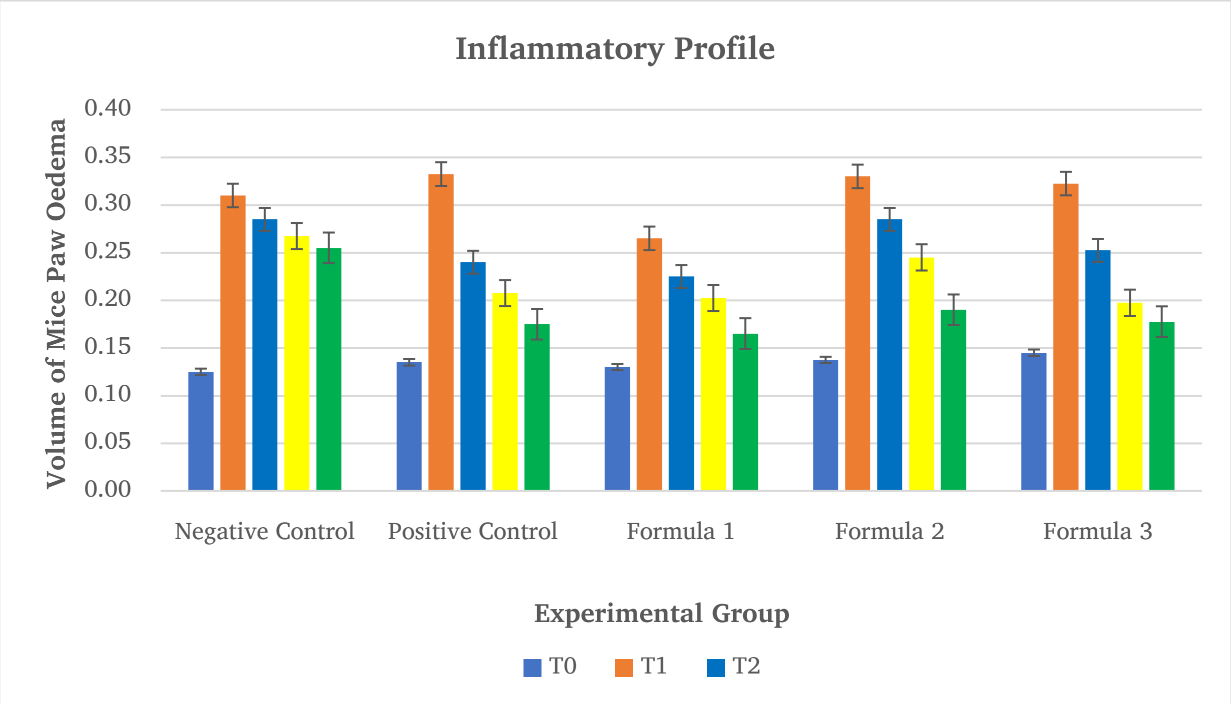

Figure 3. Average Volume of Oedema in the Test Animals Corresponding to the Treatments Administered with Test Samples. T0 = Volume of Oedema in the Test Animals Before Carrageenan Induction; T1 = Volume of Oedema in the Test Animals After Carrageenan Induction; T2 = Volume of Oedema in the Test Animals After Administration of Test Samples at 30 Minutes; T3 = Volume of Oedema in the Test Animals After Administration of Test Samples at 60 Minutes; T4 = Volume of Oedema in the Test Animals After Administration of Test Samples at 90 Minutes.

Before experimentation, the mice were acclimatized through appropriate group housing, ad libitum access to food and water, regular cage cleaning, and maintenance of optimal environmental conditions. The mice were randomly divided into five groups (n = 4 per group). Group I (negative control) received VCO, commonly used as a liniment base. Group II (positive control) received diclofenac sodium ointment, a potent COX inhibitor with anti-inflammatory, antipyretic, and analgesic effects. Groups III, IV, and V received liniment formulations I (2 g), II (3 g), and III (5 g), respectively. The mean oedema volumes recorded for each treatment group were as follows. As shown in **Figure 3,** oedema volume increased after carrageenan induction (T1), confirming inflammation in all groups. Subsequent measurements at 30, 60, and 90 minutes showed a reduction in oedema volume in all treated groups, indicating an anti-inflammatory effect.

As presented in **Figure 4**, the oedema reduction percentages were: negative control (18%), positive control (47%), Formula I (38%), Formula II (42%), and Formula III (44%). Interestingly, the negative control group treated with VCO also exhibited anti-inflammatory effects. Prior research has reported that VCO at doses of 4 g/kg and 8 g/kg body weight reduced oedema by 2.47% and 4.17%, respectively, between 180 and 300 minutes post-induction, with 8 g/kg being the optimal dose.

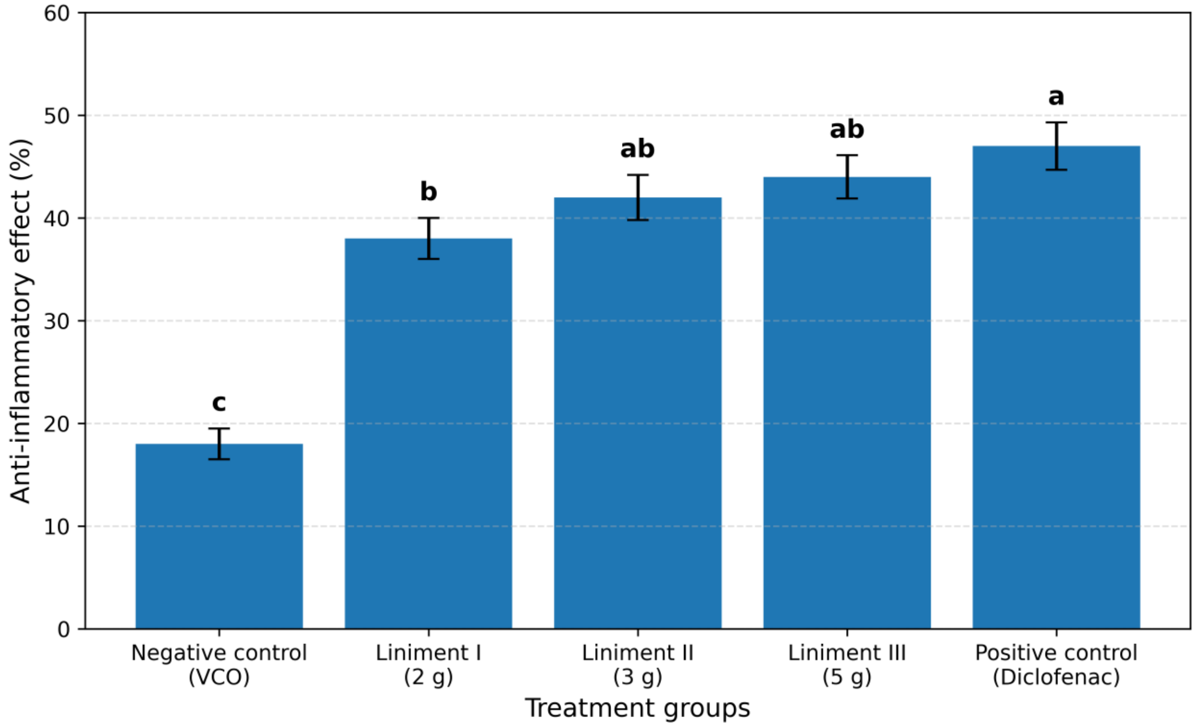

Figure 4. Anti-inflammatory effect (%) of topical liniment formulations in carrageenan-induced paw oedema in mice. Bars represent mean ± SD. Different superscript letters indicate significant differences among groups based on Tukey's HSD test at $p < 0.05$.

VCO contains bioactive constituents such as unsaturated fatty acids (oleic and linoleic acid) and flavonoids, which contribute to its anti-inflammatory properties. PUFAs exert anti-inflammatory effects by suppressing eicosanoid synthesis, reducing pro-inflammatory cytokine production, and inhibiting reactive oxygen species (ROS) formation. Flavonoids inhibit key enzymes involved in eicosanoid biosynthesis—such as phospholipase A2, cyclooxygenase, and lipoxygenase—thereby decreasing prostanoid and leukotriene levels. They also modulate transcription factors and inhibit histamine release, phosphodiesterase activity, and protein kinase functions (Nevin & Rajamohan, 2010).

Statistical analysis using one-way ANOVA revealed significant differences among the treatment groups ($p < 0.05$). Tukey's HSD test further indicated that the positive control group (diclofenac) showed a significantly higher anti-inflammatory effect compared to the negative control (VCO) ($p < 0.05$), but not significantly different from Formulas II and III. Formula I was statistically different from the negative control ($p < 0.05$), but not from the positive control. These results suggest that higher doses of *A. cepa* extract (3 g and 5 g) produced anti-inflammatory effects comparable to diclofenac. Taken together, the results indicate that the *A. cepa* extract-based liniment, especially at higher doses, exhibits significant anti-inflammatory activity and may serve as a promising natural alternative for topical anti-inflammatory therapy.

4. CONCLUSION

This study identified ten anti-inflammatory secondary metabolites in the 70% ethanol extract of Bima Brebes red onion through LC-MS/MS analysis. Topical liniments formulated with this extract in Virgin Coconut Oil (VCO) significantly reduced carrageenan-induced paw oedema in mice, with all doses (2 g, 3 g, and 5 g) showing notable anti-inflammatory effects. Although higher doses exhibited slightly greater efficacy, statistical analysis revealed no significant difference among the liniment groups, suggesting that even the lowest concentration (2 g) provided meaningful therapeutic benefits. The observed effects likely result from the combined action of flavonoids, organosulfur compounds, and VCO's bioactive constituents. These findings support the development of *A. cepa*-based liniments as cost-effective, natural alternatives for topical inflammation management and warrant further investigation into their long-term safety, mechanisms, and broader clinical potential.

AUTHOR CONTRIBUTIONS

Conceptualization: M.S., S.H., R.; methodology: K.R., R.S.R., S.H.; software: S.H.; validation: M.S., S.H., R.; formal analysis: M.S., S.H.; investigation: M.S., S.H., K.R., R.S.R., E.L.R., L.C.E.; resources: M.S., S.H., and R.; data curation: M.S., S.H.; writing—original draft preparation: M.S., S.H., and K.R., R.S.R., R.; writing—review and editing: M.S., S.H., K.R., R.S.R., E.L.R., L.C.E, R.; visualization: S.H.; supervision: R.; project administration: M.S., S.H., R.; funding acquisition: M.S. and R. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Institutional Ethics Committee of of the Faculty of Health Sciences, Universitas Pelita Harapan, Tangerang, Indonesia (Approval No. 0019/PE.KEPK-FIKes-UPH/V/2024).

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

FUNDING

This research was funded by MINISTRY OF EDUCATION, CULTURE, RESEARCH, AND TECHNOLOGY OF THE REPUBLIC OF INDONESIA, SIMBELMAWA, grant number **2546/E2/DT.01.00/2024**.

This research was funded by UNIVERSITAS PELITA HARAPAN, grant number **PM-005-H-FIKes/VII/2024**.

ACKNOWLEDGMENT

The authors would like to thank the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, Simbelmawa, Diploma of Pharmacy Study Program, Faculty of Health Sciences, Universitas Pelita Harapan, and LPPM Universitas Pelita Harapan for supporting this research. Thanks to the Faculty of Health Sciences, Universitas Pelita Harapan for facilitating the implementation of this research activity using laboratory facilities, particularly the Pharmacology and Toxicology Laboratory and the Pharmaceutical Biology Laboratory, which played a crucial role in supporting the experimental procedures and analytical assessments conducted in this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used QuillBot to assist in improving the clarity, structure, or readability of the text. After using this tool, the authors thoroughly reviewed, edited, and verified the entire content to ensure it accurately represents their own ideas and interpretations. The authors take full responsibility for the integrity and originality of the published work.

REFERENCES

Andriyono, R. I. (2019). *Kaempferia galanga* L. as an anti-inflammatory and analgesic. *Health Journal*, *10*(3), 495–500.

Audah, K. A., Ettin, J., Darmadi, J., Azizah, N. N., Anisa, A. S., Hermawan, T. D. F., Tjampakasari, C. R., Heryanto, R., Ismail, I. S., & Batubara, I. (2022). Indonesian Mangrove *Sonneratia caseolaris* Leaves Ethanol Extract Is a Potential Super Antioxidant and Anti Methicillin-Resistant *Staphylococcus aureus* Drug. *Molecules*, *27*(23), 8369. https://doi.org/10.3390/molecules27238369.

Bai, Y., Wang, H., Liu, M., Wang, Y., Lian, G., Zhang, X., Kang, J., & Wang, H. (2024). 4-Chloro-DL-phenylalanine protects against monocrotaline induced pulmonary vascular remodeling and lung inflammation. *International Journal of Molecular Medicine*, *33*(2), 373–382.

Chen, S., Liu, Y., Wang, X., Wang, H., Li, S., Shi, H., Zhu, H., Zhang, J., Pi, D., Hu, C. A., Lin, X., & Odle, J. (2016). Asparagine improves intestinal integrity, inhibits TLR4 and NOD signaling, and differently regulates p38 and ERK1/2 signaling in weanling piglets after LPS challenge. *Innate Immunity*, *22*(8), 577–587. https://doi.org/10.1177/1753425916664124.

Emelda, E., Nugraeni, R., & Damayanti, K. (2022). Review: Exploration of Indonesian herbal plants as anti-inflammatory. *Indonesian Pharmacy Natural Medicine Journal*, *6*(2), 58–64.

George, A., Chinnappan, S., Chintamaneni, M., Kotak, C. V., Choudhary, Y., Kueper, T., & Radhakrishnan, A. K. (2014). Anti-inflammatory effects of *Polygonum minus* (Huds) extract (Lineminus™) in in-vitro enzyme assays and carrageenan induced paw edema. *BMC Complementary and Alternative Medicine*, *14*, Article 355. https://doi.org/10.1186/1472-6882-14-355.

Guha, S., Alvarez, S., & Majumder, K. (2021). Transport of dietary anti-inflammatory peptide, γ-glutamyl valine (γ-EV), across the intestinal Caco-2 monolayer. *Nutrients*, *13*(5), 1448. https://doi.org/10.3390/nu13051448.

Handini, D. L. Y. (2020). The effect of maceration (soaking) time on the viscosity of betel leaf extract (*Piper betle*). *Jurnal Farmasi Tinctura*, *21*, 34–41.

Hirai, S., Horii, S., Matsuzaki, Y., Ono, S., Shimmura, Y., Sato, K., & Egashira, Y. (2014). Anti-inflammatory effect of pyroglutamyl-leucine on lipopolysaccharide-stimulated RAW 264.7 macrophages. *Life Sciences*, *117*(1), 1–6. https://doi.org/10.1016/j.lfs.2014.08.017

Indriyani, N. R., Djamaludin, A., & Helmiawati, Y. (2021). Preparation of linimentum from coconut (*Cocos nucifera* L) and horsetail (*Synedrella nodiflora*) leaf extract. *Journal of Holistic Health Sciences*, *5*(1), 57–61.

Intahphuak, S., Khonsung, P., & Panthong, A. (2010). Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. *Pharmaceutical Biology*, *48*(2), 151–157. https://doi.org/10.3109/13880200903062614.

Jose, A. M., & Vinod, A. (2024). An experimental evaluation of anti-inflammatory and analgesic activity of *Allium cepa* Linn. and *Allium ascalonicum* Linn.: A comparative study. *Journal of Ayurveda and Integrative Medicine Sciences*, *9*(5), 35–39.

Juliadi, D., & Agustini, N. P. D. (2019). Quercetin extract from red onion (*Allium cepa* L.) skin as an anti-inflammatory cream in male white mice *Mus musculus* using the hot plate method. *Jurnal Ilmiah Medica Mento*, *5*(2), 97–104.

Kim, M. H., & Kim, H. (2017). The roles of glutamine in the intestine and its implication in intestinal diseases. *International Journal of Molecular Sciences*, *18*(5), 1051. https://doi.org/10.3390/ijms18051051.

Lee, H. S., Kwon, Y. J., Seo, E. B., Kim, S. K., Lee, H., Lee, J. T., Chang, P. S., Choi, Y. J., Lee, S. H., & Ye, S. K. (2023). Anti-inflammatory effects of *Allium cepa* L. peel extracts via inhibition of JAK-STAT pathway in LPS-stimulated RAW264.7 cells. *Journal of Ethnopharmacology*, *317*, 116851. https://doi.org/10.1016/j.jep.2023.116851.

Li, X., Chen, S., Yue, G. G., Kwok, H., Lee, J. K., Zheng, T., Shaw, P., Simmonds, M. S., & Lau, C. B. (2021). Natural flavone tricin exerted anti-inflammatory activity in macrophage via NF-κB pathway and ameliorated acute colitis in mice. *Phytomedicine*, *90*, 153625. https://doi.org/10.1016/j.phymed.2021.153625.

Mangurana, W. O. I., Yusnaini, & Sahidin. (2019). LC-MS/MS (liquid chromatograph mass spectrometry) analysis and secondary metabolites and antibacterial potential of n-hexane extract of *Callyspongia aerizusa* sponge taken under different coral reef cover conditions in Staring Bay waters. *Jurnal Biologi Tropis*, *19*(2), 131–141.

Marefati, N., Ghorani, V., Shakeri, F., Boskabady, M., Kianian, F., Rezaee, R., & Boskabady, M. H. (2021). A review of anti-inflammatory, antioxidant, and immunomodulatory effects of *Allium cepa* and its main constituents. *Pharmaceutical Biology*, *59*(1), 287–302. https://doi.org/10.1080/13880209.2021.1874028.

Nevin, K. G., & Rajamohan, T. (2010). Anti-inflammatory and antinociceptive effects of virgin coconut oil. *Phytotherapy Research*, *24*(5), 762–768.

Oyovwi, M. O., & Atere, A. D. (2024). Exploring the medicinal significance of l-arginine mediated nitric oxide in preventing health disorders. *European Journal of Medicinal Chemistry Reports*, *12*, 1–9. https://doi.org/10.1016/j.ejmcr.2024.100175.

Pramitha, D. A. I., Samidya, N. W. R., Sukriani, L. D., Sasadara, M. M. V., & Wibawa, A. A. C. (2023). The quality of massage oil from a combination of VCO and Javanese chili (*Piper retrofractum* Vahl.) with varying heating temperatures during the digestion process. *Journal Scientific Medicamento*, *9*(1), 1–8.

Pranata, D., Ardiningsih, P., Rahmalia, W., Nurlina, N., & Syahbanu, I. (2020). Extraction of virgin coconut oil using stirring and cold-pressed methods. *Indonesian Journal of Pure and Applied Chemistry*, *3*(2), 11–17.

Pusparikі, J., & Suharti, S. (2019). Public perception of traditional medicine based on education in Purwakarta Regency. *Journal of Holistic Health Sciences*, *3*(1), 54–9.

Raizel, R., Leite, J. S. M., Hypólito, T. M., Coqueiro, A. Y., Newsholme, P., Cruzat, V. F., & Tirapegui, J. (2016). Determination of the anti-inflammatory and cytoprotective effects of l-glutamine and l-alanine, or dipeptide, supplementation in rats submitted to resistance exercise. *British Journal of Nutrition*, *116*(3), 470–479. https://doi.org/10.1017/S0007114516001999.

Riskianto, Andriyani, Munthe, S. W., Novia, J., Dillak, S. A., Jayanti, T., Laksana, S. M., Langi, A. J., Pattiradjawane, Z., & Sihotang, F. (2025). Antihyperglycemic activity, total phenolic, and total flavonoid contents of 96% ethanol extract of Pisang Batu (*Musa balbisiana* Colla) leaves. *Tropical Journal of Natural Product Research*, *9*(1), 348–354. https://doi.org/10.26538/tjnpr/v9i1.44.

Royal Society of Chemistry. (2025). *ChemSpider*. Retrieved August 1, 2025, from https://www.chemspider.com/.

Sarno, S. (2019). Utilization of medicinal plants (biopharmaceuticals) as a superior product for the people of Depok Village, Banjarnegara. *Abdimas Unwahas*, *4*(2), 73–76.

Soemarie, Y. B. (2016). Testing the anti-inflammatory activity of quercetin from shallot skin (*Allium cepa* L.) on male white mice (*Mus musculus*). *Jurnal Ilmiah Ibnu Sina*, *1*(2), 163–172.

Thermo Fisher Scientific. (2025). mzCloud: Advanced mass spectral database. Retrieved August 1, 2025, from https://www.mzcloud.org/.

Thomas, S., Senthilkumar, G. P., Sivaraman, K., Bobby, Z., Paneerselvam, S., & Harichandrakumar, K. T. (2015). Effect of s-methyl-L-cysteine on oxidative stress, inflammation and insulin resistance in male Wistar rats fed with high fructose diet. *Iranian Journal of Medical Sciences*, *40*(1), 45–50.

Tuuk, K. P. A., Koamesah, S., & Lidia, K. (2020). Antipyretic effect of ethanol extract of shallot (*Allium ascalonicum* L.) on Sprague-Dawley rats (*Rattus norvegicus*) that reduced DPT-HB vaccine. *Jurnal Cendana Medika*, *19*(1), 138–146.

Uraz, S., Tahan, G., Aytekin, H., & Tahan, V. (2013). N-acetylcysteine expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic acid-induced colitis in rats. *Scandinavian Journal of Clinical and Laboratory Investigation*, *73*(1), 61–66. https://doi.org/ 10.3109/00365513.2012.734859.

Vimal, A., & Kumar, A. (2018). l-Asparaginase: A feasible therapeutic molecule for multiple diseases. *3 Biotech*, *8*(6), Article 278. https://doi.org/10.1007/s13205-018-1282-3.

Yulianti, W., Ayuningtiyas, G., Martini, R., & Resmeiliana, I. (2020). Comparison of extraction methods and solvent polarity on the total phenolic content of cherry leaves (*Muntingia calabura* L). *Jurnal Applied Science*, *10*(2), 41–49.

Yunanda, V., & Rinanda, T. (2016). Wound healing activity of topical shallot (*Allium cepa*) extract on skin cuts in mice (*Mus musculus*). *Jurnal Veteriner*, *17*(4), 606–614.

Zamanabadi, S. N., Hemmati, A. A., Zalaghi, R., Sohrabi, M., Samarin, E. M., Nejaddehbashi, F., & Kiashi, F. (2025). Assessment of the anti-inflammatory effects of several native macrolichens on carrageenan-induced paw edema in rats. *Jundishapur Journal of Natural Pharmaceutical Products*, *20*(2), e158567. https://doi.org/10.5812/jjnpp-158567.

Zheng, T., Fan, M., Wei, Y., Feng, J., Zhou, P., Sun, X., Xue, A., Qin, C. X., & Yu, D. (2021). Huangbai liniment ameliorates skin inflammation in atopic dermatitis. *Frontiers in*

Pharmacology, *12*, Article 726035. https://doi.org/10.3389/fphar.2021.726035.

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