Synergistic Anti-inflammatory Activity of *Tinospora crispa L.* and *Zingiber officinale*: A BSA Assay Study

Sari Puspita Dewi, Achdi Kurnia, Shinta Dewi Permata Sari*, Muhamad Arif Budiman, Agus Rahmadi

Program Studi Pendidikan Dokter, Fakultas Kedokteran UHAMKA, Parung Serab, Tangerang, Indonesia

*Corresponding Author: Shinta Dewi Permata Sari, Program Studi Pendidikan Dokter, Fakultas Kedokteran UHAMKA, Parung Serab, Tangerang, Indonesia, E-mail: shinta.dps@uhamka.ac.id

Received : 4 Februari 2025, **Accepted** : 5 Agustus 2025, **Published** : 31 Agustus 2025

Abstrak

Ekstrak rimpang jahe *(Zingiber officinale)* dan daun brotowali *(Tinospora crispa L*) memiliki berbagai kandungan senyawa aktif yang berpotensi sebagai agen anti-inflamasi. Penelitian ini bertujuan untuk mengevaluasi aktivitas anti-inflamasi ekstrak rimpang jahe dan daun brotowali baik secara tunggal maupun kombinasi menggunakan metode uji BSA. Ekstraksi dilakukan menggunakan metode maserasi dengan pelarut etanol 70%. Uji aktivitas anti-inflamasi dilakukan secara *in vitro* pada konsentrasi 75, 100, dan 150 µg/mL dengan mengukur kemampuan ekstrak dalam mencegah denaturasi BSA yang diinduksi panas. Rendemen ekstrak brotowali mencapai 41.39% dan ekstrak jahe 25.63%. Kombinasi ekstrak 150 µg/mL menunjukkan aktivitas anti-inflamasi tertinggi (96.82%) dari semua sampel uji ekstrak. Sedangkan aktivitas anti-inflamasi terendah terdapat pada ekstrak jahe 75 µg/mL, sebesar 89.21%. Uji statistik menunjukkan perbedaan signifikan antara ekstrak jahe 75 µg/mL dan kombinasi 150 µg/mL (p=0.047). Kombinasi ekstrak jahe dan brotowali memiliki potensi untuk dikembangkan sebagai agen anti-inflamasi alami.

Kata kunci: anti-inflamasi, *Tinospora crispa L*, uji BSA, *zingiber officinale.*

Abstract

Ginger rhizome (Zingiber officinale) and Tinospora crispa L. (Tinospora crispa L.) extracts contain various active compounds that have the potential to be anti-inflammatory agents. This study aims to evaluate the anti-inflammatory activity of ginger rhizome and Tinospora crispa L. leaf extracts, both singly and in combination, using the BSA test method. Extraction was carried out using the maceration method with 70% ethanol solvent. Anti-inflammatory activity tests were carried out in vitro at 75, 100, and 150 µg/mL concentrations by measuring the extract's ability to prevent heat-induced BSA denaturation. The yield of Tinospora crispa L. extract reached 41.39% and that of ginger extract 25.63%. The combination of 150 µg/mL extracts showed the highest anti-inflammatory activity (96.82%) of all the extract test samples. In comparison, the lowest anti-inflammatory activity was found in 75 µg/mL ginger extract, at 89.21%. Statistical tests showed a significant difference between the 75 µg/mL ginger extract and the 150 µg/mL combination (p=0.047). The combination of ginger and Tinospora crispa extract has the potential to be developed as a natural anti-inflammatory agent.

Introduction

Inflammation is a fundamental physiological response to tissue injury that can be triggered by various factors, including biological agents (viruses, fungi, bacteria, and parasites), physical agents (mechanical trauma, extreme temperatures, radiation), chemical agents (acids and bases), and immunological agents (allergic reactions). This complex biological process is characterized by five cardinal clinical manifestations: rubor (redness), calor (heat), dolor (pain), tumor (swelling), and functio laesa (loss of function). Inflammation, whether acute or chronic, underlies a wide spectrum of health disorders ranging from infectious diseases like influenza and dengue fever to chronic non-communicable diseases such as autoimmune, rheumatoid arthritis, and inflammatory bowel diseases.[1]–[3] Surveillance data indicate that episodes of acute inflammation—driven by infectious disease outbreaks—remain prominent.[1], [4]

Conventional anti-inflammatory therapies, primarily non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, though effective, are often associated with substantial adverse effects such as gastrointestinal ulceration, renal impairment, cardiovascular risks, and immunosuppression with long-term use.[5]–[7] This significant limitation highlights the urgency of discovering and developing safer and more tolerable therapeutic alternatives, especially those derived from natural sources with a established history of traditional use. Natural compounds have emerged as promising anti-inflammatory agents due to their potential efficacy and reduced side effects. Ginger (*Zingiber officinale*) and Brotowali (*Tinospora crispa L.*) represent two medicinal plants with significant anti-inflammatory potential. Ginger rhizomes contain gingerol and shogaol compounds that exhibit analgesic effects through prostaglandin inhibition mechanisms. Murugesan et al studies demonstrated that ginger methanol extract at 100 µg/mL concentration could inhibit protein denaturation up to 81.68%, approaching the

positive control diclofenac[8], [9]. Additionally, Alsahli studies proved that pure 6-gingerol showed protective effects against albumin with inhibition reaching 70% at 600 µg/mL.[10]

Brotowali contains tinocrisposide, a diterpenoid alkaloid with anti-inflammatory properties, while its leaves contain alkaloids, flavonoids, tannins, and saponins. Gustina's studies have shown that brotowali leaves extract at 1.0 g/kg body weight provides anti-inflammatory effects in rats induced edema[11]. Furthermore, in silico research revealed that the Tyramine-Fe complex compound in brotowali stems demonstrates superior anti-inflammatory potential compared to single Tyramine, aspirin, and ibuprofen in COVID-19 cases.[12]. In addition, studies by Hipol et al showed that aqueous extract from *T. crispa* stem could protect against heat-induced albumin protein denaturation at 70°C.[13] The Bovine Serum Albumin (BSA) denaturation assay has emerged as an effective screening method for detecting anti-inflammatory activity. This method provides a rapid and sensitive assessment of anti-inflammatory activity compared to other protein denaturation indicators. Banerjee and Kumar Sahu validated that protein denaturation testing using BSA serves as an effective initial screening method for identifying compounds with anti-inflammatory activity.[14] Chemically, protein denaturation inhibition closely relates to protein structural stability, where compounds capable of preventing thermal stress-induced changes reflect the potential to suppress inflammatory processes.

Despite individual studies demonstrating the anti-inflammatory potential of ginger and brotowali, studies investigating their combined effects remain limited. Most previous studies focused on single-compound evaluations, lacking a comprehensive assessment of synergistic interactions between these natural agents. Therefore, this study

aims to analyze the effectiveness of combining ginger rhizome extract and brotowali leaves extract as an anti-inflammatory agent through the BSA assay. The potential synergistic effects of combining ginger and brotowali extracts could offer enhanced therapeutic efficacy and minimize adverse effects while maintaining the safety profile of natural compounds.

While previous research has established the individual anti-inflammatory properties of ginger and brotowali, as demonstrated by studies from Murugesan et al. (2020) and Gustina et al. (2024) respectively, there is a notable gap in the literature regarding their combined effects. Previous investigations have been predominantly confined to single-compound evaluations, thus failing to assess the potential synergistic interactions that may occur when these two medicinal plants are used together. Our study directly addresses this gap by being the first to comprehensively analyze the anti-inflammatory efficacy of a combined ginger and brotowali extract using the BSA assay. This novel approach shifts the focus from isolated components to a holistic, combined application, providing a scientific foundation for traditional herbal medicine practices and potentially identifying a more potent and safer therapeutic alternative than either component alone.

Research Method

This study was a true experimental design and approved by ethical clearance from the Medical and Health Research Ethics Committee of Universitas Muhammadiyah Prof. Dr. HAMKA (certificate number: KEPKK/FK/027/03/2025). This study used fresh ginger rhizomes (*Zingiber officinale*) and brotowali leaves (*Tinospora crispa* L.) extracted by maceration methods with 70% ethanol for 24 hours. The negative control was distilled

water, and the positive control was sodium diclofenac tablets (50 mg). This study consists of 13 groups of samples such as the negative control, positive control 75 µg/mL, positive control 100 µg/mL, positive control 150 µg/mL, ginger 75 µg/mL, ginger 100 µg/mL, ginger 150 µg/mL, brotowali 75 µg/mL, brotowali 100 µg/mL, brotowali 150 µg/mL, ginger-brotowali 75 µg/mL, ginger-brotowali 100 µg/mL, and ginger-brotowali 150 µg/mL. The extraction methods were used maceration with 70% ethanol for 24 hours. Maceration is a method that is often used because it does not require complex equipment and is affordable.[15] Ginger rhizomes (938.9 g) and brotowali leaves (890.4 g) were cleaned, dried at 50°C for 24 hours, ground, and then filtered through a 60-mesh sieve. The extracts were concentrated using a rotary vacuum evaporator at 40°C.[16], [17]

Sample extracts solutions were prepared by dissolving extracts (10 mg) in 10 mL of distilled water. Serial concentrations (75 µg/mL, 100 µg/mL, and 150 µg/mL) were prepared using the dilution formula. The anti-inflammatory effect was measured by the BSA assay. This assay used the TBS solution to dissolve BSA. The TBS (Tris Buffer Saline) solution was prepared by mixing tris base (2.42 g) and NaCl (17.4 g), dissolved in distilled water, and adjusted to pH 6.2-6.5. Furthermore, BSA (0.5 g) was dissolved in 250 mL TBS.[18] The BSA assay was conducted by mixed the sample extracts solutions (500 µL) with BSA, incubated at 25°C for 30 minutes, heated at 82°C for 5 minutes, cooled for 10 minutes, and measured for absorbance at 660 nm using UV-Vis spectrophotometer.[18], [19] The anti-inflammatory activity was measured by the inhibition percentage (% inhibition) that calculated by the formula [16]:

$$\frac{(Absorbance\ negative\ control - Absorbance\ sample)}{Absorbance\ negative\ control}\ x\ 100\%$$

Statistical analysis performed by SPSS software. The inhibition percentage data were tested for normality using the Shapiro–Wilk test. Statistical differences were measured

by one-way ANOVA and considered significant with a p-value <0.05. Post hoc analysis was conducted by Tukey's test.

Results

The ginger rhizome extracts and the brotowali leaves extracts were performed by the maceration method with ethanol 70% as solvent. This maceration process was repeated three times to obtain concentrated and thick extracts (Picture 1). Ginger had a much higher initial moisture content than Brotowali, as evidenced by its significantly higher weight reduction percentage during drying (91.94% vs 81.62%) (Table 1). In addition, brotowali produced over twice the amount of dry weight from a similar starting wet weight (163.7 g vs 75.7 g). Using the same amount of dried powder (60 g), the extraction process yielded substantially more thick extract from Brotowali (24.831 g) than from Ginger (15.38 g). Consequently, the calculated yield percentage was significantly higher for Brotowali (41.39%) compared to Ginger (25.63%). Brotowali gave a much better extract yield per gram of dried starting material.

(a) (b)

Picture 1. (a) Ginger rhizome extract, (b) Brotowali leaf extract

Table 1. Extract Yield and Weight Reduction of Ginger and Brotowali Extracts

Parameter	Extract	
	Ginger	Brotowali
Wet weight (g)	938.9	890.4

Dry weight (g)	75.7	163.7
Weight reduction (%)	91.94	81.62
Thick Extract Weight (g)	15.38	24.831
Simplisia powder (g)	60	60
Yields (%)	25.63	41.39

Anti-inflammatory activity of ginger extract, brotowali extract, and the combination was evaluated in vitro using the BSA assay. This assay results in an inflammation inhibition percentage that represents the anti-inflammatory activity. The positive control (sodium diclofenac) demonstrated an effective dose-dependent response, with the highest average inflammation inhibition percentage of 97.78% at 150 µg/mL (Picture 2). The ginger extracts and brotowali extracts showed significant anti-inflammatory potential, with brotowali extract achieving the highest mean inhibition of 93.33% at 150 µg/mL.

Both ginger and brotowali extracts performed dose-dependent anti-inflammatory activity. The combined extract exhibited superior performance, achieving 96.83% inhibition at 150 µg/mL, which exceeded the positive control at 75 µg/mL concentration and represented the highest anti-inflammatory activity among all test groups.

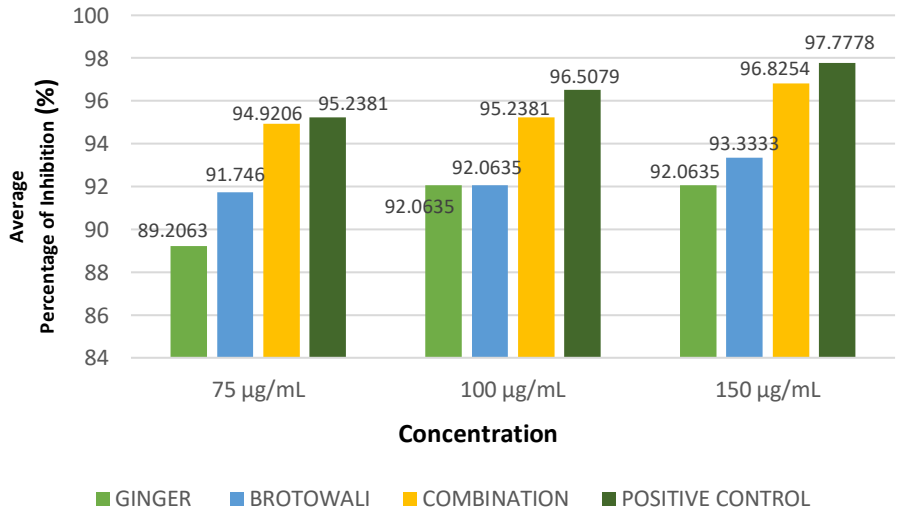

Figure 2. Inhibition Percentage of Ginger extract, Brotowali extract, and Positive Control

Statistical analysis revealed important patterns in the anti-inflammatory activity across different extract and concentrations. Normality test by Shapiro-Wilk test confirmed normal distribution for percentage of inhibition in all groups ($p>0.05$). Statistical analysis present the significance difference between percentage of inhibition among all groups test ($p<0.05$). In addition, post-hoc analysis revealed the significant difference between ginger extract 75 μg/mL and positive control 150 μg/mL. Another significant differences also showed between ginger extract 75 μg/mL and combination 150 μg/mL.

Discussion

This study demonstrates that extracts of ginger rhizome and brotowali leaf exhibit significant anti-inflammatory activity by inhibiting protein denaturation. These can be attributed to their rich content of alkaloids, flavonoids, and triterpenoids, which effectively stabilize protein structures and prevent heat-induced denaturation through non-covalent interactions. The higher extraction yield of brotowali (41.39%) compared to ginger (25.63%) further supports its superior bioactive potential, as a greater concentration of extractable compounds is likely to contribute to enhanced anti-inflammatory efficacy. Our results show that the percentage inhibition of the combination extract was higher than ginger extract alone and brotowali extract alone, in the same and higher concentration. Mechanistic synergy is critical because it shows that smaller doses of combined extracts can have the same therapeutic effect as higher doses of individual extracts. These could reduce the required dosage and associated side effects.

Our findings align with previous studies by Hipol et al. and Faradiba et al demonstrating *Tinospora crispa's* protein denaturation inhibition capabilities and showing ginger's anti-inflammatory potential.[16] Our results notably exceed Alsahli et

al.'s studies, which achieved only 72-75% inhibition with 6-gingerol at 600 μg/mL.[10] This suggests that crude extracts may offer superior bioactivity compared to isolated compounds due to multi-component interactions and the entourage effect of phytochemical complexes. The remarkable consistency of anti-inflammatory activity across different concentrations, particularly within the combination group, suggests that the efficacy is independent of the dose within the dose range. This is therapeutically advantageous for standardization purposes.

Our results suggest that combination therapy may achieve therapeutic efficacy at lowered dosages, potentially minimizing adverse effects while maintaining therapeutic benefits, as they observed a synergistic effect at a concentration of 75 μg/mL. These findings represent a significant advancement over conventional single-extract approaches, providing evidence for the development of combined natural anti-inflammatory formulations. The combinations performance of percentage inhibition approaching the value of positive controls (96.83% vs 97.78% at 150 μg/mL) indicates substantial therapeutic potential with the added benefit of natural origin and potentially better safety profiles for long-term use compared to synthetic NSAIDs. However, the study's limitations include reliance on a single BSA assay methodology, in vitro conditions that cannot fully replicate complex physiological environments, and maceration extraction, which may not be optimal for extracting all bioactive compounds. Further research needs to be conducted in vivo validation, exploration of alternative extraction methods such as ultrasonic or supercritical fluid extraction, investigation of pharmacokinetic parameters to confirm clinical applicability, and establish optimal formulation strategies for maximizing bioactive compound recovery and therapeutic efficacy from both plant materials.

Conclusion

This study successfully demonstrated the potent anti-inflammatory properties of ginger rhizome (*Zingiber officinale*) and brotowali leaf (*Tinospora crispa*) extracts, both individually and in combination. Our findings confirm that the combined extract exhibits enhanced anti-inflammatory activity, a key finding that supports the traditional use of these herbs together. The superior performance of the combined extract, which exceeded the efficacy of the positive control, highlights the potential for synergistic interactions between the compounds.

The anti-inflammatory effects were quantified using the BSA assay, which measures the inhibition of protein denaturation—a key mechanism in the inflammatory process. The dose-dependent relationship observed across all groups, especially the combined extract, provides compelling evidence for the therapeutic efficacy of these natural compounds. This suggests that the combined extract's greater inhibition percentage is a result of a synergistic effect, where the active compounds from each plant work together to produce a more powerful anti-inflammatory response than either could on their own.

To build on these promising results, future research should focus on a few key areas. First, bioassay-guided fractionation is needed to isolate and identify the specific bioactive compounds responsible for the observed synergistic effects. This will help us understand the precise molecular mechanisms at play. Second, in vivo studies are essential to validate the therapeutic efficacy and safety profiles of these extracts in a living system. This step is crucial for moving from in vitro observations to potential clinical applications. Lastly, further research should be conducted to determine optimal extraction methods and ratios to maximize anti-inflammatory activity.

The broader vision of this research is to contribute to a deeper scientific understanding of natural product pharmacology and validate traditional medicine practices. By establishing a scientific foundation for herbal combinations, this work paves the way for the development of new, effective, and safer natural alternatives to conventional anti-inflammatory drugs. Ultimately, this research aims to unlock the full therapeutic potential of medicinal plants, offering promising avenues for managing inflammation and improving health outcomes globally.

Acknowledgment

We thank Faculty of Medicine Universitas Muhammadiyah Prof. DR. HAMKA and The National Research and Innovation Agency (BRIN) for providing laboratory facilities and technical resources. We extend our sincere appreciation to all the laboratory assistants for their valuable technical assistance during the study.

References

[1] S. Fatima, "Public Health Surveillance Systems: Using Big Data Analytics To Predict Infectious Disease Outbreaks," *Int. J. Adv. Res. Eng. Technol. Sci.*, vol. 11, no. December, pp. 14–20, 2024.

[2] Y. Zhang, X. Chu, L. Wang, and H. Yang, "Global patterns in the epidemiology, cancer risk, and surgical implications of inflammatory bowel disease," *Gastroenterol. Rep.*, vol. 12, no. March, pp. 1–8, 2024.

[3] L. Hracs *et al.*, "Global evolution of inflammatory bowel disease across epidemiologic stages," *Nature*, vol. 642, no. 8067, pp. 458–466, 2025.

[4] W. Liu *et al.*, "Discovery of talmapimod analogues as polypharmacological anti-inflammatory agents," *J. Enzyme Inhib. Med. Chem.*, vol. 35, no. 1, pp. 187–198, 2020, doi: 10.1080/14756366.2019.1693703.

[5] R. Sohail *et al.*, "Effects of Non-steroidal Anti-inflammatory Drugs (NSAIDs) and Gastroprotective NSAIDs on the Gastrointestinal Tract: A Narrative Review," *Cureus*, vol. 15, no. 4, pp. 1–14, 2023.

[6] Y. Y. Hnepa, I. V. Chopey, K. I. Chubirko, and A. M. Bratasyuk, "Short- and Long-Term Effects of Nsaids on the Gastrointestinal Mucosa: Complex Analysis of Benefits and Complications Prevention," *Wiad. Lek.*, vol. 74, no. 4, pp. 1011–1018, 2021.

[7] E. J. Koshi, K. Young, J. C. Mostales, K. B. Vo, and L. P. Burgess, "Complications of Corticosteroid Therapy: A Comprehensive Literature Review," *J. Pharm. Technol.*, vol. 38, no. 6, pp. 360–367, 2022.

[8] S. Murugesan, M. R. Venkateswaran, S. Jayabal, and S. Periyasamy, "Evaluation of the antioxidant and anti-arthritic potential of Zingiber of fi cinale Rosc . by in vitro and in silico analysis," *South African J. Bot.*, vol. 130, pp. 45–33, 2020.

[9] X. Zou, Q. W. Tan, B. H. Goh, L. H. Lee, K. L. Tan, and H. L. Ser, "Sweeter than its name: anti-inflammatory activities of Stevia rebaudiana," *All Life*, vol. 13, no. 1, pp. 286–309, 2020, doi: 10.1080/26895293.2020.1771434.

[10] M. A. Alsahli *et al.*, "6-Gingerol , a Major Ingredient of Ginger Attenuates Diethylnitrosamine -Induced Liver Injury in Rats through the Modulation of Oxidative Stress and Anti-Inflammatory Activity," *Mediators Inflamm.*, vol. 2021, pp. 1–17, 2021.

[11] I. Gustina, R. Rosmaidar, R. Rinidar, M. Isa, A. Azhar, and R. Roslizawaty, "Anti-Inflammatory Effect of Brotowali (Tinospora crispa . L) Stem Extract On Carrageenan Induced Paw Edema in Rats (Rattus norvegicus)," *J. Ilm. Mhs. Vet.*, vol. 8, no. 4, pp. 188–195, 2024.

[12] W. T. Widodo, "In Silico Study of Tyramine-Fe Complex in Brotowali (Tinospora crispa) as Anti-Inflammatory," pp. 3473–3480, 2021.

[13] R. L. B. Hipol, M. F. N. M. Cariaga, and R. M. Hipol, "ANTI-Inflammatory Activities Of The Aqueous Extract Of The Stem Of Tinospora crispa (FAMILY MENISPERMACEAE)," *J. Nat. Stud.*, vol. 11, no. 1&2, pp. 88–95, 2012.

[14] M. Banerjee and S. K. Sahu, "The in-vitro anti-denaturation effects induced by substituted oxy/thioxy pyrimidine derivatives in bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds without the use of animals," *Int. J. Pharm. Sci. Res.*, vol. 11, no. 4, pp. 1885–1888, 2020.

[15] A. Octaviani, M. Zaim, and R. Avissa, "Uji Antibakterial dari Ekstrak Etanol Biji Mangga (Mangifera indica L) Terhadap Bakteri Salmonella typphimurium," *Sanus Med. J.*, vol. 5, no. 1, pp. 14–17, 2023.

[16] F. Faradiba, R. A. Syarif, A. T. M. Khaira, and T. K. Alyanti, "Pengaruh Variasi Metode Ekstraksi Jahe dan Serai terhadap Aktivitas Antiinflamasi pada Penghambatan Denaturasi Protein," *J. Fitofarmaka Indones.*, vol. 11, no. 1, pp. 33–40, 2024.

[17] Y. S. F. Tarukbua, E. De Queljoe, and W. Bodhi, "Skrining Fitokimia Dan Uji Toksisitas Ekstrak Etanol Daun Brotowali (Tinospora crispa (L.) Hook F. & T) DENGAN METODE Brine Shrimp Lethality Test (BSLT)," *Pharmacon J. Ilm. Farm.*, vol. 7, no. 3, pp. 330–337, 2018.

[18] D. F. Yani and R. Fatahillah, "Anti-Inflammatory Activity Of Ethanol Extract And Ethyl Acetate Fraction Of Kebiul (Caesalpinia Bonduc L.) Seed Coat Against Inhibition Of Protein Denaturation," *J. Kim. Ris.*, vol. 7, no. 1, pp. 1–8, 2022.

[19] Reynaldi and D. F. Yani, "Potensi Anti-Inflamasi Ekstrak Etanol Daun Cocor Bebek (Kalanchoe Pinnata L) Terhadap Denaturasi Protein Secara In vitro," *J. Kim. dan Pendidik. Kim.*, vol. 3, no. 1, pp. 12–21, 2021.