Anti-Inflammatory Integration in Vitro Leaf Extract Blumea Balsamifera and Molecular Docking Chemical Components from LCHRMS Results Againts COX-2: A Systematic Review

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ABSTRACT

Sembung plants (Blumea balsamifera (L) DC) have anti-inflammatory effects through the content of flavonoid, tannin, and alkaloid compounds by inhibiting the COX-2 enzyme. This study was conducted to analyze, separate and identify secondary metabolite compounds found in sembung leaves as anti-inflammatory using LC-HRMS instruments and also predict how the binding affinity between bioactive compounds and the COX-2 target enzyme in sembung leaves with molecular docking. The results found were Flavonoids from Blumea balsamifera ethanol extract with 13 metabolite compounds: apigenin, hesperetin, quercetin, luteolin, eriodictyol, diosmetin, sakuranetin, 5-hydroxy-7,8-dimethoxyflavonol, malvidin 3-glucoside, and 3',4',5-trihydroxy-3,7-dimethoxyflavone 5- glucoside. Blumea balsamifera showed values of 103.85 ppm for protein inhibition and 97.23 ppm for protein denaturation. Aspirin, as a positive control, showed values of 57 ppm and 62 ppm for protein inhibition and protein denaturation, respectively. These results highlight the anti- inflammatory potential of the ethanol extract of B. balsamifera. This suggests that the extract of the Sembung leaf can serve as a natural alternative or complement to synthetic drugs in managing inflammation.

Introduction

Blumea balsamifera (L.) DC. is a very well-known medicinal plant in many Asian countries, such as China (named Ainaxiang), Malaysia (named Sembung), the Philippines, Thailand, and Vietnam. Blumea balsamifera (L.) DC., a member of the Asteraceae family, Blumea balsamifera is a prominent medicinal plant in Southeast Asia, known for its traditional use in treating various ailments such as wounds, inflammation, and bacterial infections[1]. This plant contains many flavonoids, a ubiquitous group of polyphenolic compounds. Similar to the Blumea balsamifera plant, flavonoids from B. balsamifera leaves also exhibit broad pharmacological activities such as free radical scavenging, anticancer, plasmin inhibition, liver protection, and xanthine oxidase inhibition [2].

There are more than 100 volatile or non-volatile chemical components isolated from B. balsamifera [3],[4] including essential oils, flavonoids, terpenoids, phenylpropanoids, steroids, and others, where essential oils, flavonoids, and sesquiterpenes are the most abundant and are the main chemical components of B. balsamifera, with various biological activities in vitro and in vivo. There are many secondary metabolites in it and several journals prove that compounds in Blumea balsamifera have anti-inflammatory activity including flavonoid compounds, essential oils, alkaloids, terpenoids, and anthocyanins. These secondary metabolites specifically work to inhibit the cyclooxygenase (COX) enzyme. Flavonoids have anti-inflammatory activity and are considered a new class of natural antiinflammatory agents [5]. Flavonoids can inhibit the action of the COX enzyme [6]. Several flavonoids play a role in inhibiting prostaglandin formation, including hesperidin, diosmin, amentoflavone, bilobetin, morelloflavone, and ginkgetin. Several flavonoids play a role in inhibiting prostaglandin formation, including hesperidin, diosmin, amentoflavone, bilobetin, morelloflavone, and ginkgetin. Inflammation is usually caused by increased prostaglandin production facilitated by the COX-2 enzyme, so that COX-2 inhibition is a common strategy in anti-inflammatory therapy[7], COX-1 and COX-2 [6]]. The COX-2 protein specifically catalyzes the formation of prostaglandins from arachidonic acid. Through various mechanisms, including increased vasodilation, increased vascular permeability, and sensitization of pain receptors, prostaglandins produced by COX-2 contribute to the inflammatory process[9]. COX-2 significantly increased due to stimulation from

proinflammatory cytokines during inflammation [7]. This article aims to evaluate the anti-inflammatory activity of Sembung blumea balsamifera leaves through in vitro testing and to identify chemical compounds that have the potential as anti-inflammatory agents using LC-HRMS, as well as to study the molecular interactions between compounds with the COX-2 enzyme. The interaction test results are expected to provide information regarding the anti-inflammatory activity of Sembung leaves and can support the development as a candidate for natural-based anti-inflammatory agents.

Chemical composition

1. Essential oil
   The essential oil of B. balsamifera is an important chemical component due to its pharmacological activity. The main components of the essential oil include (-)-bromelain, trans-gastrolene, γeudesmol, α-eudesmol, anthocyanins, gastrolene oxide, and camphor [10], [11]. Although the volatile components in B. balsamifera are similar due to their origin, harvest time, and extraction method, there are some differences in the specific component content [12].
2. Flavonoid
   Flavonoids are a large group of phenolic plant constituents. Flavonoids from the ethanol extract of B. balsamifera contain 13 metabolite compounds: apigenin, hesperetin, quercetin, luteolin, eriodictyol, diosmetin, sakuranetin, 5-hydroxy-7,8-dimethoxyflavonol, malvidin 3-glucoside, and 3',4',5-trihydroxy-3,7-dimethoxyflavone 5-glucoside. Both fatty acyl and flavonoids play an important role in regulating inflammation. Fatty acyl can promote or inhibit inflammatory processes depending on the type and context of use. Flavonoids complement this effect by directly inhibiting inflammatory pathways and mediators [13]. Flavonoids are one of the most widely reported classes of compounds in B. balsamifera in recent years, and with various pharmacological activities have also greatly expanded the scope of clinical applications of the medicinal plant B. Balsamifera [14].
3. Terpenoid
   Terpenoids are a common class of compounds found in B. balsamifera. Its cytoskeleton types include monoterpenoids, sesquiterpenes, diterpenes, triterpenes, and so on. Sesquiterpene lactones have attracted attention due to their cytotoxicity and potential antitumor activity. Lborneol, a well-known traditional Chinese medicine, is a type of bicyclic monoterpene, and its important plant source is B. balsamifera. Currently, 5 sesquiterpenes, 51 sesquiterpenes, and 5 diterpenes have been isolated from B. balsamifera, and 2 ursolana triterpenes have been isolated from the aerial parts of B. balsamifera[13].
   In addition to the chemical components above, it was also found that B. balsamifera contains steroid compounds: β-sitosterol, stigmasterol, daucosterol, ergosterol peroxide, 4,22-stigmastadiene3-one, 2hydroxy-4,6-dimethoxyacetophenone, 2,4-dihydroxy6-methoxyacetophenone, paraben, phytol, βcarotene, lutein, hentriacontane, and others [2].

Methods

1. The process of preparation and extraction of plants
   The leaves were collected. They were thoroughly cleaned and allowed to air-dry at room temperature before being ground into a fine powder. A three-day maceration procedure was carried out using ethanol at a weight-to-volume ratio (w:v) of 1:10. The plant material and solvent were maintained unchanged throughout the maceration period, with occasional stirring to improve extraction efficiency. After 72 hours, the extract was filtered to remove plant residues. The solvent was then evaporated using a rotary evaporator (Büchi Rotavapor®, Switzerland) [13].
2. Albumin Denaturation Inhibition
   Albumin Denaturation Inhibition Albumin denaturation inhibition was assessed following the method of) with slight modifications. Four mL of 1% bovine serum albumin was added to 2 mL of sample. The pH was adjusted to 6.3 using a small amount of 1 M HCl. The mixture was allowed to stand at room temperature for 20 minutes and then heated at 57°C for 20 minutes to sufficiently destroy the secondary and tertiary structures of albumin. The resulting solution was cooled to room temperature and the absorbance was recorded at 660 nm spectrophotometrically. The negative control used was hexane. The percentage inhibition of albumin denaturation of the extract and positive control was calculated using the equation in Sakat et al. (2010): % inhibition [15].

3. Membrane Stabilization
   HRBC in Heat-Induced Hemolysis The method was performed according with slight modifications. Human blood, approximately 20 mL, was collected by a licensed physician from a healthy human volunteer who had not consumed any NSAIDs during the last two weeks prior to the study. The blood was transferred to a centrifuge tube containing heparin. The tube was centrifuged for 10 minutes at 3000 rpm and washed three times with an equal volume of normal saline. The blood volume was measured to be resuspended as a 10% v/v suspension with physiological saline. In heat-induced hemolysis, a 4 mL reaction mixture, each consisting of 2 mL of sample and 2 mL of 10% red blood cell suspension, was prepared in separate test tubes, in triplicate. All test tubes containing the reaction mixture were incubated at 56°C for 30 minutes and then cooled under running tap water. The mixture was read at 560 nm using a spectrophotometer [15].
4. Maulydia et al, 2025 Liquid chromatography-high resolution mass spectroscopy (LC-HRMS) Analysis Untargeted metabolomics profiling was performed using liquid chromatography-high resolution mass spectroscopy (LC-HRMS). This study involved metabolite identification by comparing retention times and searching the mass list using an online reference database. LCHRMS analysis used a Thermo Scientific Vanquish Horizon UH-PLC system connected to an Orbitrap Exploris 240 mass spectrometer.
5. In vitro analysis Protein inhibition assay and protein denaturation assay were used in vitro to assess the potential anti-inflammatory properties of the ethanolic extract of B. balsamifera. Protein inhibition assay To assess the inhibitory potential of the ethanolic extract of B. balsamifera against proteins involved in inflammation, a detailed protein inhibition assay was performed. This assay involved the preparation of a reaction mixture by combining 1 mL of 20 mM Tris-HCl buffer (pH 7.4) with 1 mL of the plant extract at various concentrations ranging from 100 to 500 πg/mL. A negative control (no extract) and a positive control (aspirin at an equivalent concentration range) were included to validate the inhibitory effect of the extract. To this solution, 0.06 mg of trypsin was added, and the mixture was incubated at 37 °C for 5 min to allow for stabilization of the prereaction. After this pre-incubation, 1 mL of 0.8% (w/v) casein solution was added, and the resulting mixture was further incubated at the same temperature for an additional 20 min to facilitate enzymatic activity. The enzymatic reaction was stopped by adding 2 mL of 70% perchloric acid, which precipitated the casein and unreacted proteins. The supernatant was analyzed for absorbance at 210 nm using a UV-Vis spectrophotometer (Shimadzu, UV 2500, Shimadzu Suzhou Instruments Mfg. Co., Ltd., Jiangsu, China), with buffer solution alone used as a blank to correct for background absorbance. All experiments were performed three times to ensure reproducibility [13].
6. Molecular docking analysis To evaluate the binding interactions between selected compounds and target proteins from network pharmacology, molecular docking was performed using a structured and systematic approach, including target preparation, ligand preparation, docking simulation, and analysis of docking results [11]. Post-docking assessment and analysis: Binding free energy (BFE) values were calculated for each ligand-protein complex, which reflects the strength of the interaction. Docking positions were ranked based on this score, and the top conformations were visually inspected to identify key interactions, such as hydrogen bonds, hydrophobic contacts, and stacking. Conformational visualization of selected compounds and receptors was performed using PoseView [16].

Results and Discussions

Inhibition of Albumin Denaturation Different hexane extracts and their controls were subjected to an acidic environment with the addition of HCl. Only 1% fetal bovine serum albumin was used for denaturation. The results showed that a concentration of 100 mg/mL had the highest inhibition of 30.96 ± 3.58% for the hexane extract, while the highest aspirin concentration (1.97 mg/mL) was 97.47 ± 0.77% [13].

Tukey's post hoc test showed that higher concentrations of hexane extract did not show significant differences when compared to most aspirin concentrations. Specifically, 100 mg/mL hexane extract did not show significant differences when compared to 1.97 mg/mL aspirin with p=0.981. In addition, 100 mg/mL and 75 mg/mL hexane extracts also did not show significant differences at p>0.05 in terms of aspirin with concentrations: 1.48 mg/mL, 0.98 mg/mL, and 0.49 mg/mL at adjusted p values [13].

Table 1 adjusted P-Values Of Protease Inhibition [13].

Difference of Levels	P-Value
Aspirin (1.48 mg/mL) – Extract (100 mg/mL)	0.999

Aspirin (0.98 mg/mL) – Extract (100 mg/mL)	0.999
Aspirin (0.49 mg/mL) – Extract (100 mg/mL)	0.975
Aspirin (1.48 mg/mL) – Extract (75 mg/mL)	0.092
Aspirin (0.98 mg/mL) – Extract (75 mg/mL)	0.647
Aspirin (0.49 mg/mL) – Extract (75 mg/mL)	0.856

Anti-inflammatory Activity In the albumin denaturation test, albumin denaturation by heating is a common result of inflammation and may be caused by other physical and chemical agents in the body. Further evaluation is needed to determine the potential anti-inflammatory activity [15]. In the results of the study, the hexane extract was unable to show a significant effect in inhibiting albumin denaturation, due to the difference in polarity of the solution used. showed that phytochemicals found in several plant materials are able to stabilize lysosomes, such as flavonoids, tannins, and saponins. Stabilization of the lysosomal membrane can also mean stabilization of the HRBC membrane due to its analogous structure [17]. Blumea balsamifera leaves are known to be a rich source of flavonoids, which may be the cause of its stabilizing effect on the HRBC membrane as seen in the results of the study.

Based on the research results, the percentage of inhibition and stabilization of hexane extract was below 30%, which was caused by the loss of polar phytochemicals during maceration with nonpolar hexane. It was concluded that hexane extract from Blumea balsamifera leaves had potential antiinflammatory activity in testing, stabilization of HRBC membranes, and protease inhibition, compared to aspirin. Blumea balsamifera leaves are a potential candidate as an anti-inflammatory agent, minimizing the side effects of nonsteroidal anti-inflammatory drugs (NSAIDs) [53].

B. balsamifera showed a value of 103.85 ppm for protein inhibition and 97.23 ppm for protein denaturation. Aspirin, as a positive control, showed values of 57 ppm and 62 ppm for protein inhibition and protein denaturation, respectively [13]. These results highlight the anti-inflammatory potential of the ethanol extract of B. balsamifera. Similar bioactivity has been observed for other plant-derived compounds from B. balsamifera that influence inflammatory responses, such as sesquiterpenoids, flavonoids, and essential oils [18]. The anti-inflammatory properties of mesoeudesmol B from Mesona procumbens Hemsley, which is 2-OBz proximadiol, are superior to other compounds, as evidenced by the EC50 value of 12.9 μM, which is twice as potent as quercetin [19]. This suggests that the plant extract could serve as a natural alternative or complement to synthetic drugs in managing inflammation. Further research, including mechanistic studies and in vivo trials, could strengthen its case for therapeutic applications.

Molecular docking analysis was performed using eleven receptors. Table 2 shows the binding free energy values of various proteins in relation to their ligands, with each protein identified by its name and PDB ID. The binding free energy (BFE) value, expressed in kcal/mol, reflects the strength of the interaction, with more negative values indicating more stable and stronger binding [11].

This docking study provides important information about the binding mechanism and binding affinity between the ligand and the target protein. The first step in the docking process involves predicting the orientation, shape, and location of the ligand within the protein binding site. The binding quality is then evaluated using a scoring function [20].

Table 2. Molecular docking analysis of proximadiol and eleven receptors [11].

No	No Protein name PDB ID Binding free-energy value (Kcal/mol)		
1	IL12RB2	8XRP	-6.8
2	IL22RA1	3DLQ	-5.5
3	IL23R	6WDQ	-5.9
4	IFNLR1	5T5W	-6.1
5	LEPR	3V6O	-5.9
6	IL20	4D0H	-6.1
7	IL6R	1P9M	-6.2

8	CSF3R	2D9Q	-6.0
9	IL24	6DF3	-6.2
10	IL10	2H24	-6.3
11	JAKI	6DBN	-7.1

Discussion

Based on in vitro testing results and literature review, sembung leaves (Blumea balsamifera) have potential as a natural anti-inflammatory agent. Although the hexane extract showed relatively low albumin denaturation and membrane stabilization inhibitory activity (<30%) compared to aspirin, these results indicate the presence of anti-inflammatory properties that are likely influenced by the content of bioactive compounds such as flavonoids, tannins, saponins, sesquiterpenoids, and essential oils. The low activity of the hexane extract is thought to be due to the loss of polar compounds during the extraction process using nonpolar solvents. Other studies using ethanol extracts showed better anti-inflammatory activity, which confirms the role of polar compounds in the anti-inflammatory mechanism of B. balsamifera. In addition, molecular docking analysis provides an overview of the affinity and stability of the interaction of active compounds with inflammatory target proteins, as indicated by the binding free energy value. The more negative the binding energy value, the stronger and more stable the interaction formed.

Overall, B. balsamifera has the potential to be developed as a natural-based anti-inflammatory drug candidate that could be an alternative or complement to synthetic anti-inflammatory therapies. However, further research, including identification of active compounds, studies of their mechanisms of action, and in vivo testing, is needed to confirm their effectiveness and safety

Conclusions

Based on the results of the albumin denaturation inhibition test, the hexane extract of B. balsamifera leaves showed anti-inflammatory activity characterized by its ability to inhibit protein denaturation. The highest activity was obtained at a concentration of 100 mg/mL with an inhibition percentage of 30.96 ± 3.58%. Although the inhibition value was lower than aspirin as a positive control, statistical analysis showed that several concentrations of the hexane extract were not significantly different from several concentrations of aspirin ($p > 0.05$). Blumea balsamifera showed a value of 103.85 ppm for protein inhibition and 97.23 ppm for protein denaturation. Aspirin, as a positive control, showed values of 57 ppm and 62 ppm for protein inhibition and protein denaturation, respectively. These results highlight the anti-inflammatory potential of the ethanol extract of B. balsamifera. This suggests that B. balsamifera leaf extract can serve as a natural alternative or complement to synthetic drugs in managing inflammation.

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Author Contributions

The author's contribution is to search for and collect relevant sources by identifying articles, books and other scientific sources related to the topic, analyzing and comparing research results from various sources, compiling and presenting the analysis results objectively and drawing conclusions and providing suggestions for further research.

Conflicts of Interest

The author states that there is no conflic of interest.

References

[1] Wang, J., et al. Chemical constituents and bioactivities of Blumea balsamifera (Sembung): a systematic review. 2023. https://doi.org/10.1590/fst.132322

[2] Tan et al., "Simultaneous Quantitative Determination of Polyphenolic Compounds in *Blumea Balsamifera* (Ai-Na-Xiang, Sembung) by High-Performance Liquid Chromatography with Photodiode Array Detector." *International Journal of Analytical Chemistry* 2020 (March 2020): 1–9. https://doi.org/10.1155/2020/9731327.

[3] Chen, Ming, Hui-Zi Jin, Wei-Dong Zhang, Shi-Kai Yan, and Yun-Heng Shen. "Chemical Constituents of Plants from the Genus *Blumea*." *Chemistry & Biodiversity* 6, no. 6 (2009): 809–17. https://doi.org/10.1002/cbdv.200800113.

[4] Pang, Yuxin, Dan Wang, Zuowang Fan, et al. "Blumea Balsamifera—A Phytochemical and Pharmacological Review." *Molecules* 19, no. 7 (2014): 9453–77. https://doi.org/10.3390/molecules19079453.

[5] Strzelecka M, Bzowska M, Kozie£ K, Szuba B, Dubiel O, nunez DR, Heinrich M, dan Beretaanti J. Inflammatory effects of extracts from some traditional mediterranean diet plants. Journal of physiology and pharmacology. 2005;56(1):139.156.

[6] Brahmachari G, Jash SK, Mandal LC, Mondal A dan Roy R. Cyclooxygenase (COX) – inhibitory flavonoid from Limnophila heterophylla. Rasāyan journal of chemistry. 2008;1(2):288-291.

[7] Ju, Z.; Li, M.; Xu, J.; Howell, D.C.; Li, Z.; Chen, F.-E. Recent Development on COX-2 Inhibitors as Promising Anti-Inflammatory Agents: The Past 10 Years. Acta Pharm. Sin. B 2022, 12, 2790–2807. 10.1016/j.apsb.2022.01.002

[8] Schultz, F.; Osuji, O.F.; Wack, B.; Anywar, G.; Garbe, L.-A. Antiinflammatory Medicinal Plants from the Ugandan Greater Mpigi Region Act as Potent Inhibitors in the COX-2/PGH2 Pathway. Plants 2021, 10, 351. doi: 10.3390/plants10020351

[9] Intan Wiratmini, N.; Pharmawati, M.; Sukmaningsih, A.A.S.A.; Joko Raharjo, S.; Widowati, W. Anti-Inflammatory Activity of Phyllanthus niruri L. Leaf Extract: In Vitro Study on RAW 264.7 Macrophage Cells and In Silico Analysis. HAYATI J. Biosci. 2024, 32, 287–299. https://doi.org/10.4308/hjb.32.2.287-299

[10] He, C., et al. Antibacterial effect of Blumea balsamifera DC. essential oil against Haemophilus parasuis. Archives of Microbiology, 202(9), 2499-2508. http://dx.doi.org/10.1007/s00203020-01946-4. PMid:32638056.

[11] Yang, H., et al. Antibacterial effect of Blumea balsamifera (L.) DC. essential oil against Staphylococcus aureus. Archives of Microbiology, 2021 203(7), 3981-3988. http://dx.doi.org/10.1007/s00203-021-02384-6. PMid:34032873.

[12] Yuan et al., "Variations in Essential Oil Yield, Composition, and Antioxidant Activity of Different Plant Organs from Blumea Balsamifera (L.) DC. at Different Growth Times." *Molecules* **2016**, 21(8), **https://doi.org/10.3390/molecules21081024**

[13] Maulydia et al. Metabolomic profiling and anti-inflammatory activity of Blumea balsamifera: Insights from in silico and in vitro studies. 2025. Pharmacia 72: 1–15. DOI 10.3897/pharmacia.72.e146995

[14] Pang, Y., Zhang, Y., Huang, L., Xu, L., Wang, K., Wang, D., Guan, L., Zhang, Y., Yu, F., Chen, Z., & Xie, X. (2017). Effects and Mechanisms of Total Flavonoids from Blumea balsamifera (L.) DC. on Skin Wound in Rats. International Journal of Molecular Sciences, 18(12), 2766. http://dx.doi.org/10.3390/ijms18122766. PMid:29257119.

[15] Johanna G. et al. In Vitro Anti-inflammatory Assays on Hexane Extract of Sambong (Blumea balsamifera) Leaves Johanna G. Bantol, Nice Marie R. Berdin, Zñep P. Peñascoza, and Jayfe Anthony A. Abrea. 2017. https://doi.org/10.17758/URUAE.AE09172018

[16] Schöning-Stierand K, Diedrich K, Ehrt C, Flachsenberg F, Graef J, Sieg J, Penner P, Poppinga M, Ungethüm A, Rarey M. Proteins Plus: a comprehensive collection of web-based molecular modeling tools. 2022. Nucleic Acids Research 50: W611–W615. https://doi.org/10.1093/ nar/gkac305

[17] Steenkamp, V., Nkwane, O., van Tonder, J., Dinsmore, A., Gulumian, M. (2013). "Evaluation of the phenolic and flavonoid contents and radical scavenging activity of three southern African medicinal plants." African Journal of 40 Pharmacy and Pharmacology. 7(13): 703-709.

[18] Chen, Ming, Jiang-Jiang Qin, Jian-Jun Fu, et al. "Blumeaenes A–J, Sesquiterpenoid Esters from *Blumea Balsamifera* with NO Inhibitory Activity." *Planta Medica* 76, no. 09 (2010): 897– 902. https://doi.org/10.1055/s-0029-1240800.

[19] Huang, Hung-Tse, I.-Wen Lo, Geng-You Liao, et al. "Anti-Inflammatory Sesquiterpene and Triterpene Acids from Mesona Procumbens Hemsley." *Frontiers in Chemistry* 10 (September 2022): 1003356. https://doi.org/10.3389/fchem.2022.1003356.

[20] Stanzione, F., et al. Penggunaan Alat Komputasi Docking Molekuler dalam Penemuan Obat, Progress in Medicinal Chemistry, 2021. Elsevier BV, 273–343. doi:10.1016/bs.pmch.2021.01.004.