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RESEARCH ARTICLE

Red Beetroot (*Beta vulgaris* L.) Bioactives as Promising Anaphylatoxin Receptor Antagonists for Anti-Inflammatory Therapy : A Computational Study

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Abstract

Uncontrolled inflammation, often driven by the complement system's anaphylatoxins (C3a, C5a), is a key factor in many chronic diseases, prompting the search for novel anti-inflammatory agents from natural sources. This study aimed to predict the anti-inflammatory potential of bioactive compounds from beetroot (*Beta vulgaris* L.) through a computational approach. This research employed an in silico methodology, utilizing the PubChem database for compound identification and the Way2Drug PASS Online server to predict pharmacological activity. The predictive analysis revealed that betalains possess a high probability of acting as an anaphylatoxin receptor antagonist, with a Pa (Probability to be active) score of 0.860 and a Pi (Probability to be inactive) score of 0.006. These findings strongly suggest that betalains have significant potential as a natural anti-inflammatory agent by modulating the complement system pathway. This computational evidence provides a strong foundation for further experimental validation (in vitro and in vivo) to develop betalains as a therapeutic agent for inflammation-related diseases.

Keywords: Betalains, *Beta vulgaris* L., Anti-inflammatory, Anaphylatoxin, In silico

Senyawa Bioaktif Bit Merah (Beta vulgaris L.) sebagai Antagonis Reseptor Anafilatoksin yang Menjanjikan untuk Terapi Anti-Inflamasi dengan Pendekatan Way2Drug

Abstrak

Inflamasi yang tidak terkontrol, yang sering kali didorong oleh anafilatoksin dari sistem komplemen (C3a, C5a), merupakan faktor kunci dalam banyak penyakit kronis, sehingga mendorong pencarian agen anti-inflamasi baru dari sumber alami. Penelitian ini bertujuan untuk memprediksi potensi anti-inflamasi senyawa bioaktif dari umbi bit (*Beta vulgaris* L.) melalui pendekatan komputasi. Penelitian ini menggunakan metodologi *in silico*, dengan memanfaatkan database PubChem untuk identifikasi senyawa dan server Way2Drug PASS Online untuk memprediksi aktivitas farmakologis. Hasil analisis prediktif menunjukkan bahwa senyawa betalain memiliki probabilitas tinggi untuk bertindak sebagai antagonis reseptor anafilatoksin, dengan skor Pa (*Probability to be active*) sebesar 0,860 dan skor Pi (*Probability to be inactive*) sebesar 0,006. Temuan ini memberikan indikasi kuat bahwa betalain memiliki potensi signifikan sebagai agen anti-inflamasi alami dengan cara memodulasi jalur sistem komplemen. Bukti komputasi ini menjadi dasar yang kuat untuk validasi eksperimental lebih lanjut (*in vitro* dan *in vivo*) guna mengembangkan betalain sebagai agen terapeutik untuk penyakit terkait inflamasi.

Kata Kunci: Betalains, *Beta vulgaris* L., Anti-inflammatory, Anaphylatoxin, *In silico*

INTRODUCTION

Beetroot, scientifically known as *Beta vulgaris* L., is a root vegetable originating from the Mediterranean region and North Africa and belongs to the Amaranthaceae family. Beetroot is generally classified into two main types, namely white beetroot and red beetroot. Among these, red beetroot is more commonly utilized due to its high content of bioactive compounds and natural pigments, making it particularly relevant for pharmacological and biomedical applications [1].

Beetroot has been widely utilized in various industrial sectors, including food, health, and cosmetic products. In addition to its use as a natural coloring agent, red beetroot has also been traditionally used in empirical medicine for its potential health benefits. Several studies have reported that beetroot possesses antioxidant, anti-inflammatory, and cardioprotective properties, which are primarily attributed to its bioactive compounds such as betalains, polyphenols, and nitrates. These pharmacological properties highlight the potential of red beetroot as a promising natural source for drug development and therapeutic applications [1,2].

Beetroot (*Beta vulgaris* L.) contains various antioxidant compounds beneficial for health, including betalains, polyphenols, and vitamins [3]. Betalains are natural pigment compounds predominantly found in plants from the Amaranthaceae and Cactaceae families, such as red beetroot, red spinach, and dragon fruit. The major betalain compounds identified in beetroot include betacyanins (e.g., betanin and isobetanin) and betaxanthins (e.g., vulgaxanthin), which are known for their strong antioxidant, anti-inflammatory, and potential pharmacological activities.

Anaphylatoxin is a small fragment of complement proteins produced during the activation of the complement system, which is part of the innate immune response. These molecules play an important role in inflammatory processes by recruiting and activating various immune cells, including neutrophils, macrophages, and mast cells. The main anaphylatoxins in the complement system are C3a, C4a, and C5a, which contribute to inflammation by enhancing vascular permeability and stimulating the release of inflammatory mediators such as cytokines and chemokines [4]. Considering the involvement of anaphylatoxins in inflammatory responses, identifying potential inhibitors from natural compounds such as beetroot-derived constituents is of considerable interest. Therefore, this study aims to evaluate the potential of beetroot compounds as anaphylatoxin receptor inhibitors using an in silico approach through bioinformatics-based prediction, including PASS Online analysis.

Inflammation is the body's biological response to injury or infection, involving the activation of the immune system, the release of inflammatory mediators, and the recruitment and activation of immune cells such as neutrophils, macrophages, and lymphocytes. Inflammation is triggered by various factors, including the activation of the complement system, which produces anaphylatoxins like C5a. Excessive or uncontrolled inflammation can lead to various chronic diseases, including autoimmune disorders and tissue degeneration. The causes of inflammation include pathogenic infections, tissue injury, activation of the complement system, autoimmune diseases, metabolic disorders, environmental factors, cancer, and metastasis [4].

This approach suggests that modulation of anaphylatoxin activity may represent an effective strategy for controlling inflammation without impairing immune function. Therefore, this study aims to screen and identify bioactive compounds from red beetroot with potential anaphylatoxin inhibitory activity as candidate anti-inflammatory agents. The findings are expected to contribute to drug discovery and development in pharmaceutical and biotechnology fields. To achieve this objective, the screening was performed using an in silico approach through bioinformatics-based prediction, including PASS Online analysis, enabling efficient identification of potential therapeutic compounds prior to laboratory-based validation.

MATERIALS AND METHODS

The foundational stage of our research is centered on a systematic and robust computational workflow designed for data acquisition and the subsequent prediction of biological activity. This entire process is conducted *in silico*, leveraging established and publicly accessible bioinformatics resources to efficiently screen for promising compounds before committing to laboratory experiments.

The methodology commences with the comprehensive gathering of secondary data, specifically focusing on the chemical constituents of the target plants. This data acquisition is performed using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), a vast and authoritative public repository maintained by the National Center for Biotechnology Information (NCBI). From PubChem, detailed information regarding the molecular structure of each identified plant compound is meticulously collected. A critical piece of data retrieved during this phase is the canonical Simplified Molecular Input Line Entry System (SMILES) string for each molecule. This standardized textual

representation of chemical structures is fundamental for the interoperability of various computational chemistry tools and serves as the direct input for the next stage of analysis.

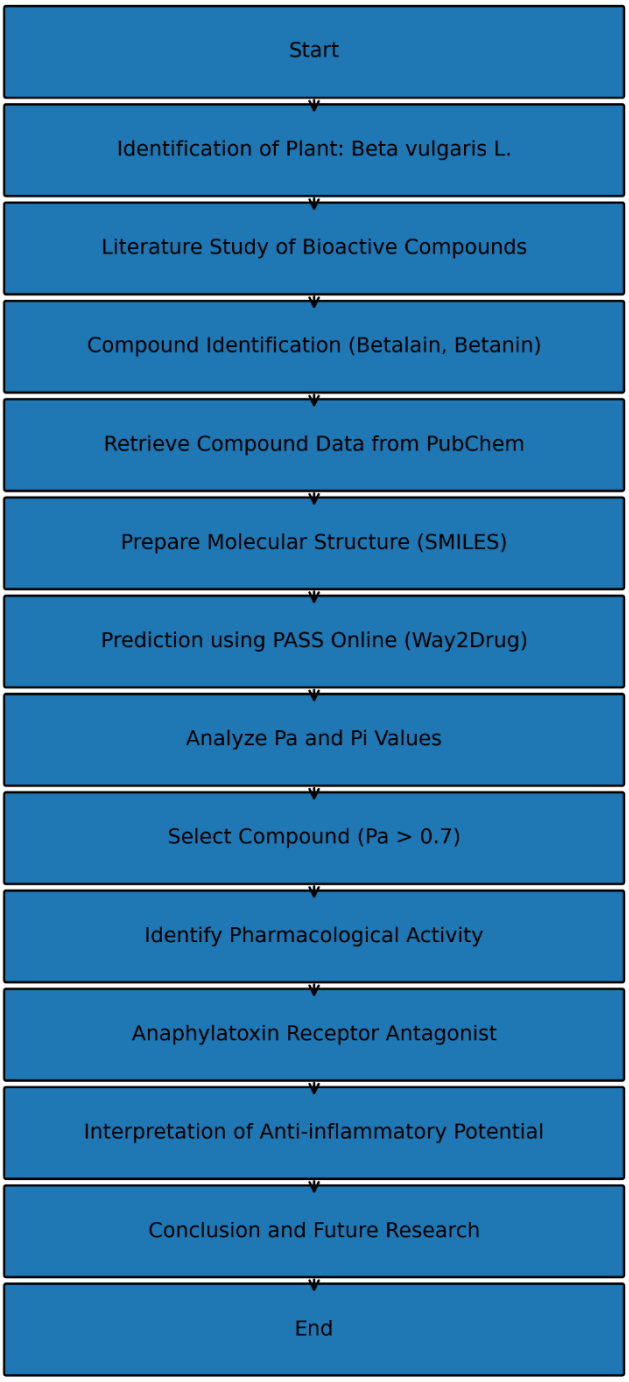

Figure 1. Flow Cart

Following the collection and organization of the compound data, the potential bioactivity of each active compound is thoroughly evaluated. This predictive analysis is carried out using the PASS Online (Prediction of Activity Spectra for Substances) web service, which is accessible via the Way2Drug portal (http://way2drug.com/passonline/). The SMILES data for each plant-derived compound is submitted to this platform, which then calculates the probability of it exhibiting various biological activities based on its structural similarity to a large database of known bioactive molecules.

The final step involves a rigorous selection process to identify the most promising candidates. The PASS Online tool provides two crucial metrics for each predicted activity: the 'Probability to be Active' (Pa) and the 'Probability to be Inactive' (Pi). The primary criterion for shortlisting a compound's potential is based on the direct comparison of

these values. However, to ensure a high level of confidence in the predictions, a more stringent quantitative threshold is applied. For a compound to be considered a highly potent candidate and selected for further investigation, its Pa value must be greater than 0.7. This criterion ensures that only compounds with a very high likelihood of being active are prioritized, effectively refining the selection to the most viable molecules for future experimental validation.

RESULTS AND DISCUSSION

Based on the analysis of bioactive compounds in beetroot (*Beta vulgaris L.*) using PubChem and bioactivity predictions from Way2Drug, various compounds were identified. Among these are potential anaphylatoxin receptor antagonists, which are believed to contribute to the plant's anti-inflammatory properties.

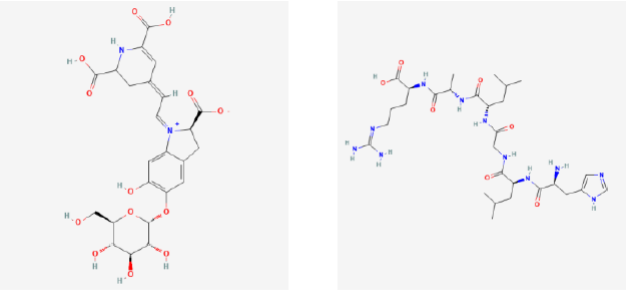

Figure 2. Structure of Betalains and Anaphyloxatin

The predicted bioactive compounds from red beetroot demonstrated potential activity related to anaphylatoxin-mediated inflammatory pathways. Anaphylatoxins, particularly C3a and C5a, are known to stimulate inflammatory responses through oxidative burst activation in immune cells such as macrophages, eosinophils, and neutrophils. In this study, several compounds identified from red beetroot showed predicted inhibitory activity toward anaphylatoxin-related targets, suggesting their potential role in modulating inflammatory responses. The interaction of C3a and C5a with their respective receptors, C3aR and C5aR2, is known to regulate inflammatory signaling pathways, including the suppression of pro-inflammatory cytokines such as TNF-α and IL-1β [4].

These findings indicate that bioactive compounds from red beetroot may contribute to anti-inflammatory effects through modulation of anaphylatoxin signaling pathways. Furthermore, the predicted activities also suggest potential involvement in regulating histamine release and immune cell activation, which are important mechanisms in inflammatory responses. Therefore, the identified compounds from red beetroot may serve as promising candidates for further investigation in the development of anti-inflammatory agents).

Table 1. Predicted Pharmacological Activity of Betalain-Derived Compounds

Compound	Pa	Pi	Pharmacological Activity
Betanin	0.860	0.006	Anaphylatoxin Receptor Antagonist

Table 1 presents the predicted pharmacological activity of specific betalain-derived compounds obtained from red beetroot (*Beta vulgaris* L.). Unlike the general term "betalains," which refers to a class of pigments, this study focused on individual betalain compounds to ensure accurate in silico prediction. Betanin, one of the major betalain constituents in red beetroot, was selected for analysis due to its well-documented presence and biological relevance. The prediction results were generated using PASS Online, a computational tool that estimates biological activity based on the structural similarity of compounds to known pharmacologically active molecules.

The prediction results indicate that betanin exhibits a high probability of acting as an anaphylatoxin receptor antagonist, with a Pa value of 0.860 and a Pi value of 0.006. A Pa value greater than 0.7 generally indicates a high likelihood of biological activity, suggesting that betanin may possess significant anti-inflammatory potential. The low Pi value further strengthens this prediction by indicating a minimal probability of inactivity. These findings suggest that betanin may modulate inflammatory pathways associated with anaphylatoxin signaling.

This result highlights the potential of betanin as a promising candidate for anti-inflammatory drug development. Given that anaphylatoxin receptors such as C3aR and C5aR play key roles in inflammatory responses, compounds capable of antagonizing these receptors may help regulate inflammation without compromising immune

function. Therefore, the predicted activity of betanin supports further investigation through molecular docking, molecular dynamics simulation, and experimental validation to confirm its pharmacological potential.

The predicted activity as an anaphylatoxin receptor antagonist suggests significant therapeutic potential for the identified betalain-derived compound. Anaphylatoxins, particularly C3a and C5a, are components of the complement system involved in innate immune responses. Although essential for host defense, excessive activation of these molecules may lead to uncontrolled inflammatory responses and contribute to chronic inflammatory and autoimmune diseases, including rheumatoid arthritis, sepsis, and vasculitis. Therefore, compounds capable of antagonizing anaphylatoxin receptors, such as C3aR and C5aR, may help regulate inflammation by preventing the transmission of pro-inflammatory signals.

Based on the PASS Online prediction, the analyzed compound demonstrated a high probability of activity with a Pa value of 0.860, which corresponds to an 86% likelihood of exhibiting anaphylatoxin receptor antagonist activity. Meanwhile, the Pi value of 0.006 indicates a very low probability of inactivity. It is important to note that Pa and Pi values are calculated independently within the PASS algorithm and are not complementary probabilities that sum to 100%. The substantial difference between Pa and Pi further strengthens the prediction that the compound may possess significant anti-inflammatory potential.

The predicted antagonistic activity may contribute to the inhibition of several downstream inflammatory processes, including immune cell recruitment, histamine release from mast cells, and oxidative burst in neutrophils and macrophages. Such targeted modulation of inflammatory pathways is advantageous in modern drug discovery, as it may reduce excessive inflammation without completely suppressing immune function. Furthermore, the use of computational screening through in silico prediction represents an efficient and cost-effective approach for identifying potential therapeutic candidates prior to experimental validation. These findings highlight the potential of betalain-derived compounds from red beetroot as promising candidates for further investigation in anti-inflammatory drug development.

C5a has a more potent effect than C3a in stimulating inflammatory responses. C3a can have anti-inflammatory effects in some conditions, while C5a is more dominant in triggering inflammation. C3a aids in B-cell differentiation and antibody production, whereas C5a plays a role in the expansion of Th1 cells and the regulation of cellular immune responses [4]. In cases of tissue injury or infection, limited activation of C3a and C5a can prevent excessive inflammation, which helps in the healing process without causing additional tissue damage. Under normal conditions, C3a and C5a help maintain the balance between osteoclasts and osteoblasts, which is important for preventing osteoporosis and excessive bone inflammation.

C5a is a key inflammatory mediator within the complement system that contributes to vasodilation, immune cell chemotaxis, cytokine release, and oxidative burst, thereby amplifying inflammatory responses. Excessive activation of C5a has been associated with various chronic inflammatory and autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus. Therapeutic strategies targeting C5a or its receptor (C5aR1), such as the clinically approved drug avacopan, have demonstrated effectiveness in reducing inflammation without completely suppressing immune function [5]. In this context, the predicted anaphylatoxin receptor antagonist activity of betalain-derived compounds from red beetroot suggests their potential to modulate inflammatory pathways through mechanisms similar to C5a inhibition. Therefore, these compounds may serve as promising candidates for further development as anti-inflammatory agents.

Despite the promising findings, this study has several limitations. The predicted pharmacological activity was obtained using an in silico approach based on two-dimensional structure–activity relationship (SAR) analysis. Therefore, the actual three-dimensional binding affinity between the identified compounds and target receptors remains unknown. Furthermore, the biological activity predicted by PASS Online does not account for pharmacokinetic properties, receptor binding stability, or biological complexity in vivo. Consequently, further validation studies, including molecular docking, molecular dynamics simulations, and in vitro or in vivo experiments, are necessary to confirm the predicted anti-inflammatory potential of betalain-derived compounds from red beetroot.

CONCLUSIONS

This in silico study successfully identified the potential of betalain-derived compounds from red beetroot (*Beta vulgaris* L.) as modulators of anaphylatoxin-mediated inflammatory pathways. The prediction results obtained using PASS Online demonstrated that the analyzed compound, betanin, exhibited a high probability of activity as an anaphylatoxin receptor antagonist, with a Pa value of 0.860 and a Pi value of 0.006. These findings indicate that betanin has a strong likelihood of inhibiting anaphylatoxin signaling, particularly involving C3a and C5a pathways, which are known to play important roles in inflammatory responses. The high probability score suggests that betalain-derived

compounds, especially betanin, may serve as promising candidates for the development of natural-based anti-inflammatory agents. This potential supports the exploration of red beetroot as a valuable natural source for drug discovery targeting inflammatory diseases. However, this study has limitations, as the findings are based solely on in silico prediction using a two-dimensional structure–activity relationship (SAR) approach. Therefore, further validation through molecular docking, molecular dynamics simulations, and in vitro or in vivo experimental studies is necessary to confirm the binding affinity and pharmacological activity of the identified compounds.

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