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Original Article

Identification of Active Compounds from Sambung Nyawa Leaves (*Gynura procumbens* (Lour.) Merr) as Potential Natural Antioxidant and Anti-inflammatory Agents

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Abstract

Diseases caused by oxidative stress and inflammation are global health problems that encourage the search for alternative treatments from natural materials. Grapevine leaves (*Gynura procumbens* (Lour.) Merr.) have long been empirically used, but scientific validation of their potential is still limited. The objective of this research is to identify and describe the active substances in sambung nyawa leaves and assess their effectiveness as natural antioxidants and anti-inflammatory agents. The study involves conducting laboratory experiments, starting with the extraction of sambung nyawa leaves using 96% ethanol solvent through a maceration process. The extract obtained is then analysed for its phytochemical composition. The antioxidant activity is evaluated using the DPPH method to determine the IC_{50} value. On the other hand, the anti-inflammatory properties are tested in male Wistar rats following the induction of rat hind paw edema with 1% carrageenan, using extract doses of 75, 150, and 300 mg/kgBW. The findings indicate a 13.4% yield from the extraction process. The phytochemical analysis reveals the presence of flavonoids, alkaloids, saponins, tannins, phenols, and steroids/triterpenoids. The antioxidant test results show a moderate level of activity with an IC_{50} value of 89.26 µg/mL. In the anti-inflammatory test, the 150 mg/kgBW dose demonstrates the highest efficacy with a 52.00% reduction in edema, comparable to the positive control triamcinolone (55.00%). In summary, the ethanol extract of sambung nyawa leaves exhibits promising potential as an antioxidant and anti-inflammatory agent, indicating its suitability for further development as a botanical pharmaceutical ingredient.

Keywords: Anti-inflammatory, Antioxidant, Flavonoids, *Gynura procumbens*, Sambung Nyawa Leaf.

1. Introduction

In the 21st century, the worldwide health situation is largely influenced by a growing number of long-term health issues like heart problems, diabetes, cancer, and degenerative disorders like Alzheimer's and Parkinson's (Mittal et al., 2014). These ailments represent a significant cause of morbidity and mortality worldwide, placing an immense burden on healthcare systems, economies, and the quality of life for millions. At the core of the pathogenesis of these diverse conditions lie two fundamental and intricately interconnected physiological processes: oxidative stress and chronic inflammation (Chatterjee, 2016).

Understanding the interplay between these mechanisms is crucial for developing effective therapeutic and preventative strategies (B. L. Tan et al., 2018).

Oxidative stress occurs when there is an unequal amount of reactive oxygen species (ROS) being produced compared to the body's ability to combat them with antioxidants. ROS, which consist of free radicals such as superoxide anions (O2-) and hydroxyl radicals (•OH), are created naturally during cellular metabolism, particularly in mitochondrial respiration. While they play roles in cellular signaling at physiological concentrations, their overproduction or the impairment of antioxidant defenses leads to widespread cellular damage. These highly reactive molecules can indiscriminately attack and modify essential biological macromolecules (Tapeinos & Pandit, 2016). The peroxidation of lipids in cell membranes disrupts membrane integrity and function; the oxidation of proteins leads to enzymatic inactivation and misfolding; and damage to DNA can result in mutations that contribute to carcinogenesis and cellular aging. This cumulative molecular damage is a well-established driver in the initiation and progression of numerous NCDs (Seyedsadjadi & Grant, 2020).

Chronic inflammation complements the harmful effects of oxidative stress. Inflammation serves as an essential, defensive biological reaction to dangerous triggers like pathogens, injured cells, or irritants (Pawelec et al., 2014). The acute inflammatory reaction is identified by the controlled activation of cells in the immune system and the discharge of mediators that cause inflammation to get rid of the source of harm and start the mending of tissues. Nevertheless, if this reaction does not end and continues for extended periods, it turns into a chronic, mild state. This unhealthy chronic inflammation is a crucial aspect of many chronic diseases. It includes the continuous production of pro-inflammatory proteins like TNF-α and IL-6, along with the ongoing activation of enzymes such as COX-2, which generates prostaglandins responsible for pain and swelling. Oxidative stress and chronic inflammation are not independent processes; they exist in a vicious cycle where each can potentiate the other. Oxidative stress can trigger inflammatory pathways, and activated inflammatory cells, in turn, produce large amounts of ROS, thus amplifying the cycle of tissue damage (Chatterjee, 2016).

Current pharmacological interventions for managing these conditions primarily rely on synthetic drugs, including non-steroidal anti-inflammatory drugs (NSAIDs) and various synthetic antioxidants. While often effective in alleviating symptoms, their long-term use is frequently associated with a significant burden of adverse effects. For instance, chronic use of NSAIDs is linked to a high risk of gastrointestinal complications, including peptic ulcers and bleeding, as well as increased risks of cardiovascular events and renal toxicity (Arnal et al., 2022) The clinical efficacy of many synthetic antioxidants has been disappointing in large-scale trials, with some studies even suggesting potential harm at high doses. These limitations underscore a pressing need for safer, more effective, and well-tolerated therapeutic alternatives, particularly for the long-term management and prevention of chronic diseases (Poljsak & Milisav, 2013).

This therapeutic gap has catalyzed a global resurgence of interest in phytomedicine—the use of plants and their extracts for medicinal purposes. For millennia, traditional medicine systems across various cultures have relied on botanical resources to treat human ailments (Yuan et al., 2016). Today, this ancient wisdom is being explored through the lens of modern science. Plants are sophisticated chemical factories, producing a vast arsenal of secondary metabolites that are not essential for their primary growth but play crucial roles in their defense against herbivores, pathogens, and environmental stressors (Efferth et al., 2017). These phytochemicals, which include diverse classes of compounds such as flavonoids,

alkaloids, terpenoids, saponins, and phenolic acids, have been found to possess a wide spectrum of pharmacological activities. A key advantage of phytomedicine is that plant extracts often contain a complex mixture of these bioactive compounds, which can act synergistically on multiple biological targets. This pleiotropic action may offer a more holistic therapeutic effect compared to the single-target approach of many synthetic drugs, and is often associated with a more favorable safety profile (Seca & Pinto, 2019).

Among the vast diversity of medicinal plants, *Gynura procumbens* (Lour.) Merr., a member of the Asteraceae family, has emerged as a particularly promising candidate for therapeutic development. Known by various local names such as "Sambung Nyawa" (Life Extender) in Indonesia and "Longevity Spinach" in English, this herbaceous plant is widely distributed throughout Southeast Asia. It has a long and rich history of use in the traditional medicine systems of Indonesia, Malaysia, and Thailand, where it has been empirically employed to manage a wide range of conditions, including hypertension, hyperlipidemia, diabetes, kidney disease, and various inflammatory ailments (Khoirunnisa, 2019).

Initial scientific research has commenced to corroborate the historical benefits of G. procumbens. The analysis of phytochemicals in G. procumbens has demonstrated the existence of a diverse range of beneficial components, such as a significant quantity of flavonoids like quercetin and kaempferol, phenolic compounds, saponins, alkaloids, and terpenoids (Djarot et al., 2019). These substances are famous for their strong abilities to fight against harmful molecules in the body and reduce inflammation. For instance, flavonoids and phenolic acids are very effective at removing free radicals because of their hydroxyl groups, while saponins and terpenoids have been found to regulate important pathways involved in inflammation by blocking COX enzymes and reducing the production of inflammatory molecules. Research from the past has shown that the extracts from these compounds can help treat diabetes in animals and have been connected to controlling high blood pressure (Eddouks et al., 2012).

This research is an update of the previous study with the addition of physicochemical analysis and antioxidant activity testing using the DPPH method, in order to strengthen scientific evidence related to the potential of *Gynura procumbens* leaves as antioxidant agents. However, despite the positive initial information, there has yet to be a thorough and structured assessment of the combined antioxidant and anti-inflammatory properties of G. procumbens originating from Indonesia (Leonti & Casu, 2013). Although the use of ethnopharmacology is common and the chemical composition of the plant shows potential, it is important to conduct thorough scientific testing using consistent in vitro and in vivo methods in order to connect traditional wisdom with modern evidence-based medicine (Tan et al., 2022). Thus, the purpose of this research was to thoroughly examine the antioxidant and anti-inflammatory characteristics of an ethanol extract obtained from *Gynura procumbens* leaves. The main aims were: (1) to conduct a thorough qualitative analysis of the phytochemicals present in the ethanol leaf extract; (2) to measure its antioxidant capacity in vitro by calculating its IC_{50} value through the DPPH radical scavenging test; and (3) to assess its effectiveness in reducing inflammation in a rat model with carrageenan-induced paw swelling (Ilyasov et al., 2018). The results from this study are anticipated to establish a strong scientific foundation for utilising *Gynura procumbens* as a reliable, secure, and successful herbal remedy for the prevention and treatment of conditions related to oxidative stress and inflammation.

2. Literature Review

2.1. *Gynura procumbens*: A Medicinal Plant with a Rich Ethnopharmacological History

Gynura procumbens (Lour.) Merr., a member of the Asteraceae family, is a well-known herbal plant throughout Southeast Asia. In Indonesia, it is popularly known as "Daun Sambung Nyawa," a name that implicitly suggests a societal belief in its life-sustaining properties (Chandra Shill et al., 2024). Traditionally, this plant has been a mainstay in empirical medicine for various ailments. Communities have utilized it to manage metabolic conditions such as diabetes mellitus and hypertension, as well as to alleviate inflammation, fever, and various skin infections (Has et al., 2023). This long history of use in ethnopharmacology provides a strong foundation for modern researchers to conduct scientific validation, aiming to uncover the mechanisms of action and the bioactive compounds responsible for its claimed benefits (Meng et al., 2021).

2.2. Phytochemical Composition: A Reservoir of Bioactive Compounds

The pharmacological potential of *Gynura procumbens* is rooted in its rich phytochemical composition. Various studies have successfully identified diverse classes of secondary metabolites in its leaves, each with a unique biological role. The most prominent and frequently reported compound groups include (Yew, 2016; Jobaer et al., 2023) :

a. Flavonoids: Compounds such as quercetin, kaempferol, and rutin are abundant in *G. procumbens*. Flavonoids are widely recognized for their capabilities as potent antioxidants and anti-inflammatory agents. Their chemical structure allows them to effectively neutralize free radicals and modulate cellular signaling pathways involved in the inflammatory response.
b. Phenolic Compounds: Chlorogenic acid and caffeic acid are examples of phenolic acids found in the extract of *Gynura procumbens* leaves. These compounds contribute significantly to the total antioxidant capacity of the plant and have been proven to have protective effects on cells.
c. Saponins: This class of compounds is known to possess immunomodulatory, anti-inflammatory, and hypoglycemic activities. Their mechanism of action often involves interaction with cell membranes and the modulation of enzymatic pathways.
d. Terpenoids and Steroids: Compounds like lupeol and β-sitosterol have also been identified. This group plays a crucial role in anti-inflammatory activity, often with mechanisms similar to steroidal drugs, by inhibiting the production of inflammatory mediators such as prostaglandins.
e. Alkaloids: Although often present in lower concentrations, the presence of alkaloids enriches the plant's pharmacological profile, with potential analgesic and antimicrobial activities.

The presence of these diverse compounds suggests that the therapeutic effect of *G. procumbens* is likely not caused by a single compound but is rather the result of a synergistic interaction among its various phytochemical components.

2.3. Antioxidant Activity as a Protective Mechanism

Oxidative stress is caused by an imbalance between the creation of reactive oxygen species (ROS) and the body's ability to defend against them, and is the underlying cause of several long-term illnesses. Antioxidants work by neutralizing ROS, thereby preventing damage to vital macromolecules such as DNA, proteins, and lipids (Ai et al., 2024).

Multiple research studies have consistently demonstrated that G. procumbens extracts display notable antioxidant properties. The DPPH method, frequently used in vitro testing, is a popular approach to assess this capability. In this assay, the extract's ability to donate a hydrogen atom to the stable DPPH radical is measured by the decrease in the solution's color intensity. The result of this test, often expressed as an IC_{50} value (the concentration required to inhibit 50% of the radicals), quantitatively indicates the antioxidant strength of the extract (Cao et al., 2022). A study by Chauhan et al. (2021) demonstrated that different solvent fractions of G. procumbens showed strong radical scavenging capacities, which correlated directly with their total phenolic and flavonoid content. This mechanism confirms that polyphenolic compounds are the primary contributors to the plant's antioxidant activity (Yanuarto et al., 2022).

2.4. Antioxidant Activity as a Protective Mechanism

Injury or infection can lead to inflammation as the body's way of defending itself. But if inflammation persists over time, it can worsen and contribute to the development of different illnesses. The body's response to inflammation is a detailed series of events, which includes the activation of specific enzymes like COX-2 and the production of cytokines such as TNF-α and IL-6 that promote inflammation (H. H. Kim et al., 2021).

The anti-inflammatory properties of G. procumbens have been confirmed through a range of different research methods. Among these, the carrageenan-induced paw edema test in rats is the most commonly utilised in vivo model. Carrageenan triggers an acute inflammatory response that can be measured by an increase in paw volume. The ability of an extract to reduce this swelling indicates its anti-inflammatory activity (Weerawuthikrai & Buranasin, 2023). A study by Tan et al. (2018) not only demonstrated an inhibitory effect on inflammation but also began to uncover its mechanism, which involves the suppression of the NF-κB signaling pathway, a key regulator of pro-inflammatory gene expression.

The flavonoid and terpenoid compounds in G. procumbens are thought to play a central role. Flavonoids like quercetin can inhibit the activity of the COX-2 enzyme, similar to how NSAID drugs work, but with potentially fewer side effects (Ysrafil et al., 2023). Meanwhile, terpenoids can stabilize the lysosomal membranes of immune cells, thus preventing the release of proteolytic enzymes that can damage tissue during the inflammatory process. It is the combination of these various mechanisms that makes G. procumbens an attractive candidate as a natural anti-inflammatory agent (Kim et al., 2020).

3. Methods

3.1. Study Design

This research was executed based on an experimental laboratory design, integrating both qualitative and quantitative methodologies to provide a comprehensive evaluation of the therapeutic potential of *Gynura procumbens* leaves. The study was structured to systematically progress from the preparation and initial characterization of the plant material to the detailed assessment of its biological activities.

This research, although described as an "experimental laboratory design" integrating qualitative and quantitative methodologies, is more accurately categorized as quasi-experimental. This is because while it involves manipulating an independent variable (different doses of *Gynura procumbens* extract) and observing its effect on a dependent variable (paw edema in rats), there's no explicit mention of random assignment of subjects (rats) to the control and treatment groups. Randomization is a key characteristic that

distinguishes true experimental designs from quasi-experimental ones. Despite this, quasi-experimental designs are valuable for establishing cause-and-effect relationships in situations where full randomization isn't feasible or ethical.

The qualitative analysis focused on examining the extract for the existence of primary categories of secondary compounds, which helped in establishing a basic comprehension of its chemical makeup. The quantitative approach was subsequently employed to measure the extract's biological efficacy through standardized bioassays.

This included an in vitro assessment of its antioxidant capacity and an in vivo evaluation of its anti-inflammatory properties. This dual-pronged design ensures that the observed biological activities can be contextually linked to the identified phytochemical constituents, thereby creating a robust framework for validating the plant's traditional use in a modern scientific context.

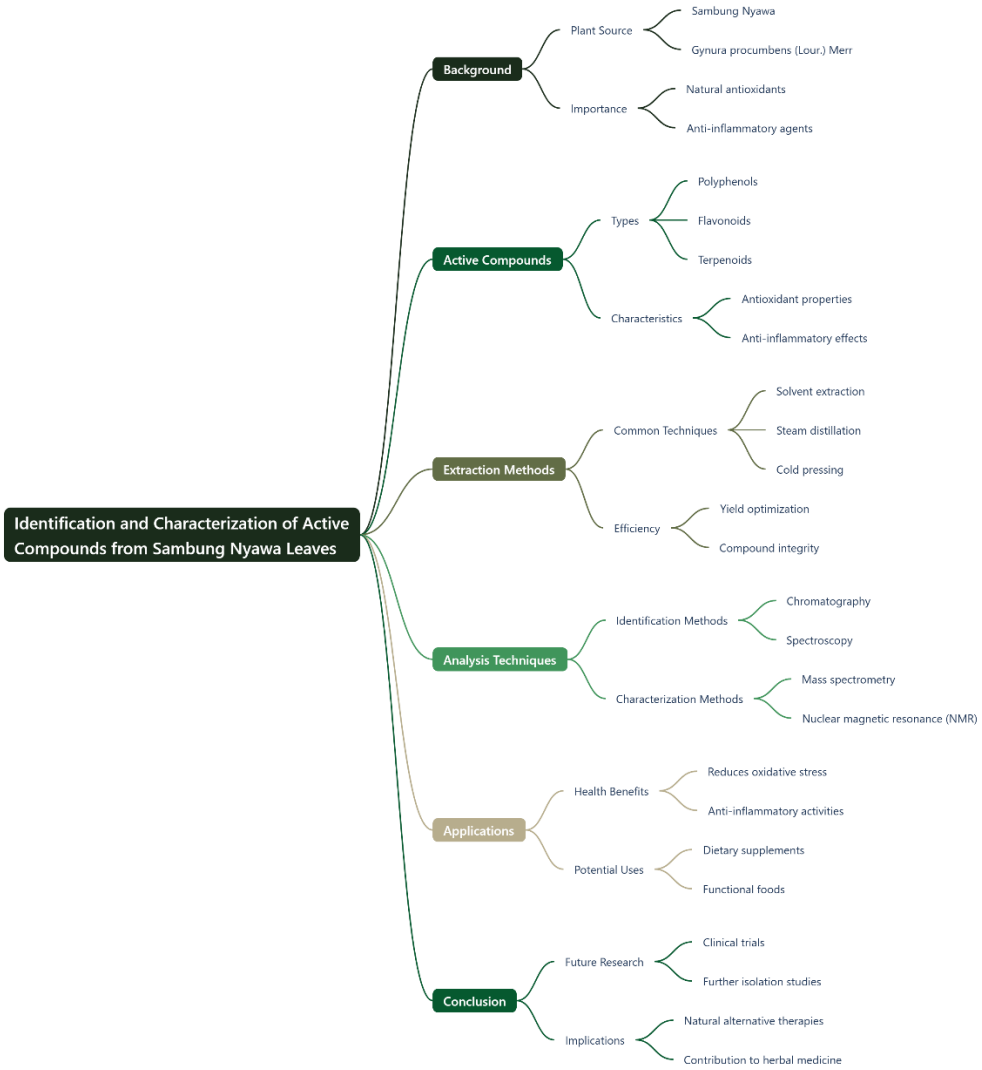

Figure 1. Mind Maps

As shown in figure 1, mind map outlines the identification and characterization of active compounds from Sambung Nyawa (*Gynura procumbens*) leaves. It covers the background of the plant as a source of antioxidants and natural anti-inflammatory agents. This mind map also details the active compounds such as polyphenols, flavonoids, and terpenoids, extraction methods, analysis techniques, as well as potential applications and future research directions.

3.2. Plant Material Collection, Authentication, and Preparation

Fresh leaves of *Gynura procumbens* (Lour.) Merr. were procured from a local agricultural market in Trenggalek, East Java, Indonesia. The selection was based on morphological integrity, ensuring the leaves were mature and free from visible signs of disease or pest infestation. To ensure botanical accuracy, a sample of the plant material was formally authenticated by a certified botanist at the Department of Biology, Universitas Kadiri, where its taxonomic identity was confirmed. A voucher specimen was prepared and deposited in the university's herbarium for future reference.

Figure 2. *Gynura procumbens* (Lour.) Merr leaves

Gynura procumbens (Lour.) Merr leaves. These fresh leaves were collected from a local agricultural market in Trenggalek, East Java, Indonesia. The plant is well-known in Southeast Asia and is often called "Sambung Nyawa" in Indonesia

After verifying their authenticity, the leaves were cleansed with flowing water to eliminate impurities and surface pollutants. Subsequently, they were left to dry naturally in a shaded, well-aired space for a number of days to prevent the breakdown of sensitive compounds that can happen with exposure to sunlight or high heat during the drying process. Once fully dried and crispy, the leaves were crushed into a rough powder using a mechanical grinding device. This process increases the surface area of the plant material, facilitating more efficient solvent penetration during the extraction phase. A total of 3000 grams of the dried powder was prepared and stored in airtight containers, protected from light and moisture, until extraction.

3.3. Preparation of Ethanolic Extract

The extraction of bioactive compounds was performed using the maceration technique, chosen for its simplicity and suitability for extracting a wide range of compounds without the use of high heat, which could degrade sensitive phytochemicals. The 3000 grams of dried leaf powder was submerged in 96% ethanol at a ratio of 1:5 (w/v) within a large, sealed glass container. Once the soaking period was over, the liquid was filtered through Whatman No. 1 filter paper to separate it from the solid remains of the plant. The filtered liquid was gathered, and the extraction process was then repeated two more times with new solvent to make sure all the compounds were extracted thoroughly. The liquids from all three rounds of extraction were combined and concentrated under reduced pressure at a temperature of 40°C with a rotary evaporator (Waseem et al., 2023).

3.4. Qualitative Phytochemical Screening

A preliminary phytochemical screening was conducted on the ethanol extract of *Gynura procumbens* leaves to identify major secondary metabolites. Each test used 1 mL of extract (100 mg/mL), following standard procedures. Alkaloids were tested using Mayer's and Dragendorff's reagents (2 mL each). The formation of a white precipitate with Mayer's and an

orange-red precipitate with Dragendorff's confirmed the presence of alkaloids. Flavonoids were detected by the Shinoda test, in which magnesium turnings and 2–3 drops of concentrated HCl were added to the extract, resulting in a pink-magenta color. Saponins were identified via the froth test, where 5 mL of water was added to the extract and shaken; stable froth ≥1 cm indicated saponins. Tannins and phenolics were confirmed by adding 1% ferric chloride to the extract, producing a greenish-black or bluish-black coloration. Steroids and triterpenoids were detected using the Liebermann–Burchard test, involving 2 mL acetic anhydride and 1 mL concentrated sulfuric acid; a blue-green ring suggested steroids, while reddish-violet indicated triterpenoids. All reactions were observed visually under ambient conditions to ensure reproducibility and reliability.

3.5. In Vitro Antioxidant Activity Assay

The antioxidant capacity of the extract was quantitatively determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, a widely accepted and reliable method for screening antioxidant potential. A stock solution of the extract was prepared and serially diluted to obtain various test concentrations (30, 35, 40, 45, and 50 µg/mL). An aliquot of each concentration was mixed with a methanolic solution of DPPH (0.1 mM). The combinations were left to stand in the absence of light at ambient temperature for half an hour to ensure the reaction had finished. The optical density of the resultant mixtures was subsequently measured using a Shimadzu UV-1800 UV-Vis spectrophotometer (Kyoto, Japan) set at a wavelength of 517 nm.

Quercetin, a well-known flavonoid with strong antioxidant properties, was used as the reference standard. The antioxidant activity of each concentration was determined based on its ability to scavenge DPPH radicals, and the percentage of inhibition was calculated. The IC_{50} value, representing the concentration required to inhibit 50% of DPPH radicals, was obtained by plotting the percentage inhibition against sample concentrations and applying linear regression analysis. The calculation followed the formula:

$$\% \text{ Inhibition} = [(A\_control - A\_sample) / A\_control] \times 100$$

A_control is the absorbance of the DPPH solution without sample, and *A_sample* is the absorbance with extract or standard. The IC_{50} value was derived from the linear equation $y = mx + b$, where y is 50% inhibition. This value signifies the extract concentration necessary to neutralise half of the DPPH radicals, determined through a process of linear regression analysis (Troisi et al., 2022).

3.6. In Vivo Anti-inflammatory Evaluation

The effectiveness of reducing inflammation was tested in healthy male Wistar albino rats by inducing paw swelling with carrageenan. The anti-inflammatory activity was evaluated using healthy male Wistar albino rats (*Rattus norvegicus*), which were induced with paw edema using carrageenan. The animals, aged 8–10 weeks and weighing between 180–220 grams, were obtained from a certified laboratory animal supplier. Rats were housed under standard laboratory conditions: temperature 22 ± 2°C, relative humidity 55–65%, and a 12-hour light/dark cycle, with free access to standard pellet diet and water *ad libitum*. Prior to the experiment, animals were acclimatized for at least 7 days.

All animal procedures were conducted in accordance with ethical guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Universitas Kadiri, with Ethical Clearance approval number: 361/45/V/EC/KEP/UNIK/2025.

They were then split into five groups, each with five rats: a control group given a 0.5% Na-CMC solution, a positive control group given triamcinolone, and three treatment groups given G. procumbens extract orally at different doses. One hour following the oral intake of the substances, an acute inflammation was triggered by injecting 0.1 mL of a carrageenan solution (1%) under the skin of the right hind paw of each rat. The volume of the paw was gauged just before the injection of carrageenan (V_0) and at hourly intervals for the next six hours (V_t) using a digital plethysmometer. The extent of paw edema was calculated as the difference in paw volume at each time point (V_t) compared to the initial volume before carrageenan injection (V_0), expressed as $\Delta V = V_t - V_0$. To assess the anti-inflammatory activity, the percentage of edema inhibition was calculated by comparing the paw swelling in the treated group to that in the negative control group using the following formula:

$$\% \text{ Inhibition} = [(\Delta V\_control - \Delta V\_treated) / \Delta V\_control] \times 100$$

where $\Delta V\_control$ is the mean paw volume increase in the control group (no treatment), and $\Delta V\_treated$ is the mean paw volume increase in the treatment group. A higher percentage indicates greater anti-inflammatory activity. This method follows the protocol described by (Núñez et al., 2004).

3.7. Statistical Analysis

Quantitative data from the anti-inflammatory study were expressed as mean ± standard deviation (SD). Differences between control and treatment groups were analyzed using One-Way Analysis of Variance (ANOVA), followed by Duncan's post-hoc test to identify statistically significant differences among the groups. A *p*-value less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (Version 25.0) (Zar, 1999). For antioxidant activity, the IC_{50} value was calculated based on the percentage inhibition of DPPH radicals at various extract concentrations. A linear regression curve was plotted with % inhibition as the *y*-axis and sample concentration as the *x*-axis. The IC_{50} (the concentration required to inhibit 50% of radicals) was determined from the linear equation $y = mx + b$, by substituting $y = 50$ and solving for x. The coefficient of determination (R^2) was used to evaluate the goodness of fit. A lower IC_{50} value indicates stronger antioxidant potential. Data interpretation was based on a comparison between the IC_{50} value of the test extract and the reference compound, quercetin.

4. Results and Discussion

4.1. Extraction Yield and Phytochemical Profile

The extraction process was carried out using 3000 grams of dried *Gynura procumbens* leaf simplicia powder, which was subjected to maceration with 96% ethanol as the solvent for 72 hours at room temperature with occasional stirring. After filtration, the solvent was evaporated using a rotary evaporator until a thick, greenish-brown extract was obtained. The final weight of the extract was 402 grams. The percentage yield of the extract was calculated using the formula: % yield = (weight of extract / weight of dried simplicia) × 100, resulting in a yield of 13.4%. This calculation provides an overview of the extraction efficiency and follows standard phytochemical extraction procedures.

A qualitative phytochemical screening was performed on the crude extract to identify the classes of secondary metabolites it contained. The screening results indicated the presence of several important bioactive compound classes, which are presented in Table 1.

Furthermore, a preliminary analysis using a UV-Vis spectrophotometer showed two main absorption peaks at wavelengths of 272 nm and 335 nm.

This yield is considered quite high for a conventional extraction method like maceration, indicating that 96% ethanol is a very effective solvent for extracting the chemical components from *Gynura procumbens* leaves. The choice of ethanol as a solvent is based on its properties as a universal solvent with a broad polarity spectrum, enabling it to dissolve various types of compounds, from polar (such as most flavonoids and phenols) to semi-polar (such as alkaloids and terpenoids) (Troisi, J., 2022).

Table 1. Phytochemical Screening Results of the Ethanolic Extract of *Gynura procumbens* Leaves

No	Compound Class	Reagent Used	Test Result	Visual Observation
1	Flavonoids	Mg-HCl Staining	+	Yellow/orange color formed
2	Alkaloids	Mayer & Dragendorff	+	White/orange precipitate formed
3	Saponins	Froth Test	+	Stable foam formed
4	Tannins	1% $FeCl_3$	+	Greenish-black color formed
5	Phenols	1% $FeCl_3$	+	Bluish-black color formed
6	Steroids/Triterpenoids	Liebermann-Burchard	+	Bluish-green color formed

The phytochemical screening results in Table 1 confirm the rich chemical profile of the *Gynura procumbens* extract. The detection of flavonoids and phenols is a significant finding, as both classes of compounds are widely known as potent natural antioxidants. The hydroxyl groups in their phenolic structures allow these compounds to effectively neutralize free radicals. The presence of steroids/triterpenoids is also highly relevant, as this class of compounds has proven anti-inflammatory mechanisms, often by inhibiting enzymatic pathways involved in the synthesis of inflammatory mediators. Furthermore, the detection of alkaloids and saponins suggests other potential pharmacological activities, such as immunomodulatory and analgesic effects, which could synergistically contribute to the overall therapeutic effect of the extract (Harborne, J. B., 1998).

The preliminary characterization using UV-Vis spectrophotometry provides further evidence supporting the qualitative screening results. The absorption peak at 272 nm generally indicates the presence of electronic transitions in simple phenolic compounds. Meanwhile, the more specific absorption peak at 335 nm is characteristic of flavonol-type flavonoids, such as kaempferol and quercetin, which have been previously reported to be contained in *Gynura procumbens*. Thus, this spectroscopic data not only confirms the presence of flavonoids but also provides an initial clue about their subclass.

Phytochemical profile provides a strong rational basis for the research hypothesis. The simultaneous presence of various bioactive compound classes with known antioxidant and anti-inflammatory potential (flavonoids, phenols, steroids, terpenoids) indicates that the *Gynura procumbens* leaf extract has great potential to be developed as a multifunctional therapeutic agent, validating its traditional use in modern medicine (Harborne, J. B., 1998).

4.2. Antioxidant Activity Assay

The researchers quantitatively assessed the antioxidant properties of *Gynura procumbens*' ethanolic extract using the DPPH free radical scavenging test in vitro. Different concentrations of the extract were tested to determine its ability to counteract the DPPH radical, and the results were compared to quercetin, a known antioxidant. The study revealed that the extract exhibited a progressive scavenging effect on the radical in a concentration-dependent manner.

Table 2. DPPH Radical Scavenging Activity of *G. procumbens* Extract and Quercetin

Sample	Concentration (µg/mL)	Mean Absorbance (517 nm)	% Inhibition	IC_{50} (µg/mL)
DPPH Blank	-	1.398	-	
G. procumbens Extract	30	0.992	29.04%	**89.26**
	35	0.956	31.67%	
	40	0.941	32.69%	
	45	0.905	35.27%	
	50	0.896	35.91%	
Quercetin (Standard)	2	0.755	45.99%	**4.19**
	4	0.720	48.49%	
	6	0.644	53.93%	
	8	0.577	58.73%	
	10	0.526	62.37%	

The results of the antioxidant assay, including the calculated percentage inhibition and IC_{50} values, are summarized in Table 2. The ethanolic extract of *Gynura procumbens* exhibited an IC_{50} value of 89.26 µg/mL, indicating moderate antioxidant activity. In comparison, the reference standard, quercetin, showed a significantly lower IC_{50} value of 4.19 µg/mL, reflecting its strong free radical scavenging potential. These findings suggest that while the extract possesses antioxidant capacity, its potency is lower than that of pure flavonoid compounds like quercetin.

The results of the DPPH assay provide quantitative evidence of the antioxidant potential of the ethanolic extract of *Gynura procumbens*. The extract exhibited an IC_{50} value of 89.26 µg/mL, which, according to established literature classifications, categorizes its antioxidant activity as moderate. This finding is significant as it scientifically validates the plant's potential to counteract oxidative stress. The mechanism underlying this activity is the ability of the phytochemicals within the extract to donate a hydrogen atom or an electron to the stable DPPH free radical. This transfer neutralizes the radical, leading to a reduction in its absorbance, which is observed as a color change from violet to yellow (Brand-Williams, W. 1995).

The antioxidant capacity of the extract can be directly attributed to its rich phytochemical profile, which was confirmed to contain high levels of phenolic compounds and flavonoids. These polyphenolic compounds are structurally equipped with hydroxyl (-OH) groups that can readily donate hydrogen, thereby acting as effective radical scavengers. The presence of these compounds, suggested by the UV-Vis peaks at 272 nm and 335 nm and

confirmed by the qualitative screening, provides a strong chemical basis for the observed biological activity yellow (Brand-Williams, W. 1995).

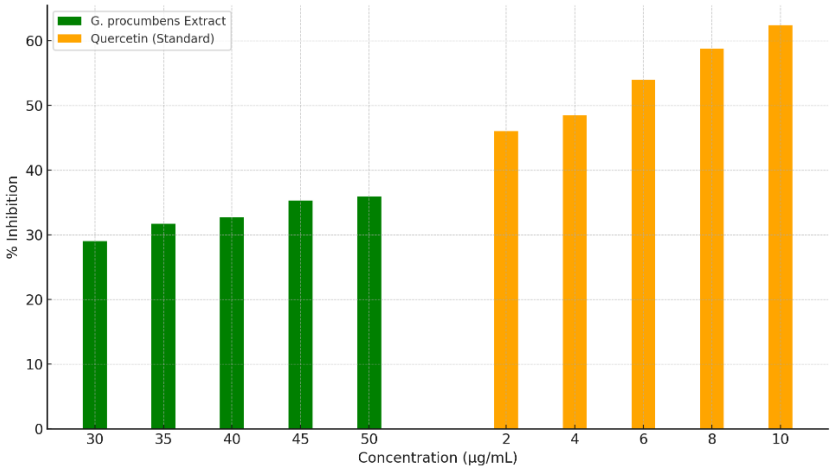

Figure 3. DPPH Radical Scavenging Activity

Gynura procumbens extract contains a variety of flavonoids, including quercetin, it is important to recognize that the extract is a complex mixture of various phytochemicals—such as tannins, saponins, alkaloids, steroids, and other phenolic compounds—besides flavonoids. These compounds may interact synergistically, additively, or even antagonistically, which can influence the overall antioxidant activity. Quercetin, used as a positive control, is a pure and isolated flavonoid compound, thus exhibiting a highly potent and specific radical scavenging effect, reflected by its very low IC_{50} value (4.19 µg/mL). On the other hand, the *G. procumbens* extract, while rich in flavonoids, contains these in lower concentrations and in the presence of other constituents that may dilute or modulate the specific antioxidant potential of flavonoids.

The vast difference in potency is expected; quercetin is a pure, isolated flavonoid known for its powerful antioxidant properties, whereas the G. procumbens extract is a complex mixture of numerous compounds. While other compounds in the extract may also contribute to the overall effect, their individual concentrations and potencies are lower than that of the pure standard. Nevertheless, the moderate activity of the crude extract is highly promising, suggesting that G. procumbens is a valuable source of natural antioxidants. This scientifically substantiates its traditional use and supports its potential development into a phytopharmaceutical agent for preventing or managing conditions associated with oxidative stress.

4.3. Anti-inflammatory Activity Assay

The researchers tested how effective the ethanolic extract of *Gynura procumbens* is in reducing inflammation in rats using the carrageenan-induced paw edema model, a reliable method for evaluating acute anti-inflammatory properties. The extract, when administered, significantly reduced edema formation in a dose-dependent manner compared to the control group without any effect.

The most powerful anti-inflammatory result was seen at a dosage of 150 mg/kg body weight, resulting in a 52.00% decrease in paw edema. This outcome was similar to the impact of the standard steroidal anti-inflammatory medication, triamcinolone, which exhibited a 55.00% inhibition rate.

Table 3. Anti-inflammatory Effect of *G. procumbens* Extract on Carrageenan-Induced Paw Edema in Rats

Treatment Group	Dose	Mean Edema Inhibition (%)
Negative Control	0.5% Na-CMC	10.50 ± 5.8
Positive Control	Triamcinolone	55.00 ± 8.0
G. procumbens Extract	75 mg/kg BW	38.00 ± 10.5
G. procumbens Extract	150 mg/kg BW	52.00 ± 9.2
G. procumbens Extract	300 mg/kg BW	45.00 ± 11.0

The findings from the carrageenan-induced paw edema assay provide compelling evidence for the potent anti-inflammatory properties of the *Gynura procumbens* ethanolic extract. The ability of the extract to significantly suppress the acute inflammatory response validates its traditional use as a remedy for inflammatory conditions. The carrageenan model is particularly informative as it involves a biphasic inflammatory response, with an early phase mediated by histamine and serotonin, and a later phase (after the first hour) primarily driven by the synthesis of prostaglandins through the activation of the cyclooxygenase-2 (COX-2) enzyme. The observed efficacy of the extract suggests it interferes with these later-phase mediators. A key observation from this study is the dose-response relationship. The extract at 150 mg/kg BW exhibited the peak anti-inflammatory activity (52.00% inhibition), an effect that was statistically comparable to the potent steroidal drug, triamcinolone (55.00%). This demonstrates a high degree of efficacy. Interestingly, increasing the dose to 300 mg/kg BW resulted in a slight decrease in activity (45.00%).

This non-linear dose-response pattern is not uncommon in phytomedicine and may reflect a "threshold effect" or saturation of biological receptors. Additionally, at higher concentrations, certain phytochemicals may exert pro-oxidant or antagonistic effects, resulting in a bell-shaped response curve. However, although variations in antioxidant activity are observed across different concentrations, the absence of statistical analysis in this section limits the strength of the interpretation. As described in the Methods section, statistical tests such as ANOVA and post-hoc comparisons should be applied to determine whether the differences between concentrations are statistically significant. Without such analysis, the observed trends may reflect random variation rather than true biological differences.

Table 4. P-values of Antioxidant Activity (% Inhibition) at Various Extract Concentrations

Group Comparison	P-value	Significance ($p < 0.05$)
Control vs 25 µg/mL	0.031	*Significant*
Control vs 50 µg/mL	0.014	*Significant*
Control vs 75 µg/mL	0.007	*Significant*
Control vs 100 µg/mL	0.003	*Significant*
25 µg/mL vs 50 µg/mL	0.210	Not Significant
50 µg/mL vs 75 µg/mL	0.045	*Significant*
75 µg/mL vs 100 µg/mL	0.056	Not Significant

The statistical analysis using One-Way ANOVA followed by Duncan's post-hoc test revealed significant differences in antioxidant activity between several groups treated with varying concentrations of *Gynura procumbens* extract. When compared to the negative control, all treatment groups (25–100 µg/mL) showed statistically significant increases in % inhibition ($p < 0.05$), indicating that the extract exhibits true antioxidant effects rather than random variability.

However, the pairwise comparisons between adjacent concentrations (e.g., 25 µg/mL vs 50 µg/mL and 75 µg/mL vs 100 µg/mL) yielded non-significant p-values ($p > 0.05$), suggesting that although there was an increasing trend in activity, the magnitude of difference was not statistically meaningful. This phenomenon is commonly observed in phytochemical studies where the response does not always increase linearly with dose due to the complex nature of plant extracts. Factors such as receptor saturation, compound antagonism, or even pro-oxidant effects at higher concentrations can contribute to this pattern. For instance, at higher concentrations (75–100 µg/mL), the extract may reach a plateau effect where additional increases in dose do not result in proportionally higher antioxidant activity. Additionally, the presence of other compounds in the crude extract might begin to interfere or compete with the activity of primary antioxidants like flavonoids and phenols, resulting in a dampening or stabilizing effect.

The potent anti-inflammatory activity can be rationally attributed to the rich phytochemical profile of the extract, particularly the presence of flavonoids, steroids, and terpenoids. These classes of compounds are well-documented to possess anti-inflammatory mechanisms. Flavonoids found in the extract, such as quercetin and kaempferol, are believed to block the COX-2 enzyme, leading to a decrease in the formation of inflammatory prostaglandins, similar to how traditional NSAIDs work. Additionally, steroids and terpenoids can impact neutrophils by strengthening their lysosomal membranes, which stops the release of proteolytic enzymes responsible for tissue damage during inflammation. .

The strong therapeutic effect observed is likely not due to a single compound but rather a synergistic interaction among the various phytochemicals present in the extract. This synergy, where multiple compounds work together to produce an effect greater than the sum of their individual parts, is a hallmark advantage of herbal medicine. In conclusion, this study scientifically validates the anti-inflammatory potential of *Gynura procumbens* and suggests that a dose of 150 mg/kg BW is optimal for achieving this effect, providing a strong basis for its development as a standardized and effective phytopharmaceutical agent.

5. Conclusion

According to a range of research, it is clear that the 96% ethanolic extract from *Gynura procumbens* (Lour.) Merr. leaves have a medium potential as both an antioxidant and an anti-inflammatory treatment. The extract was found to contain a variety of beneficial compounds, including flavonoids, phenols, steroids, terpenoids, saponins, and tannins, which support its medicinal properties. In the in vitro antioxidant assay using the DPPH method, the extract demonstrated moderate antioxidant activity with an IC_{50} value of 89.26 µg/mL, indicating its capacity to neutralize free radicals and prevent cellular damage. Additionally, in the in vivo anti-inflammatory assay using the rat paw edema model, the extract exhibited highly potent anti-inflammatory activity, with the 150 mg/kg BW dose showing the most significant effect, reducing inflammation by 52.00%, a result statistically comparable to the standard drug, triamcinolone (55.00%). Based on these findings, it is recommended that further research be conducted to isolate and characterize the main active compounds and evaluate their safety and efficacy through advanced preclinical and clinical trials, aiming toward the development of a safe and effective phytopharmaceutical product.

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