Jurnal Pijar MIPA
https://jurnalfkip.unram.ac.id/index.php/JPM

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

Antioxidant Activity and Phytochemical Screening of Ethanol Extract from *Ruellia napifera* Leaves

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Received: June 11, 2025. Accepted: June 27, 2025. Published: July 14, 2025

Abstract: Exploration of the chemical profile and antioxidant properties of ethanol extract derived from Ruellia napifera leaves is very important, considering the usefulness of these leaves for use in natural therapy products. This research investigates the identification of secondary metabolite compounds and the antioxidant activity of ethanol extract from *Ruellia napifera* leaves. The objectives are to determine the types of secondary metabolite compounds present and to evaluate the antioxidant potential using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method. Ethanol extracts were prepared using the maceration method at durations of 24 hours. Phytochemical tests revealed the presence of flavonoids, alkaloids, saponins, and steroids. Antioxidant activity was measured through IC50 values, indicating the extract's ability to inhibit 50% of the DPPH radicals. The IC50 values obtained were 10.66 µg/mL. For comparison, vitamin C as a positive control exhibited an IC50 of 2.49 µg/mL. These results suggest that the Gempur Batu leaf extract has strong antioxidant potential. These findings suggest that this extract from *Ruellia napifera* leaves holds potential for developing natural antioxidant-based therapeutic products or herbal medicine formulations.

Keywords: Antioxidant Activity; DPPH Assay; IC50 Value; *Ruellia napifera*; Secondary Metabolites.

Introduction

Plants are known to possess a wide variety of bioactive compounds, among which secondary metabolites play a pivotal role in pharmacological activities [1]. Unlike primary metabolites essential for basic plant growth and development, secondary metabolites often contribute to the plant's defence mechanisms and interactions with the environment. These metabolites, including alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids, are recognized for their potential antioxidant properties, among other bioactivities [2]. The exploration of these compounds from natural sources has garnered increasing attention due to the limitations and potential side effects of synthetic compounds [3].

One such natural source is the Gempur Batu plant, *Ruellia napifera*, a member of the Acanthaceae family, commonly found in tropical regions and traditionally used in herbal medicine. The leaves of Gempur Batu have been empirically utilized for treating kidney stones and promoting urinary tract health [4]. Despite its widespread traditional use, limited scientific literature is available regarding the bioactive compounds present in the leaves and their associated antioxidant activity. Hence, a detailed study is warranted to identify its secondary metabolite profile and evaluate its antioxidant potential. Antioxidants are molecules capable of delaying or inhibiting oxidation processes, thereby preventing cellular damage caused by reactive oxygen species (ROS) [5]. The imbalance between ROS and antioxidant defences leads to oxidative stress, which has been implicated in the pathogenesis of various chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. While endogenous antioxidants do exist within the human body, exogenous sources, particularly from dietary or plant-derived substances, are essential to maintain oxidative balance.

Currently, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in the food and pharmaceutical industries. However, their potential toxicity and carcinogenic effects have led to a growing preference for natural antioxidants [6]. Consequently, the identification of safe and effective antioxidants from medicinal plants like Ruellia napifera becomes increasingly important. Previous studies on related species, such as Ruellia tuberosa and Strobilanthes crispa, have shown promising antioxidant potential, largely attributed to their high flavonoid and phenolic content. These findings support the hypothesis that Ruellia napifera may exhibit similar bioactive properties. Moreover, the maceration extraction method, which is widely used due to its simplicity and cost-effectiveness, offers an efficient approach to isolating these compounds without the risk of thermal degradation [7].

In this study, ethanol was chosen as the solvent for extraction due to its polarity and ability to dissolve a wide range of phytochemicals. The extraction was performed for durations of 24 hours to determine the optimal condition for maximum antioxidant activity. The evaluation of antioxidant activity was conducted using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, a well-established assay that measures the ability of antioxidants to scavenge free radicals [8]. Despite these findings in related species, no studies to date have scientifically validated the antioxidant potential of Ruellia napifera. This study aims to fill that gap by providing

How to Cite:
M. R. S. Efendi, Z. Anisa, D. Setyaningrum, and A. Trisnawati, "Antioxidant Activity and Phytochemical Screening of Ethanol Extract from *Ruellia napifera* Leaves", *J. Pijar.MIPA*, vol. 20, no. 5, pp. 808–813, Jul. 2025. https://doi.org/10.29303/jpm.v20i5.9300

empirical data on its phytochemical profile and antioxidant activity.

The goals of this research are twofold: first, to qualitatively identify the types of secondary metabolites present in the ethanol extract of Ruellia napifera leaves through phytochemical screening, and second, to quantitatively assess the antioxidant activity of these extracts by determining their IC50 values. The results of this study are expected to provide scientific validation for the traditional uses of Ruellia napifera and potentially contribute to the development of natural antioxidant agents.

Research Methods

This research employed a descriptive experimental method to identify the secondary metabolite content and antioxidant activity of *Ruellia napifera* leaves. Research was conducted from February to May 2025 at the Chemistry Laboratory of Universitas Bojonegoro.

Materials and Instruments

The materials used in this research included *Ruellia napifera* leaves collected from Samberan village, Bojonegoro District. The chemicals included ethanol 70%, distilled water, magnesium, concentrated hydrochloric acid (HCl), Dragendorff reagent, $FeCl_3$ 1%, acetic anhydride, concentrated sulfuric acid (H_2SO_4), and DPPH (1,1-diphenyl-2-picrylhydrazyl). Vitamin C was used as the positive control. Instruments used were an analytical balance, rotary evaporator, UV-Vis spectrophotometer, water bath, glassware, and filter paper.

Sample Preparation

Fresh leaves of *Ruellia napifera* were washed, air-dried in the shade, and ground into fine powder using a blender. The powder was then sieved using a 40-mesh sieve to obtain uniform particle size.

Extraction Procedure

Maceration was used as the extraction method due to its simplicity and ability to preserve thermolabile compounds. 100 g of powdered leaves were soaked in 1 L of 70% ethanol for 24 hours in separate flasks. The extracts were stirred occasionally and protected from direct sunlight. After maceration, each extract was filtered and concentrated using a rotary evaporator at 45°C, followed by further evaporation using a water bath until thick extracts were obtained. The extract yield was then calculated.

Phytochemical Screening

Flavonoid Test

A total of 0.5 grams of the ethanol extract of Gempur Batu leaves was weighed and placed into a test tube. Then, 5 mL of distilled water was added and heated in a water bath. The solution was filtered, and the filtrate was dissolved in 1 mL of 70% ethanol, followed by adding 0.5 mg magnesium powder. Next, 10 mL of concentrated hydrochloric acid was added. The formation of a yellow color indicates a positive result for flavonoids. Qualitative tests were conducted to identify flavonoids, alkaloids, saponins, steroids, tannins, and terpenoids. Flavonoids were detected using magnesium and HCl; alkaloids by Dragendorff reagent; saponins by the foam test; steroids and terpenoids using Liebermann-Burchard reagent; and tannins by $FeCl_3$ [9]. All experimental procedures were conducted in triplicate to ensure reproducibility and statistical reliability of the data.

Saponin Test

0.5 grams of the ethanol extract was added to 5 mL of distilled water and heated for 5 minutes. The solution was then shaken vigorously for 5 minutes. The formation of stable foam approximately 1 cm in height after being left to stand for 10 minutes indicates the presence of saponins [10]. All experimental procedures were conducted in triplicate to ensure reproducibility and statistical reliability of the data.

Alkaloid Test

0.5 grams of the ethanol extract was added to 5 mL of 2N hydrochloric acid and heated in a water bath for 2 minutes. Then, three drops of Dragendorff reagent were added. The formation of an orange to brick-red precipitate indicates a positive result for alkaloids [8]. All experimental procedures were conducted in triplicate to ensure reproducibility and statistical reliability of the data.

Tannin Test

0.5 grams of the ethanol extract was added to 5 mL of distilled water and boiled for 5 minutes. The filtrate was collected and added to 5 drops of 1% FeCl3. The appearance of a dark blue or greenish-black color indicates the presence of tannins [11] . All experimental procedures were conducted in triplicate to ensure reproducibility and statistical reliability of the data.

Steroid and Terpenoid Test

0.5 grams of the ethanol extract was added with Liebermann-Burchard reagent, consisting of 3 drops of concentrated HCl and one drop of concentrated H2SO4. The solution was shaken gently and left to stand for a few minutes. A blue or green color indicates the presence of steroids, while a red or purple color indicates terpenoids [8]. All experimental procedures were conducted in triplicate to ensure reproducibility and statistical reliability of the data.

Antioxidant Activity Test (DPPH Assay)

The antioxidant activity was determined by the DPPH free radical scavenging method. A DPPH solution (100 ppm) was prepared, and the maximum absorption wavelength (λmax) was determined at 516 nm. Extract samples were prepared at 100, 200, 300, 400, and 500 ppm concentrations. Each 4 mL sample was mixed with 1 mL of DPPH solution, incubated at 37°C for 30 minutes, and absorbance was measured at 516 nm. The parameter used to interpret the results of antioxidant activity testing using the DPPH method is the effective concentration (EC50), commonly referred to as the IC50 value, which is the concentration required to

reduce 50% of the DPPH activity. To calculate the IC50 value, data on the percentage of inhibition from the assay must be obtained [10] [12]. All experimental procedures were conducted in triplicate to ensure reproducibility and statistical reliability of the data.

Results and Discussion

Preparation of *Ruellia napifera* Leaf Extract

Ruellia napifera leaves were extracted to isolate the bioactive compounds present in the powdered leaf material, enabling their dissolution into a suitable solvent. In this study, cold extraction by maceration was employed. This method was selected due to its simplicity and effectiveness, as it only requires a container for soaking and does not involve heating. Consequently, it helps prevent the degradation of thermolabile compounds that may be sensitive to elevated temperatures. 100 g of powdered *R. napifera* leaves were macerated with 1 L of 70% ethanol. The extraction was performed over varying durations of 24 hours. This variation aimed to evaluate the influence of maceration time on the antioxidant potential of the extract. Extractions were carried out at room temperature and protected from direct sunlight to maintain the chemical stability of the phytoconstituents. The maceration process resulted in a dark green-colored filtrate. The ethanolic extracts were subsequently concentrated using a rotary evaporator below the solvent's boiling point (50°C) to prevent the degradation of thermolabile bioactive compounds and ensure optimal preservation of phytochemicals. Following rotary evaporation, the extracts were further concentrated using a water bath at 50°C until complete evaporation of ethanol was achieved. This yielded a thick, crude ethanolic extract of *R. napifera* leaves, characterized by a dark green color and a distinct, sharp aromatic odor.

Figure 1. Extract of *Ruellia napifera* Leaves

Phytochemical Screening of *Ruellia napifera* Leaf Extract

Phytochemical screening is a qualitative analysis performed to identify the presence of secondary metabolite compounds in a sample using specific reagents as indicators. In this study, phytochemical tests were conducted to determine the chemical constituents present in the ethanolic extract of Ruellia napifera leaves.

Table 1. Phytochemical Screening of *Ruellia napifera* Leaf Ethanolic Extract.

Secondary Metabolites	Reagent	Result	Description
Flavonoid	Mg + HCl	+	Formation of yellow-colored solution
Saponins	Distilled water (Aquadest)	+	Formation of stable foam
Alkaloids	Dragendorff 's reagent	+	Formation of precipitate
Tannins	1% $FeCl_3$	-	No formation of bluish-black or greenish-brown coloration
Steroids	Liebermann –Burchard	+	Formation of green coloration
Terpenoids	Liebermann –Burchard	-	No formation of brownish or violet ring

Based on the results presented in Table I, the ethanolic extract of Ruellia napifera leaves contains flavonoids, alkaloids, saponins, and steroids. These findings are consistent with previous literature, which indicates that species within the Ruellia genus typically contain flavonoids, alkaloids, saponins, and other phytochemical constituents. The flavonoid test on the ethanol extract of Ruellia napifera leaves using HCl and magnesium metal reagents showed a positive result, indicated by the formation of a yellow-colored solution. The purpose of adding Mg and HCl is to reduce the benzopyran core present in the flavonoid structure, resulting in the formation of a red or orange-colored flavilium salt [5]. The reaction between flavonoid compounds and HCl with magnesium metal is illustrated in Figure 2.

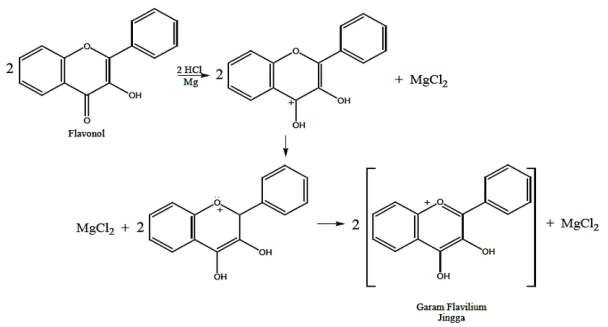

Figure 2. Reaction of Flavonoids with Magnesium Metal and HCl

The ethanol extract also tested positive for saponins, as evidenced by the persistent formation of foam when the extract was vigorously shaken with distilled water. This

foaming phenomenon indicates the presence of glycosides that hydrolyze into glucose and other compounds capable of generating foam in aqueous environments. The saponin hydrolysis reaction is illustrated in relevant literature, confirming their amphiphilic nature and surface-active properties [13].

Dragendorff's reagent was used to test for alkaloids, yielding a positive result marked by the formation of an orange precipitate, which represents a potassium-alkaloid complex. The test is based on a precipitation reaction facilitated by ion exchange between alkaloid nitrogen atoms and potassium ions. The reagent preparation involves dissolving bismuth nitrate in hydrochloric acid to prevent hydrolysis, forming bismuthyl ions (BiO^+). The subsequent reaction with potassium iodide forms a black precipitate of BiI_3, which dissolves in excess KI to produce potassium tetraiodobismuthate [3] [14]. The alkaloid nitrogen then reacts with this complex to form the observed precipitate.

Testing for tannins involved the use of 1% ferric chloride ($FeCl_3$). No blue-black or greenish coloration was observed; instead, a yellow solution formed, indicating a negative result. This suggests that the ethanol extract does not contain detectable levels of tannins. Possible explanations include: (1) low tannin concentrations in the plant sample, (2) limited solubility of tannins in ethanol, and (3) potential interaction with complex plant matrices that hinder reactivity with $FeCl_3$. These factors may have prevented the hydroxyl groups of tannins from reacting with the reagent [4] [8] [2].

The Liebermann-Burchard reagent, a combination of acetic anhydride and sulfuric acid, was used to identify steroids and terpenoids. A green coloration was observed upon addition of the reagent, indicating the presence of steroid compounds. This reaction occurs through acetylation of hydroxyl groups on the steroid structure, followed by oxidation that forms extended conjugated double bonds, leading to the green color. Sulfuric acid facilitates the hydrolysis of intermediates and stabilizes the color change. The underlying mechanism involves an electrophilic addition reaction. A hydrogen atom is removed from the steroid molecule, resulting in the formation of a carbocation and rearrangement of double bonds. This causes resonance stabilization and conjugation, contributing to the color intensity [15] [14] [16]. This reaction supports the presence of unsaturated steroidal nuclei in the extract.

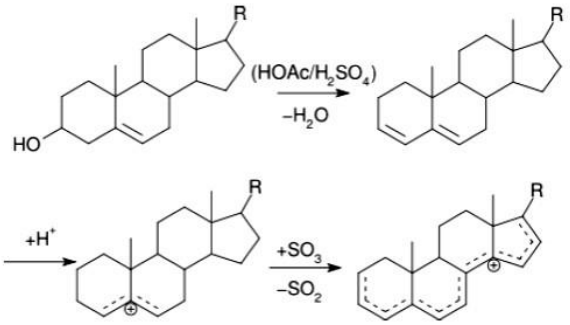

Figure 3. Steroid Reaction with *Liebermann-Burchard* Reagent

Conversely, terpenoids were not detected in the ethanol extract, as no characteristic brown or violet rings were observed upon treatment with the Liebermann-Burchard reagent. This could be due to several factors. Many terpenoids are cyclic alcohols and are thus semi-polar, which limits their solubility and interaction with the polar ethanol solvent used in this study. Consequently, these compounds may not have been efficiently extracted or reactive under the test conditions [10] [8].

Antioxidant Activity Test (DPPH Assay)

The antioxidant activity in the test solution can be observed through a qualitative assessment indicated by the fading of the solution's color. The addition of ethanol extract of *Ruellia napifera* leaves causes a discoloration of the DPPH solution from deep purple to a paler shade. A higher concentration of the extract implies a greater amount of compounds capable of donating electrons or hydrogen atoms to neutralize free radicals [16] [3] [17]. The intense purple color of DPPH will progressively fade to yellow when reacted with a sufficient amount of antioxidant compounds. This fading indicates that the DPPH free radicals have been reduced by the ethanol extract of Ruellia napifera leaves. Initially, DPPH exhibits a strong absorption at a wavelength of 516 nm. However, upon reduction, DPPH is converted into diphenylpicrylhydrazine, which gradually loses its color, resulting in a yellow hue. The absorbance decreases proportionally to the number of electrons accepted. This color change reflects the shift in energy state of the DPPH molecule. In its radical form, DPPH is unstable and highly reactive due to its unpaired electron; after electron donation, the molecule becomes more stable with lower energy [18-19]. This color transition corresponds to changes in absorbance measured at the DPPH maximum wavelength using a UV-Vis spectrophotometer. The resulting data determine the free radical scavenging activity, expressed as the IC_{50} value. After performing a qualitative assessment based on the color change of the test solution, absorbance measurements were carried out using a UV-Vis spectrophotometer at a wavelength of 516 nm. Following wavelength reading, the absorbance values of the test solutions were obtained. Based on the measured absorbance, calculations were then performed to determine the percentage of inhibition (% inhibition). After obtaining the percentage inhibition values of the samples, a regression equation was determined and calculated from the sample concentration against the percentage inhibition using Microsoft Excel. The equation was derived based on a graph with the independent variable being the sample concentration (x) and the dependent variable being the percentage inhibition (y).

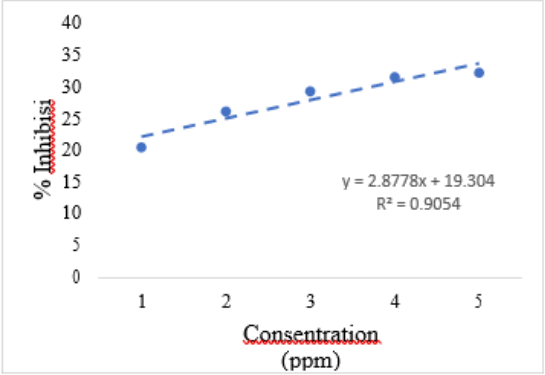

Figure 4. Linear regression equation graph of *Ruellia napifera* leaf extract

The IC_{50} value is determined based on the previously obtained linear regression equation. The IC_{50} is calculated by solving for the x value in the equation Y = a + bx, where Y is set to 50. A lower IC_{50} value indicates a higher antioxidant activity. An IC_{50} value of less than 50 ppm indicates very strong antioxidant activity; a value between 50–100 ppm indicates strong activity; 101–250 ppm indicates moderate activity; and 250–500 ppm indicates weak antioxidant activity. Based on the calculation results, the gempur batu leaf extract with a maceration time of 24 hours had an IC_{50} value of 10.66 µg/mL. The gempur batu leaf extract indicates very strong antioxidant activity. Compared to other plant extracts, such as Persea americana with an IC_{50} of 21.45 µg/mL [20] and Ageratum conyzoides with an IC_{50} of 15.88 µg/mL [21]. The IC_{50} value of 10.66 µg/mL for *Ruellia napifera* indicates a stronger antioxidant activity. However, this study is limited to in vitro analysis; further in vivo studies and toxicity evaluations are needed to confirm its efficacy and safety as a natural antioxidant agent.

Conclusion

The ethanol extract of the leaves of Ruellia napifera contains secondary metabolites such as flavonoids, alkaloids, saponins, and steroids. Based on the calculations, it has been found that the ethanol extract exhibits very strong antioxidant activity, with an IC50 value of less than 50 ppm, specifically 10.66 µg/ml, indicating its potential as a natural antioxidant source. Further studies are recommended to develop this extract into a health supplement product and to explore its other pharmacological activities through in vivo testing to better assess its efficacy and safety.

Author's Contribution

Meilisa Rusdiana Surya Efendi: contribution to this research is as a supervisor, providing direction and ideas to produce research with interesting ideas. Dyah Setyaningrum: classification of research methods and data analysis techniques. Zuffa Anisa: grammatical improvements in writing articles. Ade Trisnawati: Contributed to data analysis and prepared the results and discussion.

Acknowledgements

The authors express their gratitude to the University of Bojonegoro for facilitating and funding this research.

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