IGNATIAN
INTERNATIONAL JOURNAL FOR MULTIDISCIPLINARY RESEARCH
www.icceph.com
ISSN 2984-9942

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *CRASSOCEPHALUM CREPIDIODES* LEAF EXTRACT

Cherryver P. Lubong

School of Graduate Studies, St. Mary's University Bayombong,
Nueva Vizcaya, Philippines

ABSTRACT

This study aimed to determine the phytochemical constituents and antioxidant properties of the ethanolic leaf extract of *Crassocephalum crepidioides*, locally known as Hapon among the Ayangan community of Ifugao, collected from Barangay Halag, Aguinaldo, Ifugao, Philippines. The study was completed in May 2025. Fresh leaves were collected, authenticated, dried, pulverized, and extracted through ethanol maceration. Thin-layer chromatography (TLC) was used for qualitative phytochemical screening, while the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay was used to assess antioxidant activity. The percentage of radical-scavenging activity (%RSA) was calculated based on the absorbance of the control and sample, and the extract's activity was compared with that of ascorbic acid as a positive control. TLC results indicated the presence of carbohydrates, tannins, flavonoids, phenolic compounds, alkaloids, steroids, and coumarins. Anthraquinones, anthrones, and essential oils were not detected under the test conditions. The DPPH assay showed that the ethanolic leaf extract had a mean absorbance of 0.146 ± 0.034 and a radical-scavenging activity of 80.61%, compared with 89.79% for ascorbic acid. These findings provide preliminary evidence of the phytochemical composition and in vitro antioxidant potential of Hapon leaves. However, the study was limited to qualitative phytochemical screening and a DPPH assay, without identifying individual compounds or evaluating other biological activities. Further studies using advanced analytical methods, different extract concentrations, and additional biological and safety assessments are recommended to validate the findings and determine the plant extract's potential applications.

Keywords: *Crassocephalum crepidioides, Hapon, phytochemical screening, thin-layer chromatography, DPPH assay, antioxidant activity*

INTRODUCTION

Crassocephalum crepidioides (Benth.) S. Moore is a member of the family Asteraceae. It is commonly found in tropical areas, including parts of Asia and Africa. The plant commonly grows in disturbed areas such as yards, roadsides, gardens, and agricultural fields, including rice and corn fields. Its rapid growth and adaptability often cause it to be regarded as a weed (Silalahi, 2022). Despite this perception, *C. crepidioides* is also used as a vegetable and in traditional medicine.

In the Philippines, it is locally known as ebolo, thickhead, or “sapsapon” (Saldo, 2024) while the Ayangan community of Ifugao refers to it as Hapon. It has soft, succulent stems that can grow up to one meter in height and produces lobed leaves with a slightly hairy texture. The stems are green sometimes with purple pigmentation when the plant is young or when flowering. Its reddish-purple flowers form small clusters, and its lightweight seeds disperse easily through the wind, allowing it to spread rapidly. Traditionally, Crassocephalum crepidioides has been used in herbal medicine to manage various health conditions, including indigestion, stomach ulcers, wounds, boils, burns, and certain skin problems. Aside from its medicinal uses, the plant is also consumed as a leafy vegetable in some communities, highlighting its importance as both a food source and a traditional medicinal plant (Ologe et al., 2023). Its traditional use and accessibility make it a relevant plant for phytochemical and antioxidant research.

Phytochemicals are naturally occurring plant compounds, including phenolic compounds, flavonoids, alkaloids, and terpenes, that can exhibit biological activity; qualitative phytochemical screening is used to identify groups such as alkaloids, flavonoids, tannins, and phenols in plant materials (Misra et al., 2024; Maheshwaran et al., 2024). Identifying these groups provides initial information about the chemical composition of a plant and helps guide further studies on its possible biological effects.

Antioxidants help reduce the effects of free radicals, which are unstable molecules that may contribute to oxidative stress and cellular damage. Because of this, the antioxidant activity of plant extracts is commonly evaluated through assays that measure their ability to scavenge free radicals. Kabir et al. (2021) reported that a methanolic leaf extract of *C. crepidioides* showed measurable total phenolic content and DPPH free-radical-scavenging activity, indicating the presence of antioxidant-related constituents. Similarly, Devi et al. (2024) investigated the phytochemical constituents and antioxidant potential of *C. crepidioides* leaf extracts prepared using different solvents, further demonstrating the potential of the species as a source of bioactive compounds. More recently, Lalremruata et al. (2026) reported the presence of several phytochemical groups in different extracts of *C. crepidioides* and found that the extracts exhibited antioxidant activity against DPPH and ABTS radicals. These findings suggest that the plant may serve as a potential source of natural antioxidant compounds.

Despite these findings, the available literature remains limited regarding the phytochemical profile and antioxidant properties of the ethanolic leaf extract of locally collected C. crepidioides, particularly Hapon used by the Ayangan community of Ifugao.

Thus, findings from studies using other plant sources or extraction solvents may not fully represent the characteristics of locally collected Hapon leaves. This gap highlights the need to examine the plant using a standardized ethanolic extraction approach.

This study therefore aimed to conduct phytochemical screening and determine the antioxidant properties of the ethanolic leaf extract of *C. crepidioides*. The findings may provide baseline scientific information on the bioactive compounds and antioxidant potential of Hapon leaves and may serve as a basis for future studies on their safety, characterization, and possible food or pharmaceutical applications.

Research Questions

This study aimed to determine the phytochemical constituents and antioxidant properties of the ethanolic leaf extract of *Crassocephalum crepidioides*.
Specifically, it sought to answer the following questions:

1. What phytochemical constituents are present in the ethanolic leaf extract of *C. crepidioides* as determined through thin-layer chromatography?
2. What is the antioxidant activity of the ethanolic leaf extract of *C. crepidioides* as determined using the DPPH radical scavenging assay?
3. What is the percentage of DPPH radical scavenging activity (%RSA) of the ethanolic leaf extract of *C. crepidioides* in comparison with the positive control, ascorbic acid?

METHODOLOGY

A. Collection and Preparation of Plant Material

Fresh leaves of *Crassocephalum crepidioides* were collected from corn fields in Barangay Halag, Aguinaldo, Ifugao, Philippines. The plant specimen was authenticated by Agustin B. Lunag, Associate Professor V of the Department of Plant Science–Crop Science, College of Agriculture, Nueva Vizcaya State University, on July 30, 2025. Identification was based on the plant's observable morphological characteristics.

The collected leaves were washed thoroughly under running tap water and rinsed with distilled water to remove soil and other impurities. They were cut into small pieces and air-dried for two weeks. After drying, the leaves were pulverized using a mortar and pestle, sieved to obtain a uniform particle size, and stored in a clean, airtight container until extraction and phytochemical analysis.

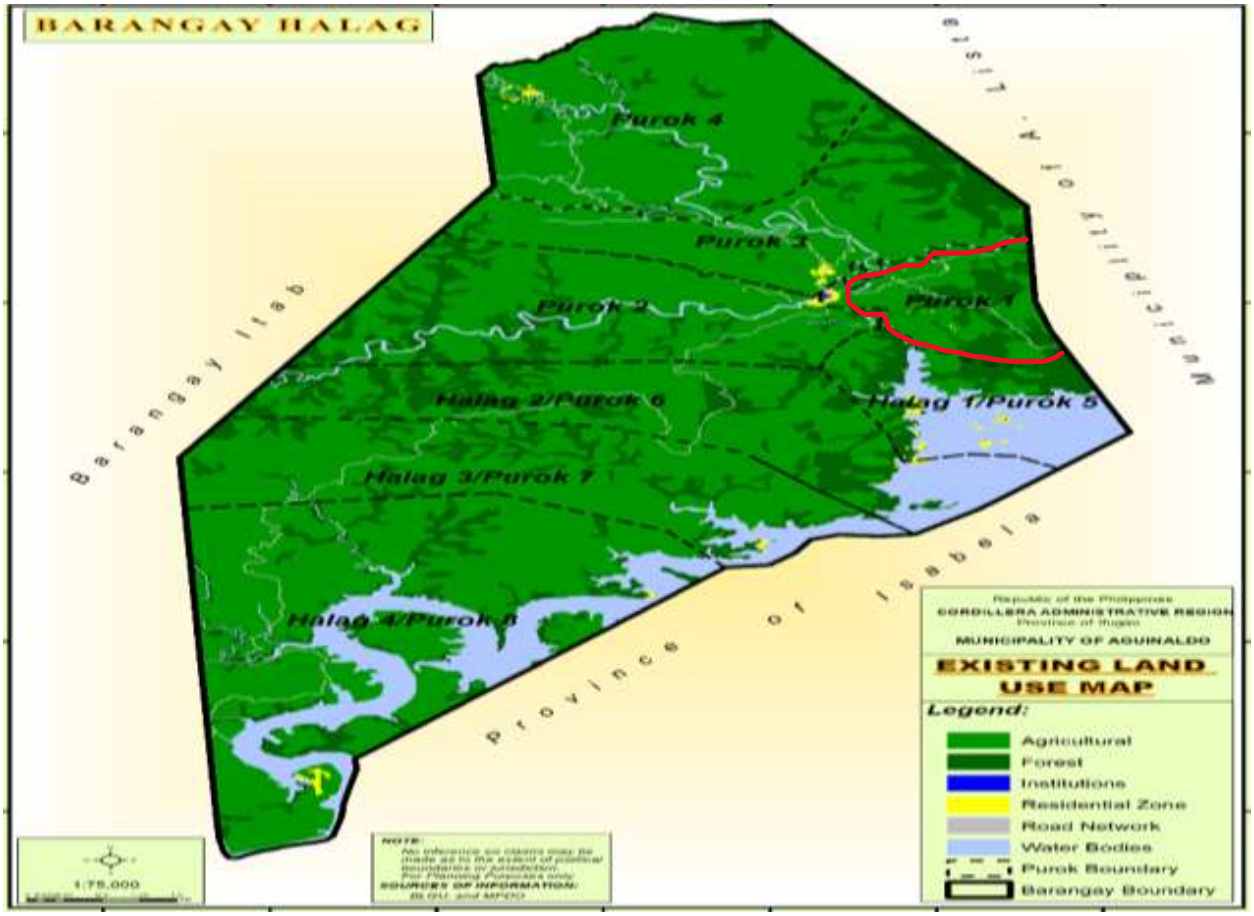


Figure 1. Geographical Location of *Crassocephalum crepidioides* specimen



Figure 2. Full view of *Crassocephalum crepidioides* specimen

B. Extraction

The dried and pulverized leaves of Crassocephalum crepidioides were extracted through maceration using ethanol as the solvent. A 1:10 ratio of plant material to ethanol was used. Specifically, 166 g of pulverized leaf sample was mixed with 1660 mL of ethanol and allowed to stand for 48 hours at room temperature. During this period, the ethanol dissolved the soluble bioactive compounds present in the leaf powder.

After 48 hours, the mixture was filtered using Whatman No. 1 filter paper to separate the liquid extract from the remaining plant residues. The filtrate was then concentrated by evaporating the solvent in a water bath maintained at 40–45 °C. The resulting crude ethanolic leaf extract was collected and stored in a clean, airtight container until it was used for phytochemical screening and antioxidant analysis.

C. Thin-Layer Chromatography

Thin-layer chromatography (TLC) was used to separate and identify the secondary metabolites present in the ethanolic leaf extract of *Crassocephalum crepidioides*. A small amount of the crude extract was applied to a TLC plate using a capillary tube. The spotted plate was placed in a developing chamber containing the appropriate mobile phase and allowed to develop until the solvent front reached the pre-marked line.

After development, the TLC plate was removed, dried, and examined using different visualizing reagents to detect specific classes of phytochemicals. The reagents used were sulfuric acid + heat; vanillin–sulfuric acid +heat; alpha-naphthol–sulfuric acid + heat; methanolic potassium hydroxide; potassium ferricyanide–ferric chloride; Dragendorff's reagent; antimony (III) chloride; magnesium acetate + heat; and ninhydrin. The appearance of characteristic spots or color changes after reagent application indicated the possible presence of specific secondary metabolites in the extract.

D. DPPH Radical-Scavenging Assay

The antioxidant activity of the *Crassocephalum crepidioides* ethanolic leaf extract was evaluated using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. The solution was filtered to obtain a clear working solution, and its absorbance was measured at 517 nm was recorded as 0.973 before the samples were added.

For each test, 3 mL of the DPPH working solution was mixed with 100 µL of the ethanolic leaf extract. The control consisted of 3 mL of DPPH solution and 100 µL of methanol, while the positive control contained 3 mL of DPPH solution and 100 µL of ascorbic acid solution. All mixtures were thoroughly mixed and incubated in complete darkness at room temperature for 30 minutes. After incubation, the absorbance of each mixture was measured at 517 nm using a UV–visible spectrophotometer. The leaf extract samples were analyzed using the designated number of replicates under the same assay conditions as the control and positive control.

The percentage of radical-scavenging activity (%RSA) was calculated using the following equation:

$$\%RSA = \frac{A_c - A_s}{A_c} \times 100$$

where $A_c$represents the absorbance of the control and $A_s$represents the absorbance of the sample or ascorbic acid standard (Baliyan, et al., 2022)

RESULTS AND DISCUSSION

Phytochemical Profiling of Crassocephalum crepidiodes Leaf Extract

Table 1. Thin Layer Chromatography Analysis

Reagent Used	Compound Tested	Observed Result	Presence/ Detection
Napthol-sulfuric acid + Heat	All carbohydrates except tetroses and trioses	Violet spot under visible light	+
Potassium ferricyanide + ferric chloride	Tannins	Dark blue color under visible light	+
	Flavonoids	Dark blue spots under visible light	+
	Phenols	Blue spots under visible light	+
Dragendorff's reagent	Alkaloids	Orange spots under visible light	+
Antimony (III) chloride	Flavonoids	Yellow spots under visible light	+
	Steroids	Fluorescent spots under visible light	+
Metanollic potassium hydroxide (KOH-MetOH)	Coumarins	Blue spots when exposed to UV light	+
	Anthraquinones	Orange spots under visible light	-
	Anthrones	Yellow spots when exposed to UV light	-
Preliminary Test (H_2SO_4+Heat)	Essential oils	Violet spots when exposed to UV light	-

Legend: (+) Detected; (-) Not Detected

The TLC screening of *Crassocephalum crepidioides* leaf extract indicated the presence of carbohydrates, tannins, flavonoids, phenolic compounds, alkaloids, steroids, and coumarins, while anthraquinones, anthrones, and essential oils were not detected under the conditions used. The positive reactions for tannins, flavonoids, and phenolic compounds are consistent with Devi et al. (2024), who detected these groups in methanolic and aqueous leaf extracts. However, their ethanolic extract tested negative for tannins and phenols, indicating that the detected profile can differ with the extraction

solvent. Similarly, Anindita et al. (2025) reported tannins, flavonoids, and alkaloids in a 70% ethanolic leaf extract, supporting the detection of these groups in the present study.

The positive alkaloid reaction also agrees with Schramm et al. (2021), who demonstrated the accumulation of the pyrrolizidine alkaloid jacobine in *C. crepidioides*, although the present TLC result does not identify this compound specifically. The detection of flavonoids, tannins, and phenolic compounds is particularly relevant to the antioxidant component of the study because recent investigations have demonstrated DPPH radical-scavenging activity in *C. crepidioides* leaf extracts (Devi et al., 2024; Lalremruata et al., 2026), providing a basis for considering the present phytochemical findings alongside the antioxidant results.

Antioxidant Property of Crassocephalum crepidiodes Leaf Extract

Table 2. Absorbance Value in the DPPH Assay (n=5)

Replicate	*Absorbance (As)*
1	0.170
2	0.111
3	0.107
4	0.171
5	0.172

Control Absorbance=0.754

The five replicate measurements of the *C. crepidioides* leaf ethanolic extract showed closely grouped absorbance values, ranging from 0.107 to 0.172, indicating consistent results across the DPPH assay. The absorbance values were substantially lower than the control absorbance of 0.754, suggesting that the extract effectively reduced DPPH radicals under the tested conditions. This finding indicates the potential antioxidant activity of the *C. crepidioides* leaf ethanolic extract.

Table 3. Percentage Radical Scavenging Activity of *Crassocephalum crepidiodes* Leaf Extract and Ascorbic Acid

Sample	*Mean Absorbance ± SD*	*% Radical Scavenging Activity*
C. crepidiodes leaf extract	0.146 ± 0.034	80.61%
Ascorbic Acid	0.077	89.79%

The ethanolic leaf extract of *C. crepidioides* exhibited 80.61% DPPH radical-scavenging activity, with a mean absorbance of 0.146 ± 0.034. Although this activity was lower than that of ascorbic acid (89.79%), the result indicates substantial antioxidant activity of the plant extract under the tested conditions. This finding is consistent with previous studies reporting DPPH radical-scavenging activity in *C. crepidioides* leaf extracts (Kabir et al., 2021; Rusli et al., 2024). Recent studies have further associated the antioxidant potential

of the plant with its phenolic and flavonoid constituents (Jahan et al., 2026; Lalremruata et al., 2026). The lower activity compared with ascorbic acid may be attributed to differences in the composition and concentration of antioxidant compounds in the crude plant extract compared with the purified standard. Overall, the findings support the potential of *C. crepidioides* leaves as a natural source of antioxidant compounds.

Conclusions

The study concluded that the ethanolic leaf extract of *Crassocephalum crepidioides*, locally known as Hapon, contained several phytochemical constituents. Thin-layer chromatography revealed the presence of carbohydrates, tannins, flavonoids, phenolic compounds, alkaloids, steroids, and coumarins. However, anthraquinones, anthrones, and essential oils were not detected under the conditions used in the study. These findings suggest that the leaves of locally collected *C. crepidioides* contain diverse secondary metabolites that may contribute to their biological activity.

The DPPH radical-scavenging assay further showed that the ethanolic leaf extract exhibited antioxidant activity, with a mean absorbance of 0.146 ± 0.034 and a radical-scavenging activity of 80.61%. Although this value was lower than the 89.79% reported for ascorbic acid, the extract demonstrated considerable radical-scavenging capacity under the conditions of the assay. Overall, the findings provide preliminary evidence of the phytochemical composition and antioxidant potential of *C. crepidioides* leaves collected from Barangay Halag, Aguinaldo, Ifugao. These results provide a basis for further investigation of the plant's chemical constituents, antioxidant activity, and possible applications.

Recommendations

Based on the findings and limitations of the study, further research is recommended to gain a deeper understanding of the phytochemical composition and antioxidant properties of *Crassocephalum crepidioides* leaves. Future studies may use more specific analytical methods to identify individual compounds and determine the total phenolic and flavonoid contents of the extract. Testing different extract concentrations is also recommended to determine their effects on antioxidant activity and establish the IC_{50} value for comparison with other studies.

Further experiments should be conducted using consistent procedures, appropriate controls, and additional replicates to verify the antioxidant results. Cytotoxicity assessment is also recommended to evaluate the potential harmful effects of the ethanolic leaf extract on cells at different concentrations. Although *C. crepidioides* is consumed as a vegetable in some communities, its use as food does not automatically establish the safety of its concentrated ethanolic extract. Therefore, cytotoxicity testing is important before considering the extract for potential food-related, nutraceutical, or pharmaceutical applications. Future studies may also compare plant samples from different locations and examine how extraction methods affect their phytochemical composition and antioxidant activity. Further biological and safety assessments are

necessary to establish the extract's potential applications and support conclusions about its safety and effectiveness.

Compliance with Ethical Standards

This study was conducted in accordance with the principles of research integrity, scientific honesty, and responsible laboratory practice. It focused on the phytochemical screening and antioxidant assessment of the ethanolic leaf extract of *Crassocephalum crepidioides* (Hapon) and did not involve human participants or animals. Therefore, informed consent and ethics clearance for human or animal research were not applicable. All laboratory procedures were carried out with due care in handling plant materials and chemicals and in preventing contamination. The findings were recorded and interpreted objectively, and the results were reported accurately without fabrication or misrepresentation. Academic integrity was maintained through proper acknowledgment and citation of all sources, and plagiarism was avoided throughout the preparation of the manuscript. No conflict of interest in the conduct of the study, and the findings were intended solely for academic and research purposes. Artificial intelligence tools were limited to language improvement and structural assistance, while the researcher retained responsibility for the accuracy, interpretation, and scientific integrity of the study.

Acknowledgments

First and foremost, the researcher expresses sincere gratitude to Almighty God for His guidance, strength, wisdom, and protection throughout the conduct of this study. Special appreciation is extended to Ma'am Cathelyn C. Mariano for generously sharing her expertise and providing valuable guidance during the laboratory procedures. The researcher also extends sincere thanks to Dr. Agustin B. Lunag, Associate Professor V of the Department of Plant Science–Crop Science, College of Agriculture, Nueva Vizcaya State University, for authenticating the plant material used in the study.

Gratitude is likewise extended to Sir Regidor Almendral for his patience, technical assistance, and guidance during the thin-layer chromatography (TLC) analysis and antioxidant evaluation. The researcher is also deeply grateful to her parents for gathering the necessary plant materials from the cornfields of Halag, Aguinaldo, Ifugao, and to Mr. Rickson G. Chang-a for his efforts, time and assistance in preparing the plant materials for laboratory analysis.

Finally, the researcher sincerely appreciates everyone who contributed their time, effort, and support to the successful completion of this study.

REFERENCES

Anindita, R., Muffida, L., Putri, I.K., Beandrade, M.U., and Futihat, C.E. (2025). Botanical, Pharmacognosy, and Phytochemical Studies of Sintrong Leaves (Crassocephalum crepidioides) Growing in Tanjung Setia Village, West Coast,

Lampung. Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati (J-BEKH), 12(1), 81-94. https://doi.org/10.23960/jbekh.v12i1.423

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C.-M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules, 27(4), 1326. https://doi.org/10.3390/molecules27041326

Devi, Y. A., Gnanasekaran, P., & Devi, H. J. (2024). Antibacterial, Antioxidant and Cytotoxicity Assessment of Crassocephalum crepidioides Leaf Extract. Journal of Pure and Applied Microbiology, 18(4), 2528–2538. https://doi.org/10.22207/jpam.18.4.24

Jahan, F., Ahmed, M., Sen, S., & Laskar, M. A. (2026). Crassocephalum crepidioides (Benth.) S. Moore: Phytochemistry, Nutritional Value, Ethnomedicinal Uses, and Pharmacological Potential. Current Chemical Biology, 20(3), 218–226. https://doi.org/10.2174/0122127968408869251117092854

Kabir, M. T., Samiha, M., Yasmin, H., Rahman, M. S., Rahman, M. S., Ashraf, G. M., & Akter, R. (2021). Free radical scavenging, Thrombolytic and cytotoxic effects of the medicinal herb, Crassocephalum crepidioides. Research Journal of Pharmacy and Technology, 2205–2210. https://doi.org/10.52711/0974-360x.2021.00391

Lalremruata, B. C., Lalrinmawia, N., & Singh, G. (2026). Phytochemical profiling and bioactive assessment of Crassocephalum crepidiodes using GC-MS and LC-MS analysis. Vegetos. https://doi.org/10.1007/s42535-026-01693-9

Maheshwaran, L., Nadarajah, L., Senadeera, S. P. N. N., Ranaweera, C. B., Chandana, A. K., & Pathirana, R. N. (2024). Phytochemical testing methodologies and principles for preliminary screening/ qualitative testing. Asian Plant Research Journal, 12(5), 11–38. https://doi.org/10.9734/aprj/2024/v12i5267

Misra, R. C., Thimmappa, R., & Bonfill, M. (2024). Editorial: Advances in discoveries of plant phytochemicals. Frontiers in Plant Science, 15, 1414150. https://doi.org/10.3389/fpls.2024.1414150

Ologe, M., Adeniyi, F., & Adeoluwa, O. (2023). A review of biological and pharmacological activities of Crassocephalum crepidioides (Benth.) S. Moore. The Tropical Journal of Health Sciences, 30(3), 01–06. https://doi.org/10.4314/tjhc.v30i3.1

Rusli, R., Nuri, I., Ramadani, M. A., Siregar, V. O., Priastomo, M., & Faisal, M. (2022). Aktivitas Antioksidan dan Tabir Surya Ekstrak Etanol Tanaman Crassocephalum crepidioides (Benth.). Jurnal Sains Dan Kesehatan, 4(3), 320–325. https://doi.org/10.25026/jsk.v4i3.1026

Saldo, Ian Jay. (2024). Phytochemical, Antibacterial, and Cytotoxicity Analyses Of Redflower Ragleaf (Crassocephalum Crepidioides) Leaves Liquid Extract. 36. 77-85.

Schramm S, Rozhon W, Adedeji-Badmus AN, Liang Y, Nayem S, Winkelmann T, Poppenberger B. The Orphan Crop Crassocephalum crepidioides Accumulates the Pyrrolizidine Alkaloid Jacobine in Response to Nitrogen Starvation. Front Plant Sci. 2021 Jul 28; 12:702985. doi: 10.3389/fpls.2021.702985. PMID: 34394157; PMCID: PMC8355542.

Silalahi, M., & Wakhidah, A. Z. (2023). Retracted Article: The food plants trade in the Kranggan Mas traditional market, West Java Province, Indonesia: food security

and local cuisine. Journal of Ethnic Foods, 10(1). https://doi.org/10.1186/s42779-023-00192-5

APA Citation:

Lubong, C. P. (2026). PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF CRASSOCEPHALUM CREPIDIODES LEAF EXTRACT. Ignatian International Journal for Multidisciplinary Research, 4(10), 478–488. https://doi.org/10.5281/zenodo.23590071

cherrylubbong21@gmail.com