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Short Communication

Unveiling the Bioactive Potential of Malaysian *Clerodendrum* Species: A Comparative Approach

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ABSTRACT

The genus *Clerodendrum* (Lamiaceae), widely distributed in tropical and subtropical regions of Asia and Africa, has traditionally been used in folk medicine to treat inflammation, skin disorders, hypertension, and infections. Despite their ethnomedicinal relevance, scientific validation of their pharmacological properties remains limited for many species. This study aimed to investigate the bioactivities of extracts from four *Clerodendrum* species: *Clerodendrum nutans*, *C. myrmecophilum*, *C. paniculatum*, and *C. disparifolium*. The extracts were evaluated for their antioxidant, antityrosinase, and anti-inflammatory activities. Antioxidant potential was assessed using the DPPH radical scavenging assay and total phenolic content (TPC), while antityrosinase and anti-inflammatory activities were determined using mushroom tyrosinase and lipoxygenase enzyme inhibition assays, respectively. Among the species tested, the leaf extract of *C. paniculatum* exhibited the strongest bioactivities, with a DPPH IC_{50} value of 79.5 µg/mL, TPC of 135.2 mg GAE/g, tyrosinase inhibition of 82.5%, and lipoxygenase inhibition of 66.8%. These results provide scientific support for the traditional use of *Clerodendrum* species in treating oxidative stress-related and inflammatory conditions. The study highlights the potential of *C. paniculatum* as a natural source of bioactive compounds that could be developed for therapeutic or cosmetic applications.

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1. INTRODUCTION

Plants have long been recognised as prolific sources of structurally diverse natural compounds that play crucial roles in human health and disease prevention. In recent decades, increasing attention has been directed toward plant-derived bioactive metabolites owing to their broad pharmacological potential and favourable safety profiles. Among the various biological properties reported, antioxidant, anti-inflammatory, and antityrosinase activities are considered particularly significant because of their close association with both therapeutic and cosmetic applications (Mucha et al., 2021). Antioxidants are essential in counteracting oxidative stress by neutralising free radicals, which are implicated in cellular damage, premature ageing, and the development of chronic disorders such as cancer, cardiovascular diseases, and neurodegenerative conditions (Allhin et al., 2025). In parallel, anti-inflammatory agents contribute to the modulation of inflammatory pathways, thereby alleviating tissue damage, pain, and swelling that underlie many acute and chronic inflammatory diseases (Salihu et al., 2024). Meanwhile, tyrosinase inhibitors play a key role in regulating melanogenesis by suppressing excessive melanin production, offering potential benefits in the management of hyperpigmentation, skin ageing, and related dermatological concerns (Zolghadri et al., 2019). There is a growing scientific interest in the systematic investigation of diverse and underexplored plant species to identify novel bioactive constituents. Such efforts not only support the development of safer and more effective therapeutic agents but also align with the increasing demand for natural ingredients in cosmetic and pharmaceutical formulations.

The genus *Clerodendrum* (Lamiaceae) comprises over 300 species of flowering plants distributed mainly across tropical and subtropical regions of Asia, Africa, and the Pacific islands. These species include shrubs, small trees, and climbers, many of which are valued for their ornamental beauty, fragrant flowers, and medicinal properties. *Clerodendrum* is well known for its diverse phytochemical composition, including flavonoids, terpenoids, phenolics, and steroids, which contribute to its wide range of biological activities such as antioxidants, anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects. Traditionally, various parts of *Clerodendrum* species such as leaves, roots, and bark have been used in folk medicine to treat ailments like asthma, fever, hypertension, and skin disorders (Wang et al., 2017).

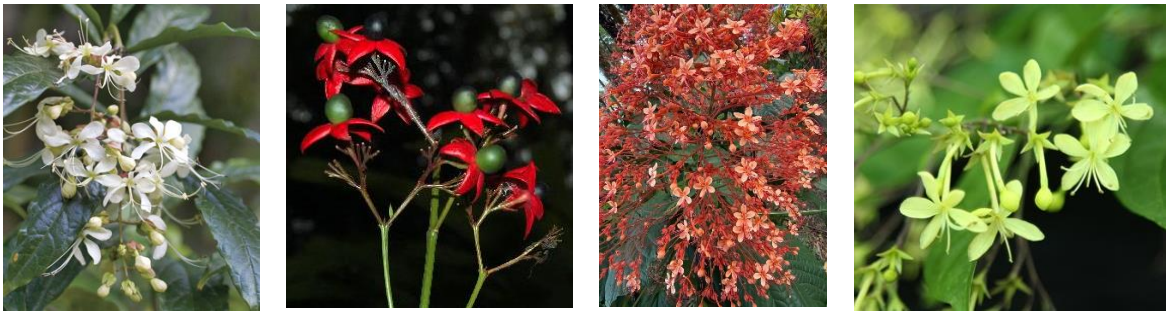
Clerodendrum nutans Wall. ex Jack, locally known in Indonesia as '*Harendong Bulu*' has traditionally been used to treat fever, inflammation, and as a general health tonic. Scientific studies have supported some of these uses, particularly its anti-inflammatory and antioxidant properties (Lim, 2012). *Clerodendrum paniculatum* L., commonly known as the '*Pagoda Flower*' is widely used in traditional medicine in Southeast Asia. It has been used for treating rheumatism, ulcers, neuralgia, typhoid, liver disorders, and wounds. Several pharmacological studies have reported that *C. paniculatum* possesses anti-inflammatory, antimicrobial, antioxidant, and antidiabetic activities, attributed to its diverse phytochemical constituents, including flavonoids, terpenoids, and phenolic acids (Mamuaja et al., 2024). *Clerodendrum disparifolium* Blume, known locally as 'Swaddling Flower' is traditionally used for treating insect bites and stings by applying crushed leaves directly to the skin (Lim, 2012). Lastly, *Clerodendrum myrmecophilum* Ridl., has limited documentation regarding its traditional uses, and there is a notable lack of scientific studies on this species, indicating that it remains largely unexplored and warrants further investigation.

In this study, we present the first report on the systematic studies of biological activities of four *Clerodendrum* species originated from Malaysia. This study focused on the analysis of antioxidant, antityrosinase, and anti-inflammatory activities of the leaf extracts of *Clerodendrum nutans*, *C. myrmecophilum*, *C. paniculatum*, and *C. disparifolium* (Figure 1).

2. METHODOLOGY

2.1. Plant Materials and Extraction Method

The leaves of *Clerodendrum* sp. were collected from Behrang Reserve Forest in September 2019 and identified by Mazatul Azrin Rahman and Nor Nafizah Mohd Noor from UPSI. The voucher specimens were deposited at the Flora of Perak (FP) herbarium, UPSI. The dried and powdered leaves of the above-mentioned *Clerodendrum* sp. species were extracted with methanol. The extracts were filtered, and the solvent was removed under vacuum using a rotary evaporator (Eyela, Germany). Percentage yields (w/w) of all plant extracts obtained are shown in Table 1.



C. nutans *C. myrmecophilum* *C. paniculatum* *C. disparifolium*

Figure 1. *Clerodendrum* species from Malaysia

Table 1. Percentage yield of *Clerodendrum* extracts

Species	Specimen	Appearance	Weight (g)	Yield (g, w/w)
C. nutans	MA-001	Dark brown	50	1.25
C. myrmecophilum	MA-002	Greenish	50	1.02
C. paniculatum	MA-003	Greenish	50	1.14
C. disparifolium	C4-2023	Dark brown	50	1.09

2.2. Solvents and Chemicals

Antioxidant: 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (Germany). Analytical grade methanol, ethanol and dimethylsulfoxide (DMSO), HPLC grade chloroform, and Folin-Ciocalteu's reagent were purchased from Merck (Germany). *Antityrosinase:* Mushroom tyrosinase enzyme (EC1.14.18.1), kojic acid and L-dopa were purchased from Sigma-Aldrich (Germany). *Anti-inflammatory:* Lipoxygenase inhibitor screening assay kit (Item No. 760700 Cayman Chemicals Co) was purchased from i-DNA Biotechnology (M) Sdn. Bhd. (Malaysia).

2.3. Antioxidant Activities

Total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteu method and expressed as gallic acid equivalents (Salleh and Ahmad, 2016; Salleh et al., 2015). Stock solutions of the extracts (1.0 mg/mL) were prepared in methanol and diluted to a final concentration of 1,000 µg/mL. An aliquot of the sample (0.1 mL) was mixed with methanol (0.9 mL), followed by the addition of Folin–Ciocalteu's reagent (0.05 mL). After 3 min, 5% Na_2CO_3 solution (0.5 mL) was added, and the mixture was incubated at room temperature with occasional shaking. Methanol (2.5 mL) was then added, and the reaction mixture was kept in the dark for 1 h. Absorbance was measured at 765 nm. Gallic acid was used as the standard, and TPC was expressed as mg gallic acid equivalent per gram of sample. All measurements were performed in triplicate and reported as mean ± SD.

The free radical scavenging activity was measured by the DPPH method with minor modifications (Salihu et al., 2023; Salleh and Khamis, 2020). Briefly, 0.1 mM DPPH (1 mL) dissolved in EtOH was added to an EtOH solution (3 mL) of the tested samples and standard (BHT) at different concentrations (200-25 µg/mL). An equal volume of EtOH was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance at 517 nm was measured with a UV–vis spectrophotometer. The percent inhibitions (I%) were calculated:

$$I\% = [A_{blank} – A_{sample} / A_{blank}] \times 100;$$

where A_{blank} is the absorbance value of the control reaction and A_{sample} is the absorbance values of the test extracts/standard. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

2.4. Antityrosinase Activity

Tyrosinase inhibition assay was carried out following the standard method (Salihu et al., 2023b; Salleh and Khamis, 2021) with slight modifications. Briefly, the extracts and kojic acid (standard) were dissolved in DMSO prepared as 1 mg/mL. The reaction was carried out using 96-well microplate and microplate reader (Epoch MicroVolume Spectrophotometer, USA) was used to measure the absorbance at 475 nm. 40 µL of extracts dissolved in DMSO with 80 µL of phosphate buffer (pH 6.8), 40 µL of tyrosinase enzyme and 40 µL of L-dopa were put in each well. Each sample was accompanied

by a blank that had all the components except for L-dopa. Kojic acid was used as reference standard inhibitor for comparison. The percentage of tyrosinase inhibition (I%) was calculated:

$$I\% = [A_{control} - A_{sample} / A_{control}] \times 100;$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the extracts/reference. Analyses were expressed as means ± SD of triplicates.

2.5. Anti-inflammatory Activity

Lipoxygenase (LOX) inhibition was determined using an enzyme immuno assay (EIA) kit (Catalog No. 760700, Cayman Chemical, USA) according to the manufacturer's instructions (Ghani et al., 2024; Shakri et al., 2020). The Cayman Chemical lipoxygenase inhibitor screening assay detects and measures the hydroperoxides produced in the lipoxygenation reaction using a purified lipoxygenase. Stock solutions of the extracts were dissolved in a minimum volume of DMSO and were diluted using the supplied buffer solution (0.1 M, Tris-HCl, pH 7.4). To a 90 µL solution of 5-LOX enzyme in 0.1 M, Tris-HCl, and pH 7.4 buffer, 10 µL of various concentrations of test samples (final volume of 210 µL) were added and the lipoxygenase reaction was initiated by the addition of 10 µL (100 µM) of arachidonic acid. After maintaining the 96-well plates on a shaker for 5 min, 100 µL of chromogen was added and the plate was retained on a shaker for 5 min. The lipoxygenase activity was determined after measuring absorbance at a wavelength of 500 nm. The percentage inhibition (I%) was calculated:

$$I\% = [A_{initial\ activity} - A_{inhibitor} / A_{initial\ activity}] \times 100;$$

where $A_{initial\ activity}$ is the absorbance of 100% initial activity wells without sample and $A_{inhibitor}$ is the absorbance of extracts/reference. All tests were carried out in triplicate and expressed as means ± SD. Quercetin was used as a positive control reference inhibitor.

Data obtained from biological activities were expressed as means ±SD and compared using the Student's t-test. Statistical analyses were carried out employing one-way ANOVA ($p<0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

3. RESULTS AND DISCUSSION

Antioxidants help protect the body from damage caused by harmful molecules called free radicals, which can lead to diseases such as cancer, heart problems, and aging-related issues. Many natural antioxidants are found in plants, especially in compounds known as phenolics, like flavonoids and phenolic acids (Lobo et al., 2010). To measure the antioxidant activity of plant extracts, two common methods are used: the TPC and the DPPH radical scavenging assay. The TPC test estimates how much phenolic compound is present, which often relates to the strength of antioxidant activity. The DPPH assay checks how well a plant extract can neutralize free radicals by changing the colour of a stable chemical (DPPH). These methods help researchers understand the antioxidant potential of different plants. The antioxidant activities of the four *Clerodendrum* species are summarised in Table 2. Among them, the extract of *C. paniculatum* exhibited the strongest DPPH scavenging activity, with IC_{50} value of 79.5 µg/mL. The extract of *C. disparifolium* also demonstrated notable radical scavenging ability, with IC_{50} value of 98.0 µg/mL. However, both values were higher than that of the positive control, BHT, which had IC_{50} value of 18.5 µg/mL. In terms of TPC, *C. paniculatum* showed the highest content (135.2 mg GAE/g), followed by *C. disparifolium* (114.5 mg GAE/g). Phenolic compounds are well-recognized as plant-based antioxidants due to their roles as reducing agents, hydrogen donors, and singlet oxygen scavengers (Liang et al., 2010). Previous studies have reported antioxidant potential in *C. paniculatum* extracts from Vietnam, with IC_{50} values in the DPPH assay of 280.0 and 588.1 µg/mL for ethanol and water extracts, respectively, and 51.7 and 72.0 µg/mL in the ABTS assay (Ngoc et al., 2023). Similarly, methanolic extracts of *C. paniculatum* from India showed high radical scavenging activity (up to 87%) (Hegde and Hungund, 2023), while leaf extracts from Indonesia exhibited strong antioxidant activity with IC_{50} value of 27.7 µg/mL in the DPPH assay (Hafiz et al., 2016).

Table 2. Antioxidant activities of *Clerodendrum* extracts

Samples	TPC (mg GA/g)	DPPH (IC_{50} in µg/mL)
C. nutans	80.4	132.4
C. myrmecophilum	96.8	128.6
C. paniculatum	**135.2**	**79.5**
C. disparifolium	114.5	98.0
Butylated hydroxytoluene (BHT)	-	18.5

Tyrosinase is a key enzyme involved in melanin production, which affects skin pigmentation. Overactivity of tyrosinase can lead to hyperpigmentation disorders such as melasma, freckles, and age spots. Therefore, tyrosinase inhibitors are widely studied for their potential use in cosmetic and pharmaceutical products aimed at skin-lightening and treating pigment-related conditions (Pillaiyar et al., 2017). Inflammation, on the other hand, is a natural immune response to injury or infection, but excessive or chronic inflammation can contribute to various health problems, including arthritis, cardiovascular diseases, and cancer. Anti-inflammatory agents work by reducing the production of pro-inflammatory mediators, such as nitric oxide (NO), prostaglandins, and cytokines, which play major roles in the inflammatory response (Chen et al., 2017). Table 3 summarizes the results of inhibition percentage of antityrosinase and lipoxygenase inhibitory activities.

Table 3. Antityrosinase and anti-inflammatory inhibitory activities of *Clerodendrum* extracts

Species	Antityrosinase (I%)	Lipoxygenase (I%)
C. nutans	60.1	45.2
C. myrmecophilum	58.6	47.3
C. paniculatum	**82.5**	**66.8**
C. disparifolium	73.2	61.2
Kojic acid	97.1	-
Quercetin	-	89.2

In this study, the antityrosinase activity of the plant extracts was evaluated using mushroom tyrosinase, a commonly used model enzyme due to its structural similarity to human tyrosinase. Among the tested samples, the extract of *C. paniculatum* exhibited the strongest inhibitory effect, with 82.5% inhibition of tyrosinase activity. This suggests that the extract contains active compounds capable of interacting with the enzyme's active site or altering its catalytic function. Although this inhibition is slightly lower than that of kojic acid, a well-known standard tyrosinase inhibitor which showed 97.1% inhibition, it still represents a significant activity. The high inhibition observed indicates the potential of *C. paniculatum* as a natural source of tyrosinase inhibitors that may be useful in the formulation of depigmenting agents or cosmeceutical products. The anti-inflammatory potential of the *Clerodendrum* extracts was evaluated using the lipoxygenase (LOX) enzyme inhibition assay. Quercetin, a well-known LOX inhibitor, was used as a positive control and showed 89.2% inhibition at a concentration of 1 mg/mL. The extract of *C. paniculatum* demonstrated a moderate inhibitory effect, with 66.8% inhibition of LOX activity. LOX catalyzes the dioxygenation of polyunsaturated fatty acids, resulting in the formation of cis, trans-conjugated diene hydroperoxides, which are inflammatory mediators (Liu et al., 2020). Notably, *C. paniculatum* also exhibited the highest TPC and strong radical scavenging activity. This aligns with previous findings that suggest a correlation between anti-inflammatory effects and the presence of polyphenolic compounds. Antioxidants, particularly phenolics, are known to inhibit plant lipoxygenases. They may interfere with the inflammatory process by blocking the arachidonic acid metabolic pathway, inhibiting LOX activity, and scavenging reactive oxygen species generated during inflammation (Muflihah et al., 2021; Abbasi-Parizad et al., 2020).

4. CONCLUSION

This study represents the first comprehensive investigation into the antioxidant, antityrosinase, and anti-inflammatory properties of extracts from *Clerodendrum* species. The results highlight the promising bioactivity of these plants, supporting their traditional medicinal use and suggesting their potential as sources of novel therapeutic agents. Given these findings, further research is necessary to identify the specific bioactive compounds responsible for the observed effects. In addition, detailed pharmacological evaluations and safety assessments are essential to fully understand their therapeutic potential and to support the development of new drugs or lead compounds based on *Clerodendrum* extracts.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION
Mazatul Azrin Rahman and Ainur Hanie Zainudin: Plant collection and experimental; Wan Mohd Nuzul Hakimi Wan Salleh, Nur Nabilah Mohd Zaini and Faezatul Alwani Mohd Rahim: Methodology and formal analysis; Fatimah Mohamed, Salam Ahmed Abed, Sherali Kuziev, Alfred Ngenge Tamfu, and Arwa R. Althaher: Review and editing. Nor Nafizah Mohd Noor: conceptualized, supervise the research, and writing original article.

DATA AVAILABILITY
The authors confirm that the data supporting the findings of this study are available within the article.

DECLARATION OF GENERATIVE AI
During the preparation of this work, the authors used ChatGPT to enhance the clarity of the writing. After using the ChatGPT, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

ETHICS
Not applicable.

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