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Phytochemical Characterization and In Vitro Antioxidant Activity of *Murraya Koenigii* Leaf Extract

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ABSTRACT

Murraya koenigii (curry leaf) is a tropical plant known for its diverse phytochemical content and potent antioxidant activity. However, limited studies have provided comprehensive quantitative data on its bioactive compounds, especially from Indonesian varieties. This study aimed to quantitatively analyze flavonoid, saponin, and tannin content in young and mature curry leaves and evaluate their in vitro antioxidant activity using the DPPH assay. An experimental design was conducted from January to March 2025. Ethanolic extracts of young and mature M. koenigii leaves from Aceh were prepared via maceration. Phytochemical quantification was performed using spectrophotometric methods, and antioxidant capacity was assessed using the DPPH radical scavenging assay with IC_{50} determination. Young leaves showed higher levels of total flavonoids (0.19%), saponins (3.34%), and tannins (5.77%) compared to mature leaves (0.13%, 2.53%, and 4.58%, respectively). However, mature leaf extract exhibited stronger antioxidant activity with an IC_{50} value of 46.77 µg/mL (very strong category), while young leaves showed 53.14 µg/mL (strong category). This suggests that antioxidant efficacy is influenced more by compound maturity and synergistic effects than total concentration. Mature M. koenigii leaves have superior antioxidant potential despite lower phytochemical concentrations, highlighting the importance of compound quality and interactions between compounds.

Key Messages:
The phytochemical content of young curry leaves was higher, but old leaves showed stronger antioxidant activity (IC_{50} = 46.77 µg/mL vs. 53.14 µg/mL), indicating that the quality of compounds and synergistic interactions are more important than the total amount of compounds.

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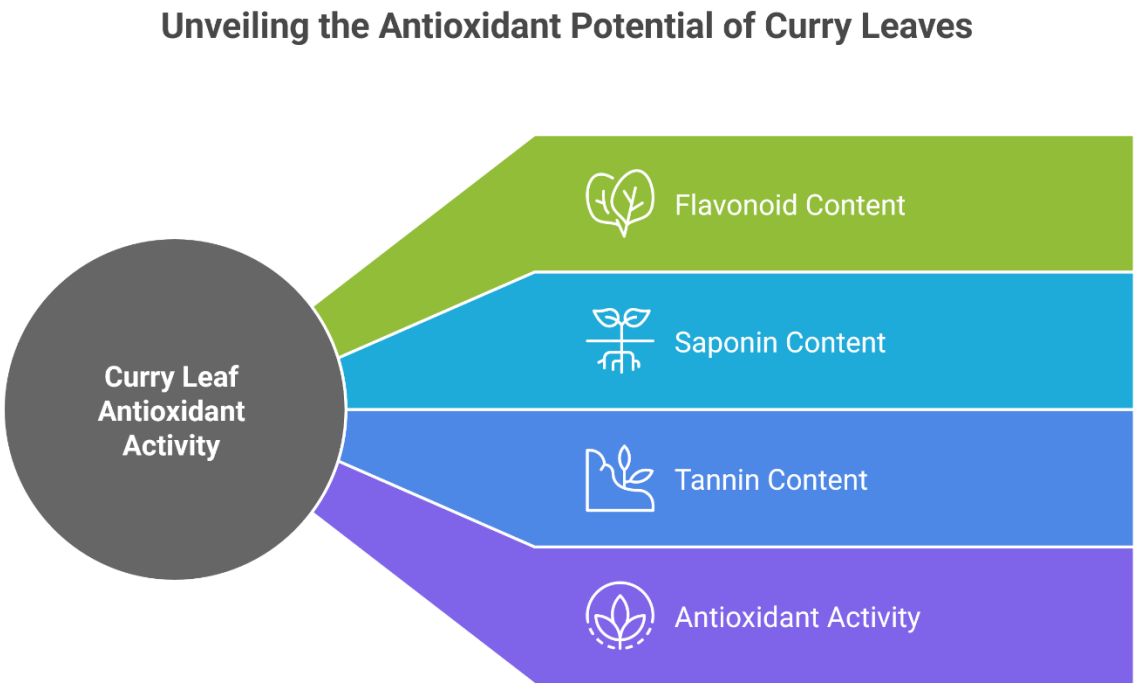
GRAPHICAL ABSTRACT



INTRODUCTION

Natural antioxidants derived from medicinal plants have gained significant attention in recent years due to their potential therapeutic applications and minimal adverse effects compared to synthetic alternatives. The escalating prevalence of oxidative stress-related diseases, including cardiovascular disorders, diabetes, and cancer, has intensified the search for potent natural antioxidant sources that can effectively neutralize free radicals and prevent cellular damage (1). Among the vast array of medicinal plants, *Murraya koenigii* (L.) Spreng, commonly known as curry leaf, has emerged as a promising candidate due to its rich phytochemical profile and demonstrated biological activities. This aromatic plant lives well in tropical and sub-tropical climates. Curry leaves belong to the Rutaceae family which has compound leaves that have 11-21 leaflets, about 2-4 cm long and 1-2 cm wide (2). Contemporary scientific investigations have revealed that *M. koenigii* possesses diverse pharmacological properties, including antidiabetic, antimicrobial, anti-inflammatory, and notably, antioxidant activities (3). In addition, the many phytochemicals found in curry leaves also have benefits such as larvicidal activity and antianxiety activity (4). The therapeutic potential of curry leaves is primarily attributed to their abundant secondary metabolites, particularly flavonoids, alkaloids, phenolic compounds, saponins, and tannins, which collectively contribute to their remarkable biological efficacy (5).

Recent phytochemical studies have demonstrated that *M. koenigii* extracts contain substantial concentrations of bioactive compounds responsible for their antioxidant properties. Aroor dkk reported that curry leaf extracts exhibited significant free radical scavenging activity, with IC50 values ranging from 50-150 µg/mL depending on the extraction method and solvent system employed (6). Similarly, Sachan et al. documented that ethanolic extracts of *M. koenigii* leaves contained appreciable amounts of flavonoids (0.8-2.1%), saponins (1.5-3.2%), and tannins (3.8-6.4%), establishing a direct correlation between phytochemical content and antioxidant efficacy (7). Furthermore, advanced analytical techniques have identified specific flavonoid compounds, including quercetin, kaempferol, and rutin, as major contributors to the antioxidant capacity of curry leaves (8). These findings have been corroborated by multiple studies demonstrating that the antioxidant activity of *M. koenigii* extracts is primarily mediated through the inhibition of lipid peroxidation, metal chelation, and free radical scavenging mechanisms. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay has been widely employed as a standard method for evaluating antioxidant activity, providing reliable and reproducible results for comparative studies across different

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plant extracts and extraction protocols (9).

Despite the extensive research conducted on *M. koenigii*, several critical gaps remain in our understanding of its phytochemical composition and antioxidant potential. Most previous studies have focused on crude extracts without comprehensive quantitative analysis of individual bioactive compounds, limiting our ability to establish structure-activity relationships and optimize extraction procedures. Additionally, there is considerable variability in reported IC50 values for antioxidant activity, ranging from 46 μg/mL to 208 μg/mL, suggesting that geographic origin, seasonal variations, and extraction methodologies significantly influence the therapeutic efficacy of curry leaf preparations (10). Furthermore, while numerous studies have qualitatively identified phytochemical constituents in *M. koenigii*, precise quantitative determination of flavonoids, saponins, and tannins using standardized protocols remains limited. This knowledge gap hampers the development of standardized herbal preparations and quality control measures essential for pharmaceutical applications. Moreover, the relationship between specific phytochemical concentrations and antioxidant activity has not been thoroughly investigated, particularly for Indonesian *M. koenigii* varieties, which may exhibit distinct phytochemical profiles due to unique environmental conditions and genetic factors.

The novelty of this investigation lies in its comprehensive approach to simultaneously quantifying multiple bioactive compounds (flavonoids, saponins, and tannins) in *M. koenigii* leaf extract using standardized spectrophotometric methods while establishing their collective contribution to antioxidant activity. Unlike previous studies that focused on individual compound classes, this research provides a holistic assessment of the phytochemical profile and its correlation with antioxidant efficacy. Additionally, the study employs rigorous extraction protocols using 96% ethanol and systematic DPPH assay procedures to ensure reproducible and reliable results. The utilization of curry leaves from the Aceh region of Indonesia adds geographical specificity to the phytochemical characterization, potentially revealing unique bioactive profiles not previously documented in the literature. This comprehensive analysis addresses existing knowledge gaps by providing precise quantitative data on multiple bioactive compounds and their synergistic effects on antioxidant activity, thereby contributing valuable information for the development of standardized *M. koenigii* preparations for pharmaceutical and nutraceutical applications.

This study aims to quantitatively determine the concentrations of flavonoids, saponins, and tannins in ethanolic extract of *M. koenigii* leaves using standardized spectrophotometric methods. Additionally, the research seeks to evaluate the antioxidant activity of the extract by determining the IC50 value through DPPH radical scavenging assay.

METHODS

Research Design

The design used an experimental study conducted from January 2025 until March 2025. The Integrated Laboratory UPT of Universitas Sebelas Maret performed the extraction of curry leaves. The Integrated Research and Testing Laboratory conducted a phytochemical investigation on curry leaf extract.

Curry leaf extraction

Curry leaves collected from Keude Siblah Village, Blangpidie, Aceh, were dried and ground using a blender. Three kilograms of leaves were macerated in 96% ethanol at a 1:10 ratio for a duration of 48 hours. The extract was filtered and then put in a water bath to evaporate until it was concentrated (11).

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Testing antioxidant activity

Preparation of Control Solution

A total of 1 ml of 0.4 mm DPPH solution was mixed with 4 ml of ethanol. The control solution was then used to determine the maximum absorption wavelength using a UV-Vis spectrophotometer, which was 517 nm.

Determination of Control Absorbance

The absorbance value of the control was determined at the peak wavelength of 517 nm.

Determination of Sample Absorbance

One milliliter of 0.4 mm DPPH solution was mixed with a specified sample volume, and ethanol was added to make a total amount of five milliliters. For half an hour, the mixture was incubated in a dark setting. The sample absorbance was measured at the peak wavelength of 517 nm after incubation.

Determination of IC50 Value

% Inhibitions = $\frac{A\ control - A\ sampel}{A\ control}$ x 100%

A regression curve was created by making the sample content vs % Inhibition (12).

Flavonoid levels

Quantification of total flavonoids with Quercetin as a reference. Add 0.3 ml of a 5% sodium nitrite solution to 10.0 mg of the quercetin standard. Add 0.6 ml of a 10% aluminum chloride solution after 5 minutes, then allow the mixture to settle for another 5 minutes. To get a final volume of 10 ml in a volumetric flask, add distilled water after adding 2 ml of 1 M sodium hydroxide. Using a spectrophotometer, measure the absorbance at 510 nm after transferring the solution to a cuvette.

Determination of total flavonoids in the sample: Weigh approximately 100 mg of the sample and add 2 ml of 4n HCl. Perform hydrolysis in an autoclave at 110 °C for 2 hours. After hydrolysis, filter the solution and add ether to the filtrate. Extract the mixture with ether and collect the ether phase. Repeat the extraction three times. After the amalgamated ether phases have been dehydrated, add 0.3 ml of 5% sodium nitrite to the anhydrous residue. After 5 minutes, add 0.6 ml of 10% aluminum chloride. Wait another 5 minutes, and then add 2 ml of 1 M sodium hydroxide. Fill a volumetric flask with distilled water until the entire capacity is 5 ml. If you need to, add extra water to make it less concentrated. Then, the solution is put into a cuvette, and the absorbance is measured at 510 nm.

Saponin levels

Quantification of total saponins: Weigh out around 100 mg of the sample, then add 2 ml of 25% H_2SO_4 and autoclave it at 110 °C for 120 minutes. Use ether to get the hydrolyzed sample out, and then dry the filtrate. Add 1 cc of distilled water and stir for 5 minutes. Then, add 50 µL of anisaldehyde, mix it up thoroughly, and let it rest for 10 minutes. Put the mixture in a water bath and heat it for 10 minutes at 60 °C after adding 2 cc of 50% H_2SO_4. Fill a volumetric flask with distilled water until it holds 10 ml. After that, make the solution 20 times less strong. Use a spectrophotometer with a wavelength of 435 nm to find the absorbance.

Using Quillaja Bark to create a Saponin Standard Curve: After 10 milligrams of saponin standard were measured, 5 milliliters of clean water were added and the mixture was vortexed for five minutes. Stir well, add 50 µL of anisaldehyde, and then let the mixture sit for ten minutes. After adding 2 cc of 50% H_2SO_4, heat for 10 minutes at 60 °C in a water bath. Using a volumetric flask, add distilled water until the total volume is 10 ml. Serial dilutions were made at concentrations of 200, 100, 50, 25, 12.5, and 6.25 µL. Determine each dilution's absorbance at a wavelength of 435 nm.

Tannin levels

The total tannin (Tannic acid equivalent) was quantified by the spectrophotometric technique; roughly 50 mg of the sample was weighed, 10 ml of diethyl ether was extracted for 20 hours, and the mixture was then filtered. To get a final volume of 10 ml, evaporate the remaining diethyl ether until it is completely dry, and then add distilled water. Add 0.1 ml of Folin-Ciocalteu reagent to 1 ml of the resulting solution, swirl the mixture, and let it stand for five minutes. Vortex in 2 ml of 20% sodium carbonate

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solution, then leave for another 5 minutes. After adding aquadest till the total volume is 10 ml, dilute the solution ten times. After 30 minutes of incubation at room temperature, use a spectrophotometer to measure the absorbance at 760 nm.

The tannic acid standard curve was created by carefully measuring the tannic acid standard, adding 10 milliliters of Folin-Ciocalteu reagent, swirling the mixture, and allowing it to stand for five minutes. Add a 20% sodium carbonate solution, then use filtered water to get the total volume down to 100 ml. Execute successive dilutions in accordance with the specified concentrations. Incubate the solutions for 30 minutes at ambient temperature, then assess the absorbance at a wavelength of 760 nm**.**

RESULTS

Proximate Analysis of Mature Curry Leaf Extract

The proximate analysis of mature curry leaf extract revealed the nutritional composition and basic chemical constituents essential for understanding the extract's overall profile (Table 1). The analysis demonstrated that carbohydrates constituted the major component with 58.29% b/b, followed by moisture content at 24.04% b/b. The lipid content was recorded at 13.28% b/b, while ash content represented 3.73% b/b of the total extract composition. Protein content was relatively low at 0.66% b/b, indicating that the extract is primarily composed of non-protein bioactive compounds.

Table 1. Proximate composition of mature curry leaf extract

Parameter	Content (% b/b)	Method
Moisture	24.04	Gravimetric
Ash	3.73	Gravimetric
Total Fat	13.28	Gravimetric
Protein	0.66	Kjeldahl
Carbohydrate	58.29	By Difference

Comparative Phytochemical Analysis of Young and Mature Curry Leaves

The quantitative phytochemical analysis revealed significant differences in bioactive compound concentrations between young and mature curry leaf extracts (Table 2). Three major secondary metabolites were successfully identified and quantified using UV-vis spectrophotometry: flavonoids, saponins, and tannins.

Table 2. Comparative phytochemical composition of young and mature curry leaf extracts

Bioactive Compound	Young Leaves (% b/b)	Mature Leaves (% b/b)
Total Flavonoids	0.19	0.13
Total Saponins	3.34	2.53
Total Tannins	5.77	4.58

The results demonstrated that young curry leaves consistently contained higher concentrations of all three phytochemical classes compared to mature leaves. Young leaves showed 46.15% higher flavonoid content (0.19% vs 0.13%), 32.02% higher saponin content (3.34% vs 2.53%), and 25.98% higher tannin content (5.77% vs 4.58%) compared to mature leaves. Tannins represented the predominant phytochemical constituent in both leaf types, followed by saponins and flavonoids in descending order.

Comparative Antioxidant Activity Assessment

The DPPH radical scavenging assay revealed distinct antioxidant capacities between young and mature curry leaf extracts (Table 3). Both extracts demonstrated strong antioxidant activity with IC_{50} values below 60 µg/mL, indicating substantial free radical scavenging potential.

Mature curry leaf extract exhibited superior antioxidant activity with an IC_{50} value of 46.772 µg/mL, categorized as "very strong" antioxidant activity. Young leaf extract demonstrated an IC_{50} value of 53.144 µg/mL, classified as "strong" antioxidant activity (13). The difference in IC_{50} values indicated that mature

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leaves required 13.6% less extract concentration to achieve 50% radical scavenging compared to young leaves.

Table 3. Comparative antioxidant activity of young and mature curry leaf extracts

Extract Type	IC_{50} Value (μg/mL)	Antioxidant Activity Category
Mature Leaves	46.772	Very Strong (IC_{50} < 50 μg/mL)
Young Leaves	53.144	Strong (IC_{50} = 50-100 μg/mL)

Correlation Analysis Between Phytochemical Content and Antioxidant Activity

Despite young leaves containing higher individual phytochemical concentrations, mature leaves demonstrated superior antioxidant activity. This apparent contradiction suggests that factors beyond total phytochemical content, such as compound maturity, structural modifications, and synergistic interactions, significantly influence antioxidant capacity.

DISCUSSION

Proximate Composition

The proximate analysis of mature curry leaf extract revealed a composition dominated by carbohydrates (58.29%), which primarily consists of structural polysaccharides and secondary metabolite glycosides. The substantial carbohydrate content indicates the presence of complex bioactive compounds in glycosylated forms, which may contribute to the extract's biological activities (14). The moisture content of 24.04% falls within acceptable ranges for plant extracts, ensuring stability while maintaining bioactive compound integrity. The lipid content of 13.28% suggests the presence of essential fatty acids and lipophilic bioactive compounds, which may enhance the extract's therapeutic potential through improved bioavailability and membrane interactions. The ash content of 3.73% indicates moderate mineral content, potentially including essential trace elements that may contribute to the extract's overall biological activity through metal cofactor functions or chelation properties. The relatively low protein content (0.66%) confirms that the extract's biological activities are primarily attributed to secondary metabolites rather than enzymatic proteins, which aligns with the phytochemical-focused therapeutic approach of plant extracts. These proximate composition results are consistent with previous studies on Murraya koenigii, where Ningappa et al. reported similar carbohydrate dominance in curry leaf extracts, emphasizing the importance of polysaccharide-bound bioactive compounds in medicinal plants (15). The balanced composition of macronutrients and bioactive compounds positions the extract as a suitable candidate for nutraceutical applications, where both nutritional value and therapeutic efficacy are desired (16).

Age-Related Variations in Phytochemical Accumulation

The comparative phytochemical analysis revealed that young curry leaves consistently accumulated higher concentrations of flavonoids, saponins, and tannins compared to mature leaves. This finding contradicts the common assumption that older plant tissues contain higher secondary metabolite concentrations due to prolonged biosynthetic activity. The 46.15% higher flavonoid content in young leaves (0.19% vs 0.13%) suggests that flavonoid biosynthesis occurs actively during early leaf development, potentially as a protective mechanism against environmental stresses including UV radiation and pathogen attacks. The 32.02% higher saponin content in young leaves (3.34% vs 2.53%) indicates that these glycosidic compounds serve crucial protective functions during vulnerable early growth stages. Saponins are known for their antimicrobial and deterrent properties, providing young tissues with essential defense mechanisms against herbivores and pathogens. Similarly, the 25.98% higher tannin content in young leaves (5.77% vs 4.58%) supports the hypothesis that phenolic compounds accumulate during active growth phases to protect developing tissues from oxidative stress and mechanical damage. These age-related phytochemical variations align with the findings of Gupta et al., who reported that young Murraya koenigii leaves contained higher concentrations of carbazole alkaloids compared to mature leaves, suggesting that secondary metabolite biosynthesis is most active during early developmental

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stages. The observed pattern may be explained by the plant's resource allocation strategy, where young tissues prioritize defense compound production to ensure survival during vulnerable growth periods (17).

Antioxidant Activity Paradox: Quality vs Quantity

The unexpected finding that mature leaves (IC_{50} = 46.772 μg/mL) demonstrated superior antioxidant activity compared to young leaves (IC_{50} = 53.144 μg/mL) despite lower total phytochemical concentrations represents a significant discovery that challenges conventional assumptions about phytochemical-antioxidant relationships. This paradox can be explained through several interconnected mechanisms that emphasize the importance of compound quality, structural maturity, and synergistic interactions over mere quantitative accumulation. The superior antioxidant activity of mature leaves may be attributed to age-related structural modifications in bioactive compounds, particularly the formation of more stable and effective antioxidant configurations. During leaf maturation, secondary metabolites undergo various chemical transformations, including glycosylation, methylation, and polymerization, which can enhance their radical scavenging capabilities. Mature tannins, for instance, may develop more complex polymeric structures with increased hydroxyl group availability, resulting in enhanced electron-donating capacity and improved antioxidant efficiency. The concept of "antioxidant synergy" provides another explanation for this phenomenon. Mature leaves may contain optimal ratios of different antioxidant compounds that work synergistically to enhance overall radical scavenging activity. Research by Surveswaran et al. demonstrated that the antioxidant activity of plant extracts depends not only on individual compound concentrations but also on their interactive effects and complementary mechanisms of action. The mature curry leaf extract may have achieved an optimal balance of flavonoids, saponins, and tannins that maximizes their collective antioxidant potential. Furthermore, the lower moisture content and altered cellular structure in mature leaves may facilitate better extraction and availability of bioactive compounds during the DPPH assay. The extended exposure to environmental stresses during the maturation process may have induced the formation of stress-responsive antioxidant compounds that are more effective in neutralizing free radicals than the constitutive antioxidants present in young leaves (18).

Mechanistic Insights into Antioxidant Activity

The strong antioxidant activity observed in both extracts can be attributed to the presence of phenolic compounds, particularly tannins, which serve as the primary contributors to radical scavenging capacity. The tannin content of 4.58% in mature leaves and 5.77% in young leaves provides substantial electron-donating capacity through their multiple hydroxyl groups, enabling efficient neutralization of DPPH radicals. However, the superior performance of mature leaf extract suggests that tannin structural maturity and polymerization degree significantly influence antioxidant effectiveness. The flavonoid content, although lower in concentration, plays a crucial role in antioxidant activity through multiple mechanisms including metal chelation, enzyme inhibition, and direct radical scavenging. The flavonoid concentrations of 0.13% in mature leaves and 0.19% in young leaves indicate that these compounds contribute to the overall antioxidant capacity through their ability to interrupt radical chain reactions and chelate pro-oxidant metal ions. Saponins, while not traditionally considered primary antioxidants, may contribute indirectly to antioxidant activity through their ability to form complex micelles that can encapsulate and neutralize free radicals. The saponin content of 2.53% in mature leaves and 3.34% in young leaves suggests that these glycosidic compounds provide additional protective mechanisms that complement the direct antioxidant effects of phenolic compounds (19).

Implications for Standardization and Quality Control

The observed age-related differences in phytochemical composition and antioxidant activity have significant implications for the standardization and quality control of Murraya koenigii preparations. The findings suggest that the selection of leaf maturity stages should be carefully considered based on the intended therapeutic application. For applications requiring maximum antioxidant activity, mature leaves appear to be the optimal choice, while applications targeting specific phytochemical concentrations may benefit from young leaf extracts. The establishment of age-specific phytochemical profiles provides a

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foundation for developing standardized extraction protocols and quality control measures. The consistent patterns observed in phytochemical distribution across different leaf ages can serve as markers for authentication and quality assessment of commercial curry leaf products. This information is particularly valuable for pharmaceutical and nutraceutical industries, where consistent bioactive compound profiles are essential for therapeutic efficacy and regulatory compliance (20). This knowledge can inform sustainable cultivation practices and contribute to the optimization of curry leaf production for commercial therapeutic applications (21).

CONCLUSION

This comprehensive study successfully characterized the phytochemical composition and evaluated the antioxidant potential of Murraya koenigii ethanolic leaf extract through quantitative analysis and DPPH radical scavenging assays. The comparative analysis between young and mature curry leaves revealed significant age-related variations in both phytochemical content and antioxidant activity. Young curry leaves consistently contained higher concentrations of all bioactive compounds: flavonoids (0.19% vs 0.13%), saponins (3.34% vs 2.53%), and tannins (5.77% vs 4.58%) compared to mature leaves. However, mature leaves demonstrated superior antioxidant activity with an IC_{50} value of 46.772 μg/mL (very strong category) compared to young leaves with an IC_{50} value of 53.144 μg/mL (strong category).

This paradoxical finding indicates that antioxidant efficacy depends on compound quality, structural maturity, and synergistic interactions rather than mere quantitative accumulation. The proximate analysis of mature curry leaf extract revealed a composition dominated by carbohydrates (58.29%), followed by moisture (24.04%), fat (13.28%), ash (3.73%), and protein (0.66%). The age-related structural modifications in bioactive compounds and optimal ratios of different antioxidant compounds working synergistically explain the superior antioxidant activity in mature leaves. For applications requiring maximum antioxidant activity, mature leaves are recommended, while young leaves may be preferred for specific phytochemical targeting. The findings validate traditional medicinal use of curry leaves and support their development as natural antioxidant supplements for nutraceutical and pharmaceutical applications. Future research should investigate molecular mechanisms underlying age-related antioxidant enhancement and develop standardized protocols for age-specific therapeutic applications.

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CONFLICTS OF INTEREST

The authors assert that there are no conflicts of interest related to this work.

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