Phytochemical profile and antioxidant potency of *Murraya koenigii* leaves extract: Influence of solvent fractionation

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Abstract. *Murraya koenigii* (L.) Spreng is renowned in Asian herbal medicine for its diverse pharmacological properties, particularly its antioxidant capabilities. While numerous studies have explored its bioactivity, there is limited comparative data on how different solvent fractions affect its phytochemical and antioxidant properties. The objective of this study was to assess the phytochemical components and antioxidant capacity of ethanol extract and its solvent fractions (n-hexane and ethyl acetate) obtained from *M. koenigii* leaves. The total concentrations of phenolics, flavonoids, terpenoids, and alkaloids were quantified spectrophotometrically, and antioxidant activity was evaluated using DPPH, ABTS, and FRAP tests. The phytochemical analysis indicated that n-hexane had the highest concentrations of flavonoids, phenolics, and terpenoids, while ethyl acetate was richest in alkaloids. The ethyl acetate fraction demonstrated the most potent antioxidant activity, as evidenced by its lowest IC_{50} values in DPPH (5.10 ± 0.49 mg/L) and ABTS (94.96 ± 0.17 mg/L) assays, along with the highest FRAP reducing power (279.00 ± 1.00 mg AAE/g). Pearson correlation analysis showed significant correlations between alkaloids and DPPH ($r = -0.952$, $p < 0.01$) and FRAP ($r = 0.961$, $p < 0.01$) activities. These results highlighted that metabolite polarity and assay conditions greatly influence antioxidant behavior.

1 Introduction

The curry leaf plant, *Murraya koenigii* (L.) Spreng belongs to the Rutaceae family and has been used for centuries in South and Southeast Asian traditional medicine and cooking [1]. The plant's complex phytochemical composition, which contains phenolic chemicals,

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flavonoids, and alkaloids, attracted a lot of scientific interest. Numerous pharmacological benefits, including antioxidant, anti-inflammatory, antibacterial, and anticancer qualities, have been linked to these phytochemicals. Because of this, *M. koenigii* is a prospective source for finding new therapeutic compounds [1]. Among these constituents, carbazole alkaloids are particularly noteworthy due to their structural diversity and potent biological effects [2]. Found mainly in the leaves, bark, and roots, these compounds have demonstrated antitumor, antidiabetic, and anti-inflammatory activities in previous studies [1, 3]. Furthermore, *M. koenigii* is also a rich source of terpenoids, which contribute significantly to its distinctive aroma and medicinal properties. Terpenoids, including monoterpenes, sesquiterpenes, diterpenes, and triterpenes, have been linked to various biological activities, including insecticidal and other therapeutic effects [4]. Flavonoid and phenolic compounds further contribute to the plant's antioxidant capacity [1].

While the antioxidant properties of *M. koenigii* have been widely reported [1], most studies have relied on crude extracts or isolated compounds and have not compared fractions derived from solvents with different polarities. Previous research has not simultaneously quantified the four major groups of secondary metabolites, including alkaloids, flavonoids, phenolics, and terpenoids, across these plant fractions. Moreover, the quantitative correlation between these metabolite groups and their antioxidant performance remains poorly characterized. In particular, the contribution of semi-polar and nonpolar constituents to total antioxidant activity has not been comprehensively assessed. A polarity-based fractionation approach is crucial to delineate how metabolite distribution changes with solvent characteristics and how these changes affect antioxidant performance.

Therefore, this study aimed to observe the antioxidant activity and phytochemical composition of *M. koenigii* ethanol extract and its solvent fractions (n-hexane and ethyl acetate). By quantitatively linking the levels of major secondary metabolites with their corresponding antioxidant responses, this research offers a mechanistic insight into the relationship between chemical composition and antioxidant potency, and provides a more accurate foundation for the targeted utilization of *M. koenigii* in natural product and functional food applications.

2 Materials and methods

2.1 Plant collection and extraction

M. koenigii leaves were obtained from Wuluhan District, Jember City, East Java Province, Indonesia, and identified at the Laboratory of Plant Taxonomy, Structure, and Development, Department of Biology, Faculty of Mathematics and Natural Science, Brawijaya University (identification no. 00902/UN10.F0916/B/TA.00.02.3/2024). Extraction and fractionation were carried out using a modified technique from earlier research [5]. Dried leaf powder (100 g) was macerated with 90% ethanol (1 L) for 24 h and filtered. After concentrating the filtrate at 50°C with a rotary evaporator, any remaining solvent was removed by immersing it in a water bath at 60°C.

Ethyl acetate and n-hexane were used consecutively for fractionation. First, water and n-hexane (1:1, v/v) were used to partition the ethanol extract until the n-hexane layer turned colorless. Ethyl acetate (1:1, v/v) was then used to extract the residual aqueous phase. The n-hexane and ethyl acetate fractions were obtained by evaporating each organic component under reduced pressure at 45°C (50 rpm). Before being used again, all extracts were kept at 4°C.

2.2 Antioxidant activity assays

2.2.1 DPPH assay

Each extract was made into a stock solution (1,000 ppm) in ethanol and serially diluted. After combining 0.5 mL of each sample with 1.5 mL of a 0.1 mM DPPH solution, the samples were incubated for 30 min at 37°C in the dark. A UV-Vis spectrophotometer was used to detect absorbance at 515 nm. The following formula was used to get the inhibition percentage [6]:

$$\%\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (1)$$

The IC_{50} value was calculated by plotting a calibration curve of % inhibition vs. concentration. The positive control was ascorbic acid (5–25 ppm).

2.2.2 ABTS assay

To perform the ABTS experiment, 7 mM ABTS and 2.45 mM potassium persulfate were combined to make the $ABTS^+$ solution, which was then incubated in the dark for 12–16 h. Then, methanol was added to the mixture until its absorbance at 745 nm was 0.70 ± 0.02. 2.85 mL of $ABTS^+$ solution was mixed with 0.15 mL extract (1,000 ppm) and incubated at room temperature for 30 min. At 745 nm, absorbance was measured. Regression analysis was used to determine IC_{50} values, and radical inhibition activity was computed as a percentage of inhibition. The standard was ascorbic acid at values between 10 and 80 ppm [7].

2.2.3 FRAP assay

To prepare fresh FRAP reagent, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ (10:1:1, v/v/v) were mixed. After mixing 3 mL of the FRAP reagent with 1 mL of extract at a concentration of 100 ppm, the mixture was incubated for 30 min at 37°C. FRAP activity was measured in mg of ascorbic acid equivalents per gram of extract (mg AAE/g), and absorbance was measured at 596 nm [8].

2.3 Quantitative phytochemical analysis

2.3.1 Total flavonoid content (TFC)

Quercetin was used as the standard when measuring the total flavonoid content using the aluminum chloride colorimetric technique. In order to create a standard curve, a quercetin stock solution at a concentration of 1,000 ppm was first made using 96% ethanol and diluted to reach concentrations ranging from 20 to 100 ppm. In the experiment, 0.2 mL of 10% $AlCl_3$ and 0.2 mL of 1 M Na_2CO_3 were mixed with 0.1 mL of extract at 10,000 ppm, and distilled water was added to make a total volume of 10 mL. The absorbance was measured at 320 nm after 30 minutes of room temperature incubation. Using the calibration curve equation ($y = mx + c$), the flavonoid concentration was measured as mg of quercetin equivalent per gram of extract (mg QE/g).

2.3.2 Total phenolic content (TPC)

Gallic acid was used as a reference when measuring the total phenolic content using the Folin–Ciocalteu technique. To create the calibration curve, a 1,000 ppm gallic acid stock solution was made in distilled water and then diluted to concentrations between 20 and 120 ppm. In the experiment, 1 mL of 500 ppm extract and 0.4 mL of Folin-Ciocalteu reagent were combined, and the mixture was allowed to stand for three minutes. After adding 4 mL of 5% Na_2CO_3, the liquid was diluted with distilled water to a total volume of 10 mL. The mixture was incubated at room temperature for 40 minutes before the absorbance at 758 nm was measured. The total phenolic content was determined using gallic acid equivalent (mg GAE/g).

2.3.3 Total alkaloid content (TAC)

The bromocresol green (BCG) complexation method was used to measure the concentration of alkaloids, with caffeine serving as the reference standard. The BCG reagent (10^{-1} M) was made by dissolving 0.007 g of BCG in 0.3 mL of 2 N NaOH and 0.5 mL of distilled water, heating the mixture for 15 min at 60°C, and then diluting it to a volume of 100 mL. Citric acid (0.2 M) and disodium phosphate (2 M) were combined to create a phosphate buffer (pH 4.7).

In the test, 2 mL of extract (500 ppm), 2 mL of BCG, and 2 mL of phosphate buffer were mixed. Then, 3 mL of chloroform was used for extraction. After isolating the chloroform layer, it was moved to a volumetric flask, and chloroform was used to adjust the volume to 10 mL. At 289 nm, the absorbance was measured. A calibration curve was created using caffeine standard solutions ranging from 50 to 500 ppm, and the results were expressed as µg caffeine equivalent per mL (µg/mL).

2.3.4 Total terpenoid content (TTC)

Linalool was used as a reference standard for the terpenoid content measurement. After thoroughly mixing 1.5 mL of 1,000 ppm extract with 4.5 mL of chloroform, the mixture was incubated for three minutes. The inner tube wall was then gradually filled with 750 μL of H_2SO_4 while being kept cold in an ice bath. A reddish-brown precipitate formed after the mixture was left at room temperature in the dark for four hours. After carefully removing the supernatant, the residue was dissolved in methanol to a final amount of 4 milliliters. 538 nm was used to quantify absorbance. A standard calibration curve was prepared using linalool concentrations ranging from 5 to 160 ppm, and the terpenoid concentration was reported as μg of linalool equivalent per mL (μg/mL) [9].

2.4 Statistic analysis

Each experiment was run three times, and the results were presented as the mean ± standard deviation (SD). Pearson's correlation coefficients for TPC, TFC, TAC, TTC, and antioxidant activities were computed using the SPSS 22.0 software.

3 Results

3.1 Extraction yield

To evaluate solvent efficiency, extraction yields were calculated based on 100 g of dried *M. koenigii* leaf powder. The ethanol extraction produced 80.034 g of crude extract (80.03% yield), indicating a high recovery of polar to semipolar constituents (Table 1). Fractionation of the crude extract resulted in 5.50 g of the n-hexane fraction (5.50%) and 3.58 g of the ethyl acetate fraction (3.58%). The markedly lower yields of these fractions reflect the smaller abundance of nonpolar and semipolar metabolites compared with the predominantly polar compounds extracted by ethanol. Reporting these yields highlights the differential efficiency of each solvent system and provides essential context for interpreting subsequent phytochemical and antioxidant results.

Table 1. Antioxidant analysis of *M. koenigii* ethanol extract and its fractions.

Sample/Fraction	Mass obtained (g)	Yield (%)
Crude ethanol extract	80.034	80.03
n-Hexane fraction	5.50	5.50
Ethyl acetate fraction	3.58	3.58

3.2 Antioxidant activity

DPPH, ABTS, and FRAP tests were used to assess the antioxidant capacity of the crude ethanol extract, n-hexane fraction, and ethyl acetate fraction from *M. koenigii*. Together, these methods evaluate the capacity to scavenge free radicals and lower oxidative stress.

In every assay, the ethyl acetate fraction outperformed the other two samples in terms of antioxidant activity. In the DPPH assay, it showed an IC_{50} of 5.10 ± 0.25 mg/L, demonstrating the strongest radical scavenging activity, which was even more potent than the positive control ($p<0.05$), ascorbic acid (IC_{50} = 9.85 ± 0.04 mg/L). The n-hexane fraction also displayed significant DPPH activity (IC_{50} = 30.14 ± 0.30 mg/L), while the crude ethanol extract had the lowest activity (IC_{50} = 122.40 ± 0.70 mg/L). In the ABTS assay, the ethyl acetate fraction again demonstrated superior activity with an IC_{50} of 74.17 ± 0.52 mg/L, followed by the crude ethanol extract (IC_{50} = 94.96 ± 0.17 mg/L) and the n-hexane fraction (IC_{50} = 123.19 ± 1.78 mg/L). In the FRAP assay, which quantifies reducing power, the ethyl acetate fraction recorded the highest value (279 ± 1.00 mg AAE/g extract), significantly surpassing the n-hexane fraction (141.83 ± 0.76 mg AAE/g extract) and the crude ethanol extract (91.33 ± 2.25 mg AAE/g extract) (Table 2).

Table 2. Antioxidant analysis of *M. koenigii* ethanol extract and its fractions.

Sample/Fraction	DPPH (mg/L) IC_{50} ± SD	ABTS (mg/L) IC_{50} ± SD	FRAP (mg AAE/g) ± SD
Ascorbic acid	9.85 ± 0.04^{b}	50,01 ± 0.25^{a}	-
Crude ethanol extract	122.40 ± 0.70^{d}	94.96 ± 0.17^{c}	91.33 ± 2.25^{a}
n-Hexane	30.14 ± 0.30^{c}	123.79 ± 1.44^{d}	141.83 ± 0.76^{b}
Ethyl Acetate	5.10 ± 0.49^{a}	74.17 ± 0.52^{b}	279.00 ± 1.00^{c}

Note: Distinct notations denote a significant difference among the groups.

The superior antioxidant level of the ethyl acetate fraction is primarily attributed to its enriched content of phenolic and flavonoid compounds, which are well-known for their radical-neutralizing properties through hydrogen donation and electron transfer mechanisms. The notable activity of the n-hexane fraction in DPPH and FRAP assays, despite its lower ABTS activity, suggests the presence of nonpolar terpenoid and flavonoid compounds that may be more effective in less polar radical environments. The fractionation process clearly enhanced antioxidant activity by concentrating specific bioactive compounds, as the crude extract, containing a broader mix, consistently showed the lowest activity.

3.3 Quantitative phytochemical analysis

Quantitative analysis revealed distinct profiles for flavonoids, total phenolics, alkaloids, and terpenoids across the crude extract and its fractions, reflecting the selectivity of the extraction solvents. The n-hexane fraction exhibited the highest flavonoid content (167.21 ± 3.81 mg QE/g) and the highest phenolic content (111.0 ± 1.00 mg GAE/g). It also contained the highest concentration of terpenoids (204.43 ± 4.64 μg/mL), indicating its efficiency in extracting lipophilic and moderately polar compounds. Conversely, the ethyl acetate fraction showed the highest alkaloid content (216.88 ± 2.34 μg/mL) and substantial amounts of phenolics (63.30 ± 0.43 mg GAE/g) and flavonoids (107.59 ± 4.12 mg QE/g). The crude ethanol extract generally contained lower concentrations of these phytochemicals compared to the fractionated extracts (Table 3).

Table 3. Quantitative phytochemical analysis of *M. koenigii* crude extract and fractions.

Sampel	Flavonoid (mgQE/g ± SD)	Fenol (mg GAE/g) ± SD)	Alkaloid (μg/mL ± SD)	Terpenoid (μg/mL ± SD)
Crude extract ethanol	137.30 ± 0.88[b]	49.61 ± 0.22[a]	77.99 ± 0.78[a]	9.31 ± 1.80[a]
n-Hexane	167.21 ± 3.81[c]	111.0 ± 1.00[c]	153.47 ± 0.81[b]	204.43 ± 4.64[c]
Ethyl Acetate	107.59 ± 4.12[a]	63.30 ± 0.43[b]	216.88 ± 2.34[c]	105.74 ± 0.65[b]

Note: Distinct notations denote a significant difference among the groups.

These results underscore that fractionation effectively segregates secondary metabolites based on their polarity. The n-hexane fraction preferentially enriched lipophilic compounds like terpenoids and nonpolar flavonoids, while the semipolar ethyl acetate fraction concentrated alkaloids and a significant portion of semipolar flavonoids and phenolics.

3.4 Correlation between phytochemical content and antioxidant activity

The correlation analysis revealed unique associations between the primary phytochemical groups and the three antioxidant tests (DPPH, ABTS, and FRAP), highlighting that each metabolite plays a distinct role based on the assay mechanism and solvent used. Flavonoids demonstrated a very strong positive correlation with ABTS ($r = 0.932$, $p < 0.01$), suggesting that they significantly contributed to ABTS radical scavenging activity (Table 4). The ABTS assay is more sensitive to both polar and moderately nonpolar antioxidants, which aligns with the intermediate polarity of many flavonoid compounds. Conversely, flavonoids showed no significant correlation with DPPH ($r = 0.252$) and a moderate negative correlation with FRAP ($r = -0.708$, $p < 0.05$). This indicates that flavonoids in *M. koenigii* are more effective as hydrogen or electron donors in the ABTS system rather than strong reducers in the FRAP assay.

Phenolics exhibited a notable positive correlation with ABTS ($r = 0.789$, $p < 0.01$), reinforcing their established function as effective radical scavengers. In contrast, phenolics had a weak and negative correlation with DPPH ($r = -0.504$) and showed an almost nonexistent relationship with FRAP ($r = -0.050$). This pattern suggests that the phenolic constituents present in *M. koenigii* fractions were more reactive toward the ABTS radical, likely due to their solubility and steric compatibility rather than toward DPPH or iron-reducing conditions in the FRAP assay.

A very strong negative correlation with DPPH ($r = -0.952$, $p < 0.01$), indicating that higher alkaloid content was associated with stronger DPPH-scavenging activity (lower IC_{50} = better activity). Alkaloids also show a very strong positive correlation with FRAP ($r = 0.961$, $p < 0.01$), suggesting they contribute substantially to reducing power in the iron-based FRAP assay. Their correlation with ABTS was weak and nonsignificant ($r = -0.411$), implying that alkaloids in *M. koenigii* are more suited to electron-transfer mechanisms (FRAP, DPPH) than hydrogen atom transfer (ABTS). Terpenoids demonstrate a moderate negative correlation with DPPH ($r = -0.731$, $p < 0.05$), indicating a meaningful contribution to DPPH scavenging. Their correlation with ABTS is weaker (r = 0.586) and nonsignificant, while the correlation with FRAP is minimal (r = 0.240). This suggests that nonpolar terpenoids participate modestly in free-radical scavenging but play a limited role in reducing power compared with alkaloids and phenolics.

Table 4. Pearson's correlation between phytochemical content and antioxidant activity in *M. koenigii* fractions.

	DPPH	ABTS	FRAP
Flavonoid	0.252	0.932**	-0.708*
Phenolic	-0.504	0.789**	-0.05
Alkaloid	-0.952**	-0.411	0.961**
Terpenoid	-0.731*	0.586	0.24

Note: **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level

4 Discussion

The results reveal that *M. koenigii* is a potent source of diverse phytochemicals, supporting its ethnomedicinal relevance and potential as a source of natural therapeutic agents. Fractionation significantly affected the concentration and distribution of secondary metabolites, resulting in distinct antioxidant capacities among the ethanol extract and its fractions. DPPH, ABTS, and FRAP assays were used to measure antioxidant activity, and the results consistently indicated that the ethyl acetate fraction had the highest activity of any sample. This strong antioxidant ability, evident from its lowest IC_{50} values for DPPH and ABTS and highest FRAP value, indicates a superior capacity for radical scavenging and reducing power [10, 11].

The main observation warranting further discussion was the ethyl acetate fraction's superior antioxidant activity, even though quantitative analysis showed the n-hexane fraction had higher total flavonoid and phenolic content. This discrepancy was attributed to the significant quantities of terpenoids and specific nonpolar flavonoids preferentially isolated by n-hexane. Nonpolar terpenoid compounds, which are abundant in the essential oil of curry leaves, are known for their antioxidant properties and may exhibit enhanced efficacy in neutralizing nonpolar radicals, but are ineffective in the polar system [4]. The ethanol extract displayed lower activity due to compound complexity and matrix effects that attenuate radical-scavenging efficiency. These findings demonstrate that fractionation is effective in concentrating active phytochemicals and enhancing antioxidant potency.

The differing antioxidant activities observed among the solvent fractions in this study can be largely explained by the character of each fraction and solvent polarity-driven selectivity, and the resulting chemical composition of each fraction. Firstly, the ethyl acetate fraction contained the highest alkaloid content. Higher alkaloid concentrations led to more effective DPPH radical scavenging, according to correlation analysis, which revealed a substantial negative association between alkaloid content and DPPH IC_{50}. Additionally, alkaloid content and FRAP activity were found to be strongly positively correlated. While terpenoids that are highest in the n-hexane fraction and show a significant negative correlation with DPPH IC_{50} indicate their role in radical scavenging, particularly in less polar environments. However, their overall impact did not surpass the combined effect of alkaloids and specific polar phenolics/flavonoids in the ethyl acetate fraction. This result strongly suggests that alkaloids, particularly the carbazole alkaloids known in *M. koenigii* [1, 3], play a significant role in the ethyl acetate fraction's antioxidant capacity. Alkaloids are known to act as antioxidants through several mechanisms, including direct free radical scavenging, sequestering catalysts involved in oxidation, and metal chelation due to their nitrogen-containing heterocyclic structures [12].

Secondly, while the n-hexane fraction contained higher total flavonoids and phenolics, it is plausible that the specific types or structural variations of these compounds isolated by ethyl acetate possess greater individual antioxidant potency. Different solvents selectively extract compounds based on their polarity, leading to variations not just in quantity but also in the chemical structure and efficacy of the isolated constituents. In order to neutralize free radicals and stop chain reactions, phenolic substances and flavonoids mainly function as antioxidants by donating hydrogen atoms or electrons [13]. The specific arrangement of hydroxyl groups and other functional moieties in the flavonoids and phenolics present in the ethyl acetate fraction might confer superior electron-donating or radical-stabilizing capabilities.

Lastly, polar solvents such as ethyl acetate preferentially extract moderately polar phenolics and flavonoid aglycones that are both sufficiently soluble in the assay medium and intrinsically strong electron/hydrogen donors, which explains the high FRAP and low DPPH IC_{50} values observed for the ethyl acetate fraction. In contrast, non-polar solvents like n-hexane concentrate lipophilic constituents (terpenoids, non-polar phenolics, lipids) that may be less soluble or less reactive under the polar conditions of typical in-vitro assays (methanolic/aqueous media), producing lower apparent antioxidant activity despite measurable total phenolic. This solvent polarity that can affect assay compatibility is well documented in studies surveying extraction efficiency and antioxidant outcomes across solvent systems [14].

Beyond simple solubility effects, the polar paradox provides a conceptual framework for the apparent paradox that our antioxidant activity depends not only on chemical abundance but on the solvent type where the radical reactions occur. The polar paradox originally posits that polar antioxidants can be more effective in non-polar matrices and vice versa because of preferential partitioning to the reactive interface. When assays are conducted in polar media, non-polar antioxidants frequently fail to access or neutralize radicals efficiently. Recent re-evaluations and experimental work emphasize that while the polar paradox remains a useful heuristic, real systems include additional modifiers of concentration dependence, association colloids, and interfacial phenomena that alter the expected outcomes. Thus, the low activity of the n-hexane fraction in polar assays is consistent with polar paradox behavior, while the superior performance of the ethyl acetate fraction reflects favorable polarity and interfacial positioning in the assay solvent medium. The nonpolar n-hexane fraction was enriched with lipophilic constituents and showed the highest concentrations of flavonoids and terpenoids [15].

These findings emphasize the critical role of fractionation in separating and enriching distinct phytochemical classes, thereby potentially increasing their inherent biological activities. The pronounced antioxidant activity of the ethyl acetate fraction, significantly correlated with its high alkaloid content, specific phenolic, and flavonoid constituents, suggests its considerable promise for development in pharmaceutical and functional food applications [6]. In the same way, the n-hexane fraction's rich terpenoid and flavonoid profile emphasizes its suitability for uses that call for these particular substances. Future study should focus on isolating and precisely identifying the particular molecules responsible for the described actions in each fraction in order to establish their therapeutic relevance. Comprehensive mechanistic studies and in vivo assessments should come next.

5 Conclusion

This study demonstrates that *M. koenigii* extract possesses a rich and diverse phytochemical profile, with the distribution of phenolics, flavonoids, alkaloids, and terpenoids strongly influenced by solvent polarity. Among the examined fractions of *M. koenigii*, the ethyl acetate fraction consistently exhibited the strongest antioxidant performance, indicated by its lowest IC_{50} values in both DPPH and ABTS assays and its highest FRAP reducing power. This superior activity was supported by correlation analysis showing strong associations between alkaloid content and both DPPH and FRAP responses, as well as significant contributions from phenolics and flavonoids in the ABTS assay. These findings emphasize that antioxidant efficacy is not solely dependent on total metabolite abundance but also on chemical structure, polarity compatibility, and the assay environment. Future research should focus on isolating individual active constituents and validating their mechanisms and bioactivities in biological systems.

References

1. R. Balakrishnan, D. Vijayraja, S.H. Jo, P. Ganesan, I. Su-Kim, D.K. Choi, Medicinal profile, phytochemistry, and pharmacological activities of *Murraya koenigii* and its primary bioactive compounds. Antioxidants. **9**, 101 (2020). https://doi.org/10.3390/antiox9020101
2. A. Tiwari, B. Mishra, Diverse pharmacological actions of potential carbazole derivatives by influencing various pathways of molecular signaling. Future J. Pharm. Sci. **10**, 77 (2024). https://doi.org/10.1186/s43094-024-00650-0
3. A. Mehreen, S. Kamal, S.M. Vagif, M. Qaisar, S. Urainab, A. Ullah, Novel carbazole alkaloid from *Murraya koenigii* (L.) Spreng. Int. J. Second. Metab. **10**, 354 (2023). https://doi.org/10.21448/ijsm.1193419
4. B.R.R. Rao, D.K. Rajput, G.R. Mallavarapu, Chemical diversity in curry leaf (*Murraya koenigii*) essential oils. Food Chem. **126**, 989 (2011). https://doi.org/10.1016/j.foodchem.2010.11.106
5. E.M. Satyavarapu, P.K. Sinha, C. Mandal, Influence of geographical and seasonal variations on carbazole alkaloids distribution in *Murraya koenigii*: Deciding factor of its in vitro and in vivo efficacies against cancer cells. BioMed Res. Int. **2020**, 7821913 (2020). https://doi.org/10.1155/2020/7821913

6. N.I. Ramli, F. Abas, I.S. Ismail, Y. Rukayadi, S.M. Nor, Determination of antioxidant activity, phenolic compounds, and toxicity of methanolic and ethanolic extracts of pink pigmented facultative methylotrophs (PPFM) bacteria pigment. Pertanika J. Trop. Agric. Sci. **46**, 1407 (2023). https://doi.org/10.47836/pjtas.46.4.21
7. M. Rivaldi, A. Frediansyah, S.A.A. Aziz, A.P. Nugroho, Active biomonitoring of stream ecosystems: Untargeted metabolomic and proteomic responses and free radical scavenging activities in mussels. Ecotoxicology. **34**, 425 (2025). https://doi.org/10.1007/s10646-024-02846-9
8. F.P. Nonglang, A. Khale, W. Wankhar, S. Bhan, Pharmacognostic evaluation of *Eranthemum indicum* extracts for its in-vitro antioxidant activity, acute toxicology, and investigation of potent bioactive phytocompounds using HPTLC and GCMS. Beni-Suef Univ. J. Basic Appl. Sci. **11**, 129 (2022). https://doi.org/10.1186/s43088-022-00311-2
9. A.M. Khalifa, M.A. Eid, R.M. Gaafar, K.M. Saad-Allah, D. Gad, Induction of bioactive constituents and antioxidant enzyme activities in *Achillea fragrantissima* (Forskal) callus cultures using ZnO nanoparticles. Vitro Cell. Dev. Biol. Plant. **59**, 808 (2023). https://doi.org/10.1007/s11627-023-10388-8
10. R. Addis, S. Cruciani, S. Santaniello, E. Bellu, G. Sarais, C. Ventura, M. Maioli, G. Pintore, Fibroblast proliferation and migration in wound healing by phytochemicals: Evidence for a novel synergic outcome. Int. J. Med. Sci. **17**, 1030 (2020). https://doi.org/10.7150/ijms.43986
11. G. Aquino, M.G. Basilicata, C. Crescenzi, V. Vestuto, E. Salviati, M. Cerrato, T. Ciaglia, F. Sansone, G. Pepe, P. Campiglia, Optimization of microwave-assisted extraction of antioxidant compounds from spring onion leaves using Box–Behnken design. Sci. Rep. **13**, 14923 (2023). https://doi.org/10.1038/s41598-023-42303-x
12. A. Thawabteh, S. Juma, M. Bader, D. Karaman, L. Scrano, S. Bufo, R. Karaman, The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins. **11**, 656 (2019). https://doi.org/10.3390/toxins11110656
13. M. Leopoldini, N. Russo, M. Toscano, The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chem. **125**, 288 (2011). https://doi.org/10.1016/j.foodchem.2010.08.012
14. I. El Mannoubi, Impact of different solvents on extraction yield, phenolic composition, in vitro antioxidant and antibacterial activities of deseeded *Opuntia stricta* fruit. J. Umm Al-Qura Univ. Appl. Sci. **9**, 176 (2023). https://doi.org/10.1007/s43994-023-00031-y
15. W.Y. Oh, S. Liu, J. Lee, Revisiting polar paradox: Antioxidant activity in bulk oil using selected phenol lipids. Food Sci. Biotechnol. **33**, 3491 (2024). https://doi.org/10.1007/s10068-024-01605-0