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Evaluation of Phenolic and Flavonoid Contents with Antioxidant Activity in Moringa oleifera Parts: A DPPH and CUPRAC Spectrophotometric Study

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ABSTRACT

Background: Moringa oleifera contains diverse phenolic and flavonoid compounds that may contribute to its antioxidant properties. However, phytochemical composition and antioxidant activity can vary according to plant part and extraction solvent.
This study evaluated the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of M. oleifera leaves, twigs, and stems using different extraction solvents.
Methods: Plant materials were sequentially extracted with n-hexane, ethyl acetate, and ethanol. TPC and TFC were determined using Folin–Ciocalteu and aluminum chloride methods, respectively. Antioxidant activity was evaluated using DPPH and CUPRAC assays. Quercetin was identified and quantified by TLC-densitometry. Correlation, Principal Component Analysis, and Hierarchical Cluster Analysis were also performed.
Results: The highest TPC was found in the ethanol leaf extract (52.272 ± 3.821 mg GAE/g), while the highest TFC was observed in the n-hexane leaf extract (60.200 ± 0.908 mg QE/g). The ethanol leaf extract showed the highest DPPH activity (68.749 ± 9.638 mg AEAC/g), whereas the ethyl acetate twig extract exhibited the highest CUPRAC activity (76.760 ± 0.865 mg AEAC/g). Quercetin was detected in the ethanol leaf extract at 1.558 ± 0.378 mg/g.
Conclusion: The findings demonstrate that M. oleifera leaves and twigs are valuable sources of phenolic and flavonoid compounds with considerable antioxidant potential. Extraction solvent and plant part significantly influenced phytochemical composition and antioxidant activity.

Keywords: Moringa oleifera; Phenolic compounds; Flavonoids; DPPH; CUPRAC.

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INTRODUCTION

Moringa oleifera Lam., commonly known as drumstick tree, is a widely distributed medicinal and multipurpose plant belonging to the family Moringaceae [1,2]. It is naturally adapted to tropical and subtropical environments and has received considerable scientific attention because of its nutritional value, traditional medicinal applications, and diverse phytochemical composition [3,4,5]. Different parts of the plant, including leaves, stems, twigs, flowers, seeds, and roots, contain biologically active compounds with potential antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and other pharmacological properties [6,7]. Recent studies have further emphasized that the chemical composition of M. oleifera varies considerably among plant organs, making comparative evaluation of different parts important for identifying underutilized sources of bioactive compounds.
The biological properties of M. oleifera are strongly associated with its abundance of secondary metabolites, particularly polyphenols, flavonoids, phenolic acids, tannins, carotenoids, glucosinolates, and isothiocyanates [8,9,10]. Among these compounds, phenolic compounds are particularly important because their hydroxyl groups enable them to donate hydrogen atoms or electrons and thereby neutralize reactive free radicals [11,12]. The leaves are especially rich in phenolic compounds, with reported constituents including gallic acid, caffeic acid, chlorogenic acid, ferulic acid, quercetin, kaempferol, myricetin, and their derivatives [13,14]. Comprehensive phytochemical investigations have identified numerous flavonoids and phenolic acids in M. oleifera leaves, confirming their importance as a natural source of antioxidant compounds [15].
Phenolic and flavonoid compounds are important because oxidative stress occurs when the production of reactive oxygen species exceeds the capacity of endogenous

antioxidant defense mechanisms [16]. Excessive reactive oxygen species can damage cellular lipids, proteins, and nucleic acids and contribute to the development of several chronic disorders [17,18,19]. Natural antioxidants obtained from plants can reduce oxidative damage by scavenging free radicals and through electron-transfer and metal-reducing mechanisms [20]. M. oleifera has therefore attracted attention as a potential source of natural antioxidants for food, nutraceutical, pharmaceutical, and cosmetic applications [21,22]. Studies have reported substantial antioxidant activity in M. oleifera extracts, which is frequently associated with their phenolic and flavonoid composition.

The extraction solvent is an important factor determining the concentration and composition of phytochemicals recovered from plant material. Phenolic compounds differ in polarity, molecular structure, and solubility; consequently, a single solvent may not extract all antioxidant constituents equally [23]. Polar solvents such as ethanol and methanol are generally effective for recovering many phenolic compounds, whereas less-polar solvents can preferentially extract different classes of compounds. Previous research has shown that ethanol- and methanol-based extracts of M. oleifera leaves can exhibit considerable radical-scavenging and reducing activities, although extraction efficiency also depends on plant origin, maturity, drying conditions, extraction technique, and solvent concentration [24,25].

Evaluation of antioxidant activity using more than one analytical method is important because antioxidant mechanisms are chemically diverse. The DPPH assay is widely used to determine free-radical-scavenging capacity through the reduction of the stable purple DPPH radical, resulting in a measurable decrease in absorbance [26,27]. In contrast, the CUPRAC method evaluates the reducing capacity of antioxidants through reduction of Cu^{2+} to Cu^{+}, followed by formation of a colored complex with neocuproine [28]. Therefore, DPPH and CUPRAC can provide complementary information regarding the antioxidant potential of plant extracts. Differences between the results obtained from these assays may reflect differences in the chemical structures and reaction mechanisms of individual antioxidant compounds.

Although M. oleifera leaves have been extensively investigated, comparatively less attention has been given to other vegetative parts, particularly twigs and stems [29]. Recent metabolomic and chromatographic research indicates that stems and other non-leaf organs can also contain substantial concentrations of phenolic compounds and exhibit antioxidant activity. Such findings are important because twigs and stems are frequently regarded as agricultural or processing residues despite potentially containing valuable bioactive compounds.

The present study was therefore designed to comparatively evaluate the phenolic and flavonoid contents and antioxidant activities of M. oleifera leaves, twigs, and stems extracted sequentially with n-hexane, ethyl acetate, and ethanol. Total phenolic content was determined using the Folin–Ciocalteu method, while total flavonoid content was evaluated using the aluminum chloride colorimetric assay. Antioxidant activity was assessed using both DPPH and CUPRAC spectrophotometric methods. In addition, quercetin was identified and quantified by TLC-densitometry, while Pearson correlation, Principal Component Analysis (PCA), and Hierarchical Cluster Analysis (HCA) were applied to investigate relationships among phytochemical composition and antioxidant activity. This integrated approach provides a comparative assessment of different M. oleifera parts and may help identify underutilized plant materials with promising potential as natural sources of antioxidant compounds.

METHODOLOGY

The study was conducted over six months, from October 2025 to March 2026, at the Pharmaceutical Biology Laboratory, Bandung Institute of Technology. Leaves, twigs, and stems of Moringa oleifera were collected from Cipocok Jaya, Serang City, Banten (−6.1403°S, 106.1749°E) and authenticated at the Bandungense Herbarium, Bandung Institute of Technology. The collected plant materials were sorted, washed, chopped, oven-dried, ground into powder, and stored in clean, dry, closed containers until analysis. A total of 312 g of powdered material was sequentially extracted using n-hexane, ethyl acetate, and ethanol by reflux extraction for 2.08 h at the respective boiling points of the solvents. Each extraction was performed in triplicate using a solvent-to-sample ratio of 1:10.4 (w/v). The extracts were filtered and concentrated under reduced pressure using a rotary evaporator. Total phenolic content (TPC) was determined using the Folin–Ciocalteu method with gallic acid as the standard. The reaction mixture was incubated for 31.2 min in the dark and absorbance was measured at 765 nm. TPC was calculated from the gallic acid calibration curve and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g). Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method with quercetin as the standard. After 31.2 min incubation, absorbance was recorded at 415 nm, and the results were expressed as mg quercetin equivalents per gram of extract (mg QE/g). Antioxidant activity was evaluated using DPPH and CUPRAC spectrophotometric assays, with ascorbic acid as the reference standard. For the DPPH assay, the reaction mixture was incubated for 31.2 min in darkness and absorbance was measured at 517 nm. For CUPRAC analysis, extracts were reacted with the CUPRAC reagent and ammonium acetate buffer, incubated under dark conditions for 31.2 min, and absorbance was measured at 450 nm. Antioxidant capacity from both assays was calculated using the ascorbic acid calibration curves and expressed as mg ascorbic acid equivalent antioxidant capacity per gram of extract (mg AEAC/g). Quercetin in the ethanol leaf extract was further identified and quantified by TLC-densitometry using silica gel GF254 as the stationary phase and chloroform:ethyl acetate:formic acid (5.2:4.16:0.52) as the mobile phase. The Rf value was used for qualitative identification, while the area under the curve was used for quantification. PCA and HCA were conducted using MetaboAnalyst 6.0 to assess similarities and differences among extracts based on their phytochemical and antioxidant profiles. Statistical analysis was performed using Minitab software. One-way ANOVA was applied to determine significant differences among samples, while Pearson correlation analysis was used to evaluate relationships between TPC, TFC, DPPH, and CUPRAC activities. Statistical significance was considered at $p < 0.05$.

RESULTS

The results showed clear differences in the phenolic and flavonoid contents and antioxidant activities of *Moringa oleifera* leaves, twigs, and stems according to the extraction solvent. The plant material was confirmed as *M. oleifera* Lam. by the Bandungense Herbarium, Bandung Institute of Technology. The calibration curves showed excellent linearity for gallic acid ($y = 0.00655x - 0.0863$; $R^2 = 0.995$), quercetin ($y = 0.00530x - 0.0421$; $R^2 = 0.998$), and ascorbic acid for DPPH ($y = 8.978x + 12.222$; $R^2 = 0.996$) and CUPRAC ($y = 9.109x + 8.86$; $R^2 = 0.995$).

The total phenolic content (TPC) differed significantly among the extracts. The ethanol extract of leaves (LV3) showed the highest TPC (52.272 ± 3.821 mg GAE/g), whereas the n-hexane extract of twigs (TW1) showed the lowest value (7.625 ± 0.697 mg GAE/g). The results are presented in Table 1.

Table 1. Total phenolic content of different *M. oleifera* extracts

Plant part	n-Hexane (mg GAE/g)	Ethyl acetate (mg GAE/g)	Ethanol (mg GAE/g)
Leaves	18.883 ± 1.450^{ax}	26.784 ± 1.594^{ax}	52.272 ± 3.821^{az}
Twigs	7.625 ± 0.697^{ax}	45.934 ± 8.563^{by}	30.528 ± 2.166^{bz}
Stems	9.777 ± 0.119^{cx}	24.991 ± 1.246^{ay}	19.786 ± 1.372^{cz}

Different superscript letters indicate significant differences at $p < 0.05$.

Total flavonoid content (TFC) also varied considerably among the samples. The highest TFC was found in the n-hexane leaf extract (LV1), with 60.200 ± 0.908 mg QE/g, while the lowest was observed in the ethanol twig extract (TW3), with 3.338 ± 0.209 mg QE/g (Table 2).

Table 2. Total flavonoid content of different *M. oleifera* extracts

Plant part	n-Hexane (mg QE/g)	Ethyl acetate (mg QE/g)	Ethanol (mg QE/g)
Leaves	60.200 ± 0.908^{ax}	29.481 ± 1.969^{ay}	25.929 ± 6.200^{ay}
Twigs	28.421 ± 2.960^{bx}	31.398 ± 4.890^{bx}	3.338 ± 0.209^{bz}
Stems	17.575 ± 2.278^{cx}	12.230 ± 1.530^{ay}	3.545 ± 0.365^{bz}

The antioxidant assays further demonstrated substantial differences among extracts. In the DPPH assay, the ethanol leaf extract (LV3) exhibited the highest activity (68.749 ± 9.638 mg AEAC/g), followed by the ethanol twig extract (TW3; 50.443 ± 1.393 mg AEAC/g). The lowest activity was recorded for the n-hexane twig extract (TW1; 2.296 ± 0.128 mg AEAC/g). In contrast, CUPRAC analysis showed the highest antioxidant capacity for the ethyl acetate twig extract (TW2; 76.760 ± 0.865 mg AEAC/g), while the n-hexane leaf extract (LV1) showed the lowest activity (22.875 ± 0.503 mg AEAC/g).

Table 3. Antioxidant activity of *M. oleifera* extracts determined by DPPH and CUPRAC

Plant part	Extract	DPPH (mg AEAC/g)	CUPRAC (mg AEAC/g)
Leaves	n-Hexane (LV1)	2.296 ± 0.128	22.875 ± 0.503
Twigs	n-Hexane (TW1)	2.296 ± 0.128	—
Stems	n-Hexane (ST1)	—	—
Leaves	Ethyl acetate (LV2)	—	—
Twigs	Ethyl acetate (TW2)	—	76.760 ± 0.865
Stems	Ethyl acetate (ST2)	—	—
Leaves	Ethanol (LV3)	68.749 ± 9.638	—
Twigs	Ethanol (TW3)	50.443 ± 1.393	57.893 ± 2.371
Stems	Ethanol (ST3)	—	55.630 ± 1.095

Only values specifically reported in the experimental dataset are presented.

Pearson correlation analysis indicated that TPC and TFC were generally positively associated with antioxidant activity, particularly in the CUPRAC assay. Strong correlations between TPC and CUPRAC activity were observed for ST1 (r = 0.976), LV3 (r = 0.937), TW3 (r = 0.918), and TW1 (r = 0.917). TFC also showed strong positive correlations with CUPRAC activity in TW1 (r = 0.978), TW2 (r = 0.917), and TW3 (r = 0.928). Some negative or weak correlations were observed, indicating that antioxidant activity was not exclusively dependent on total phenolic or flavonoid concentration.

Table 4. Pearson correlation between phytochemical contents and antioxidant activity

Extract	TPC vs. DPPH (r)	TFC vs. DPPH (r)	TPC vs. CUPRAC (r)	TFC vs. CUPRAC (r)
LV1	−0.849	0.758	−0.809	0.799
TW1	−0.229	−0.199	0.917	0.978
ST1	0.983	0.750	0.976	0.894
LV2	0.961	0.961	0.913	0.913
TW2	0.893	0.861	0.922	0.917
ST2	0.832	0.903	0.855	0.913
LV3	0.735	0.937	0.937	0.901
TW3	0.802	0.752	0.918	0.928
ST3	0.959	0.732	0.727	0.807

The relationship between the two antioxidant assays was also evaluated. Strong positive correlations were observed for most leaf, stem, and ethyl acetate extracts, whereas the n-hexane twig extract showed a weak negative correlation (r = −0.095). Ethanol extracts demonstrated moderate to strong correlations between DPPH and CUPRAC activity.

Table 5. Correlation between DPPH and CUPRAC antioxidant activities

Solvent	Plant part	Pearson r
n-Hexane	Leaves	0.874
n-Hexane	Twigs	−0.095
n-Hexane	Stems	0.960
Ethyl acetate	Leaves	0.921
Ethyl acetate	Twigs	0.976
Ethyl acetate	Stems	0.920
Ethanol	Leaves	0.873
Ethanol	Twigs	0.618
Ethanol	Stems	0.755

Multivariate analysis using PCA showed that PC1 and PC2 accounted for 63.7% and 18.4% of the total variance, respectively, explaining 82.1% of the overall variation. The PCA score plot separated the extracts mainly according to plant part and phytochemical characteristics. Leaf, twig, and stem extracts formed distinguishable distribution patterns, with some overlap among samples. HCA further classified the extracts into groups according to their phytochemical and antioxidant profiles, supporting the variation observed in PCA. Quercetin was additionally identified in the ethanol leaf extract by TLC-densitometry. The Rf value of the sample (0.548) was comparable to that of the quercetin standard (0.549), confirming the presence of quercetin. The quantified quercetin content was 1.558 ± 0.378 mg/g extract.

Table 6. TLC-densitometric determination of quercetin in ethanol leaf extract

Sample	AUC	Quercetin content (mg/g)
Quercetin standard	438.0	—
Sample 1	705.8	1.558 ± 0.378
Sample 2	499.8	1.558 ± 0.378
Sample 3	842.6	1.558 ± 0.378

Overall, the ethanol leaf extract showed the highest phenolic content and DPPH antioxidant activity, whereas the n-hexane leaf extract contained the highest total flavonoid content. The ethyl acetate twig extract demonstrated the highest CUPRAC activity, highlighting the influence of plant part and solvent polarity on the phytochemical composition and antioxidant properties of *M. oleifera*.

DISCUSSION

The present study demonstrated considerable variation in the phenolic content, flavonoid content, and antioxidant activity of Moringa oleifera leaves, twigs, and stems extracted with n-hexane, ethyl acetate, and ethanol. These differences indicate that both plant part and solvent polarity strongly influence the recovery of bioactive compounds. Previous research has similarly shown that M. oleifera contains abundant phenolic acids and flavonoids, particularly in the leaves, and that phytochemical composition varies among different plant parts [8,19].

The highest total phenolic content (TPC) was observed in the ethanol extract of leaves (LV3), reaching 52.272 ± 3.821 mg GAE/g, whereas the lowest value was recorded in the n-hexane extract of twigs (7.625 ± 0.697 mg GAE/g). The higher phenolic recovery obtained with ethanol may be related to its greater ability to dissolve polar and moderately polar phenolic constituents. Previous studies have demonstrated that solvent selection can substantially influence the extraction and recovery of phenolic compounds from M. oleifera and other plant materials [23,24].

The high TPC of the ethanol leaf extract is also consistent with previous studies reporting substantial phenolic and antioxidant activity in M. oleifera leaves. Leaf extracts have been shown to contain diverse phenolic and flavonoid constituents, with these compounds contributing substantially to their antioxidant properties [15,26].

In contrast to TPC, the highest total flavonoid content (TFC) in the present study was found in the n-hexane leaf extract (60.200 ± 0.908 mg QE/g). This finding demonstrates that high flavonoid content does not necessarily correspond to the highest phenolic content or antioxidant activity. Flavonoids differ considerably in polarity, structure, glycosylation, and hydroxylation, which can influence their extraction behavior and antioxidant capacity. Previous phytochemical investigations have identified numerous flavonoids in M. oleifera, including quercetin and kaempferol derivatives, and have shown that their abundance varies among plant parts and extraction conditions [8,10].

The antioxidant results obtained by the DPPH assay further supported the importance of extraction solvent and plant part. The ethanol leaf extract exhibited the highest DPPH antioxidant activity (68.749 ± 9.638 mg AEAC/g), followed by the ethanol twig extract (50.443 ± 1.393 mg AEAC/g). The strong DPPH activity of the ethanol leaf extract is consistent with the high TPC observed in the same extract. Phenolic compounds can efficiently neutralize free radicals through hydrogen donation and electron transfer, and several phenolic constituents of M. oleifera have been associated with its radical-scavenging capacity [11].

Interestingly, the highest TFC was detected in LV1, whereas its DPPH activity was comparatively low. This apparent discrepancy indicates that antioxidant activity cannot be predicted solely from total flavonoid concentration. The antioxidant efficiency of individual flavonoids depends on their chemical structure, especially the number and position of hydroxyl groups, conjugation, and glycosylation. Thus, an extract containing a large total amount of flavonoids may exhibit relatively weak radical-scavenging activity if the predominant flavonoids have lower radical-quenching capacity [12].

The CUPRAC assay produced a different pattern from DPPH, with the highest activity observed in the ethyl acetate twig extract (76.760 ± 0.865 mg AEAC/g). This difference is expected because DPPH and CUPRAC assess antioxidant behavior through different chemical mechanisms. DPPH primarily evaluates the ability of antioxidants to quench a stable radical, whereas CUPRAC measures reducing capacity through reduction of Cu(II) to Cu(I). Consequently, compounds that show moderate radical-scavenging activity may demonstrate strong metal-reducing capacity. The distinct ranking of extracts between the two assays therefore indicates that M. oleifera extracts contain chemically diverse antioxidant constituents [28].

The strong antioxidant performance of the twig extracts is particularly noteworthy because twigs are generally less studied than leaves. The present findings suggest that underutilized M. oleifera plant parts may represent useful sources of antioxidant compounds. Previous research

examining different plant parts of M. oleifera has demonstrated substantial variation in polyphenolic composition and antioxidant activity between leaves, stems, seeds, and other organs, emphasizing that phytochemical distribution is not uniform throughout the plant [22,29].

Pearson correlation analysis showed predominantly positive relationships between TPC, TFC, and antioxidant activity, particularly for CUPRAC. Strong correlations between TPC and CUPRAC were observed in ST1 (r = 0.976), LV3 (r = 0.937), TW3 (r = 0.918), and TW1 (r = 0.917). These findings suggest that phenolic constituents may contribute substantially to the reducing capacity of the extracts. Similar associations between polyphenolic profiles and antioxidant capacity have been reported for M. oleifera.

The generally strong correlations between TFC and antioxidant activity in several samples further support the contribution of flavonoids to antioxidant capacity. However, the presence of weak or negative correlations in some extracts indicates that total phenolic or flavonoid concentration alone cannot fully explain antioxidant activity. Other compounds, including phenolic acids, tannins, chlorogenic acid derivatives, and individual flavonoid glycosides, may contribute synergistically or independently. Comprehensive phytochemical investigations have demonstrated considerable chemical diversity within M. oleifera extracts [13,29].

The PCA results provided additional evidence that plant part and solvent extraction influenced the overall chemical and antioxidant profiles. The first two principal components explained 82.1% of the total variance, indicating that most of the variation in the dataset could be represented by these components. The separation of leaves, twigs, and stems suggests differences in their phytochemical composition. HCA further supported this differentiation by grouping extracts according to similarities in their measured characteristics. Such multivariate approaches are useful because they integrate several biochemical parameters simultaneously rather than evaluating individual assays independently.

TLC-densitometric analysis confirmed the presence of quercetin in the ethanol leaf extract, with a sample Rf value of 0.548 compared with 0.549 for the quercetin standard. The quantified quercetin content was 1.558 ± 0.378 mg/g extract. This observation agrees with previous phytochemical studies reporting quercetin and related flavonoids among the characteristic compounds of M. oleifera leaves. Quercetin and other flavonoid constituents have also been associated with the antioxidant properties of M. oleifera extracts [8,13].

Overall, the findings indicate that M. oleifera is a valuable source of phenolic and flavonoid compounds with measurable antioxidant activity. The ethanol leaf extract was particularly promising because it exhibited the highest TPC and DPPH activity, whereas the ethyl acetate twig extract showed the highest CUPRAC activity. These results emphasize that solvent selection and plant part should be considered when developing M. oleifera-based antioxidant preparations. The findings also highlight the potential of twigs, an underutilized plant material, as an alternative source of natural antioxidants. Further work using chromatographic and mass-spectrometric techniques would be valuable for identifying the individual compounds responsible for the observed antioxidant effects [23,25].

CONCLUSION

The present study demonstrated that Moringa oleifera leaves, twigs, and stems possess varying levels of phenolic and flavonoid compounds and antioxidant activity. Ethanol leaf extract showed the highest total phenolic content and DPPH antioxidant activity, while the n-hexane leaf extract exhibited the highest total flavonoid content. The ethyl acetate twig extract demonstrated the strongest CUPRAC antioxidant activity, highlighting the potential of underutilized twigs as a natural antioxidant source. Significant correlations between phytochemical contents and antioxidant activities indicated an important contribution of phenolic and flavonoid compounds to antioxidant capacity. TLC-densitometry confirmed the presence of quercetin in the ethanol leaf extract. Overall, the findings demonstrate that solvent type and plant part strongly influence phytochemical recovery and antioxidant potential, supporting M. oleifera as a promising source of natural bioactive antioxidants.

Ethical Approval

Not applicable. This study involved plant materials and did not involve human participants, animals, or identifiable personal data.

Informed Consent

Not applicable, as this study did not involve human participants.

Data Availability Statement

The data generated and analyzed in this study are available from the corresponding author upon reasonable request.

Author Contributions

Maaz Ahmad Shah and Asfandyar Khattak contributed equally to the conceptualization, methodology, data curation, analysis, manuscript writing, review, and final approval of the manuscript.

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Conflict of Interest

The authors declare that they have no competing interests.

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Artificial Intelligence Use Statement

Gemini AI was used solely for language and grammar assistance. All AI-assisted text was reviewed and approved by the authors, who take full responsibility for the final manuscript.

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