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Research Article
Unveiling the Antioxidant Potential of Underutilized *Moringa oleifera* Parts: A DPPH and CUPRAC-Based Study

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Abstract

Background and Objective: *Moringa oleifera*, a plant rich in flavonoids and phenolic compounds, is widely recognized for its antioxidant potential. This study aimed to evaluate the antioxidant activity of underutilized parts of *M. oleifera* (twigs and stems) compared to leaves (well-utilized part), expressed as ascorbic acid equivalents. Additionally, the relationships between total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity were investigated. **Materials and Methods:** TPC and TFC were determined using UV–Visible spectrophotometry. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assays. Correlations among TPC, TFC and antioxidant activity were analyzed using Pearson's correlation, principal component analysis (PCA) and hierarchical cluster analysis (HCA). Flavonoid compounds were further characterized using TLC densitometry. Statistical significance among the data was analyzed using One-Way ANOVA in Minitab software at a significance level of $p<0.05$. **Results:** The ethanolic extract of *M. oleifera* leaves exhibited the highest antioxidant activity in both DPPH assays, while the ethyl acetate of *M. oleifera* twigs extract showed the highest activity using the CUPRAC assay. It is possible to develop twigs *M. oleifera* as a potential antioxidant agent resource. **Conclusion:** Among the tested plant parts, leaves demonstrated the highest antioxidant activity compared to twigs and stems. However, twigs also showed promising potential activity to be developed as source of antioxidant agent.

Key words: Antioxidant, underutilized parts, *M. oleifera*, DPPH, CUPRAC

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Free radicals are highly unstable molecules contain unpaired electron. It can induce oxidative stress represented by reactive oxygen species (ROS). Free radicals can cause chain reactions leading to cellular damage through the oxidation of biomolecules. Antioxidants are compounds that can prevent by neutralizing or capturing the free radicals. Antioxidants mechanism by giving electron to free radicals so the chain reaction will break[1].

Moringa oleifera commonly known as kelor has antioxidant activity. In addition, *M. oleifera* has other pharmacological effect such as anti-microbial, anti-fungi, anti-cancer and anti-inflammatory activity due to secondary metabolites contain[2]. Flavonoid are the most abundant phenolic compounds in *M. oleifera*. The presence of hydroxyl groups provides antioxidant activity[3].

Moringa oleifera can grows well in Indonesia with temperature and environment conditions that support optimal growth, so it is not difficult to obtain. People generally use *M. oleifera* traditionally as foods, wound healing, constipation, fever and source of vitamin C[4]. The study research proposes to investigate the potential of underutilized parts (twigs and stem) of *M. oleifera* as a source of natural antioxidant.

The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the Cupric Ion Reducing Antioxidant Capacity (CUPRAC) method. This study also aims to examine the total phenol and flavonoid content, the correlation of antioxidant from both test methods and to identify and determine the flavonoid content in selected extract.

MATERIALS AND METHODS

Study area: The study was conducted over a period of approximately six months, from October 2025 to March 2026, at the Pharmaceutical Biology Laboratory, Bandung Institute of Technology.

Chemicals: Gallic acid, quercetin, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and neocuproine from Sigma-Aldrich (MO, USA). Analytical grade compounds were also used.

Plant collection and determination: *Moringa oleifera* parts are leaves (LV), twigs (TW) and stem (ST) was collected from Cipocok Jaya in Serang City, Banten (GPS coordinates: -6.1403°S, 106.1749°E). The plant was determined at the Bandungense Herbarium, Bandung Institute Technology, then sorted, washed with water, chopped and dried using an oven. After that, dried sample was ground into powder with grinder and stored in a dry and closed container.

Extraction: The extraction was carried out using the reflux method. A 300 g sample of *M. oleifera* parts was sequentially extracted with n-hexane, ethyl acetate and ethanol at the respective boiling points of the solvents for 2 hrs. Each extraction step was performed in triplicate using a solvent-to-sample ratio of 1:10 (w/v) to ensure reproducibility and maximize yield. The resulting liquid extracts were filtered and concentrated under reduced pressure using a rotary evaporator to obtain viscous crude extracts.

Total phenolic content (TPC): Gallic acid was used a reference standard in determining total phenol content. First, was prepared a stock solution of gallic acid 1000 µg/mL. The stock was diluted to 60-130 µg/mL. For extract was following the same protocol with standard. In to Eppendorf tube, was added 50 µL gallic acid or sample, 400 µL of 1 M sodium carbonate and 500 µL of 10% Folin-Ciocalteu reagent. A blank solution was prepared by 50 µL methanol, 400 µL of 1 M sodium carbonate and 500 µL of 10% Folin-Ciocalteu reagent. The mixture was incubated for 30 minutes protected from light, the absorbance was measured using UV-vis spectrophotometry at λ 765 nm. A minimum of six concentration used to plot a gallic acid calibration curve. The total phenolic content was quantified using linear regression equation of gallic acid and showed as a milligram of gallic acid equivalent (GAE) per gram extract (mg GAE/g)[4].

Total flavonoid content (TFC): The total flavonoid content was used quercetin as a reference standard. Quercetin was prepared a stock solution 500 µg/mL dissolved with methanol, then diluted to 40 to 100 µg/mL. For extract was following the same protocol with a standard. A 100 µL of quercetin or extract was added by 400 µL methanol, 560 µL distillated water, 20 µL of 10% aluminum chloride and 20 µL of 1 M sodium acetate in Eppendorf tube. The mixture was incubated for 30 minutes, the absorbance was measured using UV-vis spectrophotometry at λ 415 nm. The absorbance values of quercetin were used to obtain a quercetin calibration curve. Each extract was measured six times to analyzed flavonoid content. Total flavonoid content was determined using linear regression equation of quercetin and displayed as a milligrams of quercetin equivalent per gram extract (mg QE/g)[5].

Antioxidant activity using DPPH method: The method was used ascorbic acid as a standard to determine antioxidant activity. The DPPH 50 µg/mL was prepared. The standard solution was prepared in variation concentration. Each concentration was added with methanol until the volume was 125 µL, then added by 750 µL of DPPH solution. In dark condition and protected from light, the mixture was incubated for 30 minutes. To measure the absorbance was used UV-vis spectrophotometry at λ 517 nm. The calibration curve of the ascorbic acid inhibition (%) was made from a percentage of inhibition each concentration. The extract was made by dissolving in methanol, filtered and applied with the same process for a standard. The antioxidant activity was showed as milligrams of ascorbic acid equivalent antioxidant capacity (mg AEAC) per gram extract[6].

Antioxidant activity using CUPRAC method: The CUPRAC method was used ascorbic acid as a standard to calculate antioxidant activity. The CUPRAC stock solution was prepared of neocuproine and cupric chloride. The CUPRAC solution 100 µg/mL was made from the stock solution. Each concentration was added with 750 µL of the CUPRAC solution, then added by ammonium acetate buffer 250 µL. The solution was incubated for 30 minutes in dark conditions, then the absorbance was measured with UV-vis spectrophotometry at λ 450 nm. The extract was performed by dissolving in methanol, filtered and applied the same protocol for a standard. The antioxidant activity was quantified with the regression equation of standard solution and expressed in mg AEAC/g extract[7].

Determination of quercetin content by TLC-densitometry: The flavonoid compound in *M. oleifera* extract was identified and quantified using thin layer chromatography. Quercetin was used as a marker compound. The mobile phase was consisted of chloroform - ethyl acetate - formic acid (5:4:0.5) and stationary phase was silica gel GF_{254} plate. Retardation factor (Rf) was used as a qualitative analysis to evaluate the separations. The AUC value was used to quantified quercetin content in extract with one point method.

Statistical analysis: Multivariate statistical analysis was performed by using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) with the Metaboanalyst 6.0 web platform. The data sets consisted of total phenolic content, total flavonoid content, antioxidant activity DPPH and CUPRAC method. A PCA was used to identify variations and relationship of the extract. Meanwhile, HCA was used to classify the samples into clusters which were visualized using a dendrogram.

Minitab software was conducted the significance between the data. It was performed using One-way ANOVA method ($p<0.05$) to see statistically significant differences each data. To determine the correlation between TPC, TFC and antioxidant activity testing was used a Pearson correlation method.

RESULTS AND DISCUSSION

Plant determination: The sample was identified at the Bandungense Herbarium, Bandung Institute of Technology, based on its morphological characteristics. The results confirmed that the plant was *Moringa oleifera* Lam., a member of the Moringaceae family.

Total phenolic content (TPC): The TPC was quantified using the equation of the calibration curve gallic acid ($y = 0.0063x-0.0863$) with $R^2 = 0.995$. The values of TPC assay were presented in Table 1. In this experiment, the highest TPC value was from LV3 (50.262±3.821 mg GAE/g) and the lowest TPC value was from TW1 (7.332±0.697 mg GAE/g). Research by Sachan *et al.*[8], the TPC value of aqueous extract was 47.50±0.82 mg GAE/g, the TPC value u sing ethanol 70% was 97.98±0.54 mg GAE/g and the TPC result using acetonitrile solvent was 50.27±0.26 mg GAE/g The other study reported that the TPC value of stem from methanol extract was 29.18±0.26 mg GAE/g and n-hexane extract was 25.75±1.45 mg GAE/g[9]. It showed the total phenolic content in *M. oleifera* extracts vary depending on the solvent used for extraction.

The TFC was calculated using a quercetin calibration curve ($y = 0.0051x-0.0421$) with $R^2 = 0.998$. The highest TFC was from LV1 (57.885±0.908 mg QE/g) and the lowest TFC was from TW3 (3.21±0.209 mg QE/g). The result of TFC value from n-hexane, ethyl acetate and ethanol extracts of *M. oleifera* leaves, twigs and stem can be seen in Table 2. According to Uno *et al.*[10], total flavonoid content in *M. oleifera* leaves using maceration methods exhibited 19.97 mg QE/g. The total flavonoid content (TFC) of *M. oleifera* leaves varied depending on the extraction method, even when the same solvent (70% ethanol) was used. The Soxhlet extract showed a TFC of 24.5 mg Isoquercetin Equivalent (IQE)/g, followed by the UAE extract (24.23 mg QE/g), while the maceration extract exhibited a lower value (18.62 mg GAE/mL)[11,12].

The results in this study are in line with previous studies reported in the literature. The differences results in TFC and TFC between experiments and literature was affected by several conditions such as, solvent used for extractions,

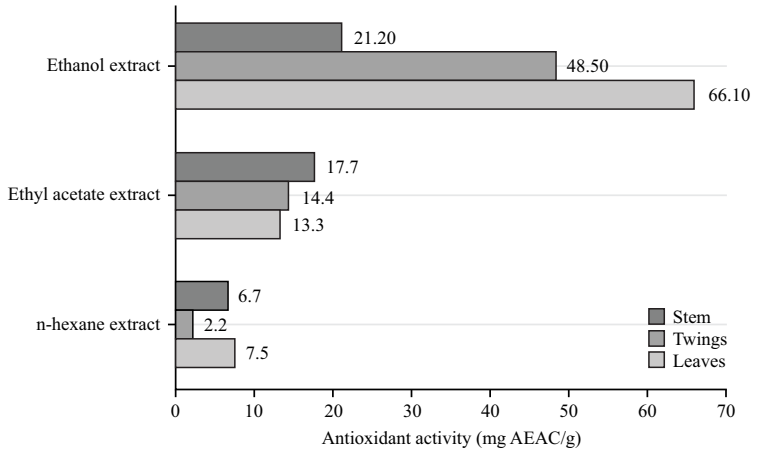

Fig. 1: Antioxidant activities of *M. oleifera* extracts using DPPH method

Table 1: Total phenolic content in different parts of *M. oleifera* extract

	Total phenolic content (mg GAE/g)		
Sample	n-Hexane extract	Ethyl acetate extract	Ethanol extract
Leaves	18.157±1.45^{ax}	25.754±1.594^{ax}	50.262±3.821^{az}
Twigs	7.332±0.697^{ax}	44.167±8.563^{by}	29.354±2.166^{bz}
Stem	9.401±0.119^{cx}	24.03±1.246^{ay}	19.025±1.372^{cz}

$^{a-c}$Distinct letter in the same column indicates significant difference ($p<0.05$), $^{x-z}$Distinct letter in the same row indicates significant difference ($p<0.05$)

Table 2: Total flavonoid content in different parts of *M. oleifera* extract

	Flavonoid total content (mg QE/g)		
Sample	n-Hexane extract	Ethyl acetate extract	Ethanol extract
Leaves	57.885±0.908^{ax}	28.347±1.969^{ay}	24.932±6.2^{ay}
Twigs	27.328±2.96^{bx}	30.19±4.89^{bx}	3.21±0.209^{bz}
Stem	16.899±2.278^{cx}	11.76±1.53^{ay}	3.409±0.365^{bz}

$^{a-c}$Distinct letter in the same column indicates significant difference ($p<0.05$) and $^{x-z}$Distinct letter in the same row indicates significant difference ($p<0.05$)

extraction methods and solvent ratio for extraction[13]. Furthermore, other factors that affect differences in the result also include plant parts, growing conditions, harvest time, plant age, growing location[14].

The determination of TPC assay was carried out using the Folin-Ciocalteu reagent, which reacts with the hydroxyl group in phenolic compounds to form a blue-molybdenum-tungsten complex. The flavonoid content can be measured using aluminum chloride and quercetin as a standard. The reaction involved was the formation of a complex between aluminium chloride and the hydroxyl group (C3' and C4') or ketone group with hydroxyl group. A positive flavonoid reaction was indicated by resulting yellow color can be determined using spectrophotometry at λ 415 nm. Sodium carbonate was added to maintain alkaline conditions, as the aluminium chloride complex with hydroxyl groups (C3 and C4) is unstable and degrades under acidic conditions[15,16].

The phenolic and flavonoid compounds contain hydroxyl group acting as hydrogen atom donors. This contributes to their antioxidant activity by neutralizing free radicals by donating hydrogen atoms to becoming stable conditions. Flavonoids is the most common phenolic compounds in *M. oleifera*. The existence of phenolic hydroxyl groups provides antioxidant activity[17].

Antioxidant activity (DPPH and CUPRAC method): Ascorbic acid calibration curve was used to calculate antioxidant activity using DPPH method ($y = 8.633x+12.222$), with $R^2 = 0.996$. The result of antioxidant activity value with DPPH method was presented in Fig.1. The LV3 had the highest value for DPPH method (66.105±9.638 mg AEAC/g), followed by TW3 (48.503±1.393 mg AEAC/g) and meanwhile the lowest value was found in TW1 (2.208±0.128 mg AEAC/g). It can be predicted that twigs (underutilized part) of *M. oleifera* have

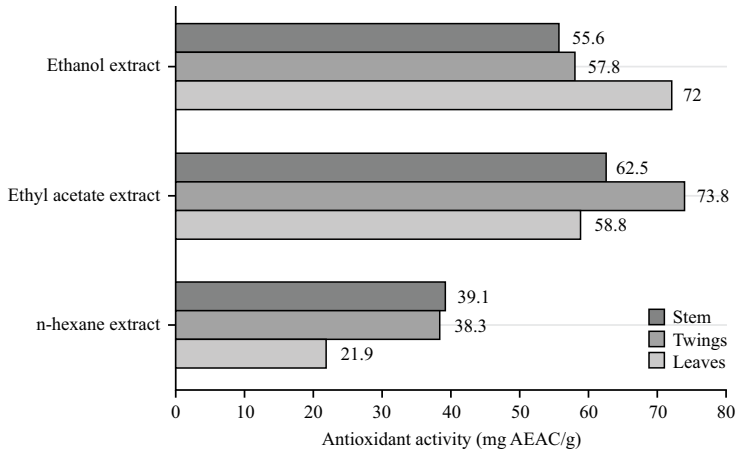

Fig. 2: Antioxidant activities of *M. oleifera* extracts using CUPRAC method

the potential to be developed as an antioxidant source. A study by Pradana *et al.*[18] using the DPPH method reported antioxidant activity expressed as an IC_{50} value, with *M. oleifera* leaves extract showing an IC_{50} of 217.21 mg/g. The IC_{50} value of *M. oleifera* methanol extract varies depending on the parts. The IC_{50} value of the bark was 40 µg/mL, while those of the stem and leaves were 720 µg/mL and 320 µg/mL, respectively[19]. Another research[20], the SC_{50} values for different extracts of *M. oleifera* were as follows: Stem 62.31 µg/mL and leaves 27.02 µg/mL. The experimental results in this study are in line with the literature, indicating that *M. oleifera* exhibits antioxidant activity.

The result for CUPRAC method was showed in The CUPRAC method was quantified using ascorbic acid as a standard. The linear regression equation of ascorbic acid for CUPRAC method was ($y = 8.759x+8.86$), with $R^2 = 0.995$. The result of CUPRAC methods in *M. oleifera* was presented in Fig. 2. Based on CUPRAC method assay, the highest antioxidant activity was found in TW2 (73.808±0.865 mg AEAC/g) and the lowest value was found in LV1 (21.995±0.503 mg AEAC/g). It can be supposed that twigs part of *M. oleifera* have potential as natural antioxidant source. Previous studies have shown that the antioxidant activity of *M. oleifera* leaves varies depending on the solvent used, with IC_{50} values of 80.49±7.36 µg/mL for methanol, 164.37±8.28 µg/mL for 50% ethanol and 170.22±7.36 µg/mL for 70% ethanol[21]. Using the CUPRAC method, it can be concluded that extract with semipolar or polar solvents such as ethyl acetate and ethanol have potential antioxidant activity in *M. oleifera* extract.

The different result between DPPH and CUPRAC methods based on each mechanism. The DPPH methods is a combination of the Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) methods. Mechanism of HAT works by donating hydrogen atom to free radicals, while SET mechanism by donates one electron through a protonation process. A DPPH radical reagent has a purple color and will change to yellow or colorless due to mechanism in addition of hydrogen atom or electron which means has antioxidant activity[22]. Many studies express antioxidant activity in terms of IC_{50}. The IC_{50} value represents the concentration required to scavenge 50% of DPPH radicals. A lower IC_{50} value indicates a stronger ability to neutralize DPPH radicals, reflecting higher antioxidant activity. In this study, antioxidant activity was expressed as ascorbic acid equivalent antioxidant capacity (AEAC), which reflects free radical scavenging activity by comparison with the standard, ascorbic acid[23].

The determination using CUPRAC classified in to Single Electron Transfer (SET) mechanisme by donating one electron. CUPRAC method is based on the reduction of Cupric (Cu^{2+}) to cuprous (Cu^{+}) which will form a colored complex with neocuproine. The complex will perform a yellow to orange color and can measured using a spectrophotometer[24].

The TPC values of TW3 was 29.354±2.166 mg QE/g and using CUPRAC methods value was 57.893±2.371 mg AEAC/g. Meanwhile, the TPC values of ST3 was 19.025±1.372 mg QE/g and antioxidant values using CUPRAC methods was 55.630±1.095 mg AEAC/g. The results of the study showed that TPC values do not always align with results from the CUPRAC assay. The CUPRAC method is based on the reaction between the CUPRAC chromogenic reagent [Cu(II/I)-neocuproine] and antioxidant. Phenolic compounds act as reducing agents. This method measures the ability of a compound to reduce metal ions based on their standard reduction potential. A compound exhibits antioxidant activity if it is capable of reducing the Cu^{2+}-neocuproine/Cu^{+}-neocuproine with an E^0 value 0.6 V[25]. Therefore, a high value in the CUPRAC assay is determined when a compound has a reduction potential below 0.6 V.

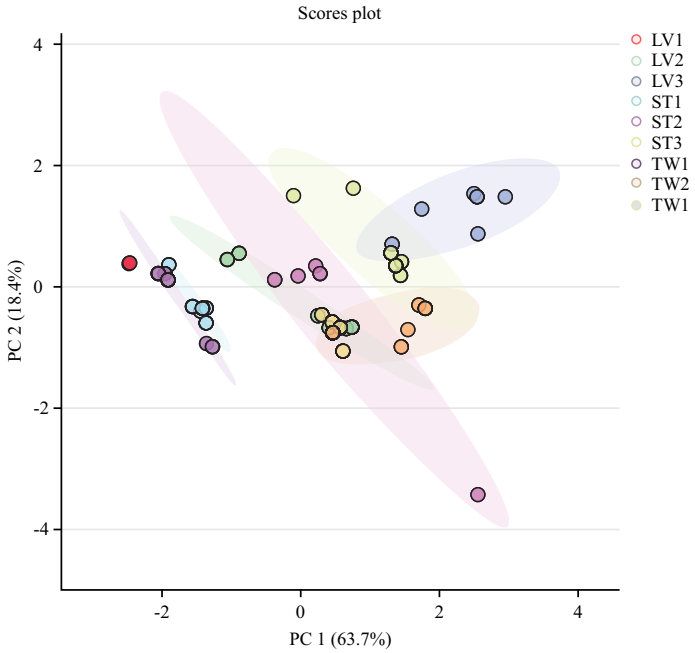

Fig. 3: PCA scores plot of *M. oleifera* extract

Based on the experiment, the TFC values of TW1 has the greatest value. However, the extract showed different result in the antioxidant activity using the DPPH methods. TFC value of TW1 was 57.885±0.908 mg QE/g, meanwhile antioxidant capacity values using DPPH was 2.209±0.128 mg AEAC/g. It can conclude the high TFC values in sample does not always has strong antioxidant activity. Referred to Masek *et al.*[26], flavonoids that possess hydroxyl groups at C3 and C4 and a double bond between C2 and C3 generally exhibit strong antioxidant activity. The DPPH assay mechanism involves hydrogen atom donation, which depends on the presence of hydroxyl groups. Therefore, samples with high total flavonoid content (TFC) but low DPPH antioxidant activity may contain flavonoids lacking hydroxyl groups at the C3-C4 positions.

Multivariate statistical analysis: The result of PCA can be seen in Fig. 3. The total of principal component was 82.1% (PC1 was 63.7% and PC2 was 18.4%) indicating these components represent the variability of the data. PC1 represent variations, particularly the contribution oh phenolic and flavonoids compounds along with antioxidant capacity. Meanwhile, PC2 represent additional among samples unexplained by PC1 such as extraction solvents and plant parts.

The PCA score plot showed that the samples are clustered into three main groups: LV, ST and TW. The LV group spans both negative and positive regions of PC1, with closely clustered samples indicating similar antioxidant properties. The ST group occupies the central region, suggesting intermediate characteristics. In contrast, the TW group is located on the positive side of PC1, indicating distinct chemical and antioxidant profiles compared to the LV group. However, several samples are closely positioned and exhibit overlap, indicating that the differences are not statistically significant and that they share similar chemical compositions and antioxidant profiles. Therefore, LV3 can be considered the extract with the most prominent antioxidant characteristics among the samples tested.

Hierarchical cluster analysis (HCA) was conducted to validate and support the grouping patterns observed in the PCA analysis. The HCA results, presented in Fig. 4, showed that greater distances reflect larger differences among samples. The dendrogram revealed two main clusters based on the solvent used. Cluster 1 is further divided into two sub-clusters, with LV1, ST1 and TW1 grouped closely, indicating similar antioxidant profiles, while LV2, ST3 and TW2 form a separate sub-cluster. Furthermore, LV3 and TW3 are grouped together, suggesting similarity between these samples but distinct differences from the others.

Differences between HCA and PCA results may be attributed to the extraction solvent and the distribution of bioactive compounds in *M. oleifera*. Solvent polarity affects the types and amounts of compounds extracted, thereby influencing antioxidant profiles. Hernandez *et al.*[27] demonstrated that solvent polarity significantly impacts phytochemical properties based on the principle of 'like

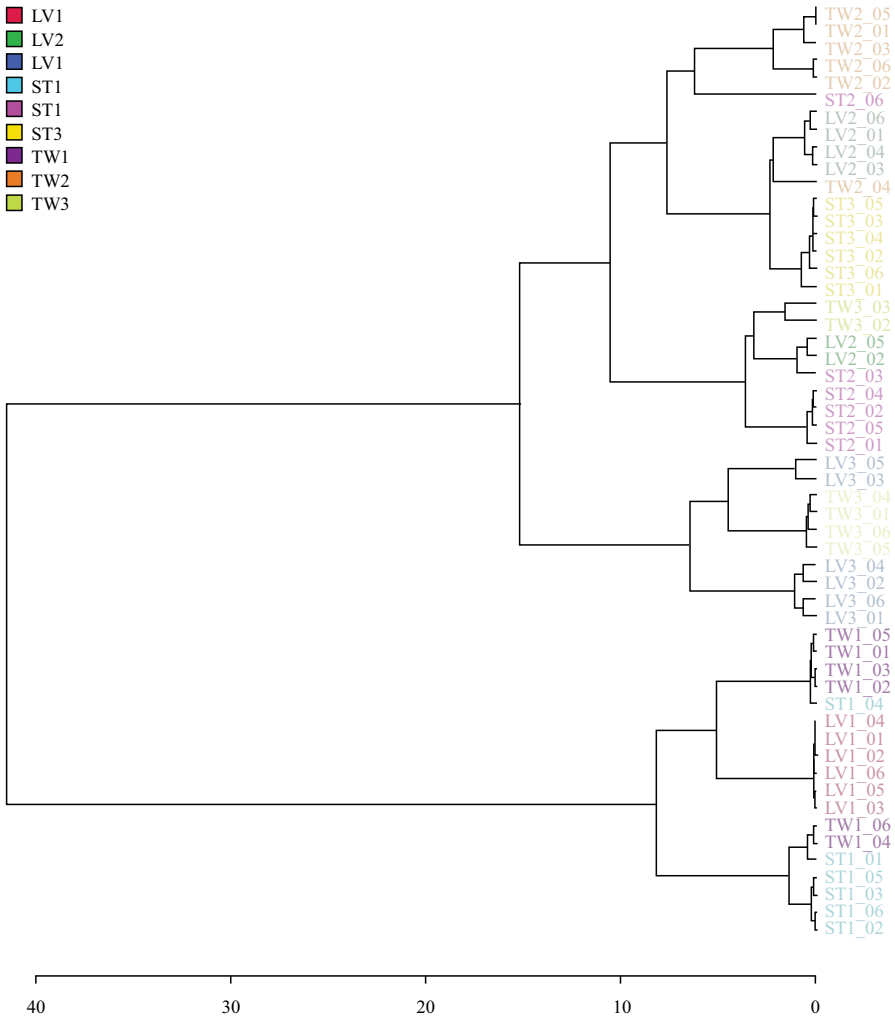

Fig. 4: HCA dendrogram of *M. oleifera* extract

dissolves like'. Other studies have reported that leaves contain higher bioactive compounds than seeds or other organs, explaining their stronger antioxidant activity[28], with polyphenols being most abundant in leaves and stems[29].

Determination of flavonoid using thin layer chromatography: The identification and quantification of flavonoid content in the selected extract were performed using TLC densitometry. The ethanol extract of *M. oleifera* leaves was selected based on its antioxidant activity determined by the DPPH assay. Common flavonoids reported in *M. oleifera* include quercetin-3-O-glucoside, kaempferol and rutin[30]. Similarly, in the Moringaceae family, quercetin, myricetin and kaempferol are the predominant flavonoids[31]. Therefore, quercetin as selected as the standard compound for quantification in this research.

Thin layer chromatography was used to identify and quantify quercetin in ethanol leaves extract. The mobile phase was used are chloroform-methanol-formic acid (5:4:0.5) and stationary phase was used silica gel GF_{254}. The TLC chromatogram of *M. oleifera* extract and standard can be seen in Fig. 5. Based on determination of the Rf (retention factor) value of quercetin is 0.549 and sample 0.548, it can be concluded quercetin was contain in extract. Retention factor was used as the parameter of qualitative analysis. Meanwhile, quercetin content in sample was quantified using AUC value with one point method. As presented in Table 3, the value of quercetin content was 1.498±0.378 mg/g. A study conducted by Thomas *et al.*[32] reported that the quercetin content in the ethanol extract of *M. oleifera* leaves was 993.5 µg/g. Another study by Laksmiani *et al.*[33] found that the quercetin content in extracts obtained by maceration and Soxhlet methods was 24.5% w/w and 20.95% w/w, respectively. These findings

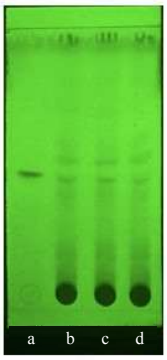

Fig. 5: TLC chromatogram patterns of quercetin standard and ethanol extract of *M. oleifera* leaves, a: Quercetin standard and b, c, d: Ethanol extract of *M. oleifera* leaves; under UV λ 254 nm

Table 3: Flavonoid content in ethanol extract of *M. oleifera* leaves

Sample	AUC	Content (mg/g)
Quercetin	438	1.498±0.378
Sample 1	678.7	
Sample 2	480.6	
Sample 3	810.2	

Table 4: Correlation of TPC and TFC with antioxidant activities

	Pearson correlation coefficient (r)	
Antioxidant parameter	Total phenol	Total flavonoid
DPPH LV1	-0.849	0.758***
DPPH TW1	-0.229	-0.199
DPPH ST1	0.983****	0.750***
DPPH LV2	0.961****	0.961****
DPPH TW2	0.893***	0.861***
DPPH ST2	0.832***	0.903****
DPPH LV3	0.735***	0.937****
DPPH TW3	0.802***	0.752***
DPPH ST3	0.959****	0.732***
CUPRAC LV1	-0.809	0.799***
CUPRAC TW1	0.917****	0.978****
CUPRAC ST1	0.976****	0.894***
CUPRAC LV2	0.913****	0.913****
CUPRAC TW2	0.922****	0.917****
CUPRAC ST2	0.855***	0.913****
CUPRAC LV3	0.937****	0.901****
CUPRAC TW3	0.918****	0.928****
CUPRAC ST3	0.727***	0.807***

Values represent Pearson correlation coefficients (r) between antioxidant parameters and total phenol or total flavonoid contents. Positive values indicate a positive correlation, while negative values indicate an inverse correlation. *** and **** denote statistically significant correlations at $p<0.01$ and $p<0.001$, respectively, values without asterisks are not statistically significant. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, CUPRAC: Cupric Reducing Antioxidant Capacity, LV: Leaves, TW: Twigs, ST: Stem and 1, 2,3: Indicate the respective extraction/treatment groups as defined in the methodology

Table 5: Correlation of DPPH and CUPRAC methods of n-hexane, ethyl acetate and ethanol extract

		Pearson correlation coefficient (r)	
		DPPH antioxidant	CUPRAC antioxidant
n-Hexane	Leaves		0.874***
	Twigs		-0.095
	Stems		0.960****
Ethyl acetate	Leaves		0.921****
	Twigs		0.976****
	Stems		0.920****
Ethanol	Leaves		0.873***
	Twigs		0.618**
	Stems		0.755***

**Moderate correlation, ***Strong correlation and ****Very strong correlation

suggest that the quantification of flavonoid compounds in extracts can vary depending on the extraction method, growing region, plant age and harvest time[34,35].

Statistical analysis was purposed to identify each data contributed to the antioxidant activities. Two antioxidant activity method (DPPH, CUPRAC), TPC and TFC was observed linearity using Pearson's correlation. The result was represented in Table 4. The correlation of each data was showed by Schober scale[36], (0.4-0.6: Moderate, 0.6-0.8: Strong, 0.8-1.0: Very strong).

The correlation between total phenols and flavonoids in *M. oleifera* LV, TW, ST on the antioxidant activity (DPPH and CUPRAC) was identified using Pearson's method. The result was represented in Table 5. A positive correlation indicated total phenol and flavonoid in extract was contribute antioxidant activity.

A previous study demonstrated a significant correlation (r = 0.95) between total phenolic content (TPC) and total flavonoid content (TFC) in *M. oleifera* leaves[37]. Similarly, the statistical analysis in this study indicated that both TPC and TFC exhibited strong to very strong correlations with antioxidant activity. However, no statistically significant correlation was observed between TPC, TFC and antioxidant activity determined using the DPPH method.

CONCLUSION

The DPPH value of leaves, twigs and stem extracts were in the range of 2.28-66.105 mg AEAC/g, while the CUPRAC value of leaves, twigs and stem extracts were in the range of 21.995-73.808 mg AEAC/g. The TW2 has the second highest of TPC and TFC value with 44.167 mg/g and 30.19 mg QE/g, respectively. The DPPH assay indicated that TW3 demonstrated high antioxidant activity potential, while in the CUPRAC assay TW3 exhibited the highest value of antioxidant activity. The *M. oleifera* twigs exhibited greater antioxidant activity than other underutilized parts. The twigs of *M. oleifera* showed promising potential for use as a source of antioxidant agents.

SIGNIFICANCE STATEMENT

Moringa oleifera are commonly known for their use as health supplements and consumed as foods. *M. oleifera* contain flavonoid compounds that play an important role in antioxidant activity. The unused parts (twigs and stem) have been studied for their activity. This study employed the DPPH and CUPRAC methods. The result showed that *M. oleifera* has potential as a natural source of antioxidant, particularly focusing on unused parts. The study result showed that the twigs are potential to be developed as a natural source of antioxidants.

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