A Comparative Study on Phytochemical Screening and Antioxidant Activity of Aqueous Extract from Various Parts of *Moringa oleifera*

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**Abstract**

In this work, we reported a comparative study on the phytochemical screening and antioxidant activity of aqueous extract from different parts of *Moringa oleifera* plant, i.e., leaves, twigs, stem barks, and woods. The phytochemical content of each extract was screened through qualitative analysis and followed by quantitative analysis for the determination of total phenolic content and total flavonoid content. The results showed that different parts of *Moringa oleifera* plant would give different extracted natural products. Both leaf and twig parts of *Moringa oleifera* contained alkaloids, flavonoids, saponins, carbohydrates, polyphenols, proteins, amino acids, and phenolics. Meanwhile, only flavonoids, carbohydrates, and phenolics were found in the aqueous extract of the stem barks and wood parts of *Moringa oleifera*. In agreement to the phytochemical assay, the aqueous extract of leaf part of *Moringa oleifera* gave the highest total phenolic content (684 ± 16.7 µg GAE·mL⁻¹) and the highest flavonoid content (514 ± 26.1 µg CE·mL⁻¹) than other parts. Consequently, the leaf extract exhibited the highest antioxidant activity (64.6 ± 0.69%) which was close to the antioxidant activity of ascorbic acid (71.7 ± 0.56%) as the positive control. This finding is critical for the utilization of *Moringa oleifera* leaf part as the most potent source of antioxidant agents in the future.

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**INTRODUCTION**

Herbalists worldwide have long been intrigued by studies on medicinal plants and traditional medicines. The holistic philosophy of traditional medicine focuses on promoting well-being, preventing diseases, regulating immunity, and maintaining personalized care. More than 60% of drugs have been documented to be produced from plants due to their mildness on the human body with little or no side effects, low cost of manufacture, and ease of availability. Therefore, medicinal plants offer great health benefits for the human body.

The most common and most cultivated species in the Moringaceae family and the Moringa genus is *Moringa oleifera* (*M. oleifera*) [1–3]. A native of this plant is predicted...
to come from Northern India, Pakistan, Afghanistan, Bangladesh, and Nepal’s sub-Himalayan mountains. From tropical to subtropical areas, *M. oleifera* is widely cultivated throughout the world. It is fast-growing and resists extreme drought as well as moderate frost [4]. All components of *M. oleifera*, including the leaves, flowers, seeds, pods, and roots have been evaluated [5–10]. It was reported that the dry leaves contain a large amount of macro and micronutrients (protein 29.4 percent, fat 5.2 percent, calcium 21.85 mg · g⁻¹, phosphorus 2.52 mg · g⁻¹, magnesium 4.48 mg · g⁻¹, iron 2.56 mg · g⁻¹, potassium 12.36 mg · g⁻¹, vitamin B complex 3.11 mg · g⁻¹, vitamin C 1.58 mg · g⁻¹, vitamin E 1.10 mg · g⁻¹) [11,12]. Furthermore, it also contains a lot of natural products such as tannins, sterols, saponins, terpenoids, phenolics, alkaloids and flavonoids like quercetin, isoquercitrin, kaemfericitin, isothiocyanates, and glycoside compounds [13–15].

In several ancient cultures, *M. oleifera* was used as a traditional medicine to treat various diseases and infections in infusions or tea and be consumed fresh in recent salads [16]. Moreover, its application in food, pharmaceutical, cosmetic and agricultural industries has been also investigated [17–20]. Shih et al. [21] identified the effect of the chemical composition and antioxidant activity of *M. oleifera* grown in Taiwan in different parts (leaf, stem, and stalk). The *M. oleifera* samples were extracted with methanol. As a feature of the Moringa section, the antioxidant activity pattern was leaf > stem > stalk extracts. Except for the stalk portion, the Moringa extract demonstrated intense hydrogen peroxide scavenging activity and high Superoxide Dismutase (SOD) activity. Farooq and Koul [22] have studied a comparative study of the antioxidant and antibacterial properties of aqueous and ethanolic leaves and seed extracts of *M. oleifera*. The comparative study shows that *M. oleifera* aqueous and ethanolic leaves and seed extracts have good antioxidant and antibacterial properties with a high total phenolic content and total flavonoid content.

The purpose of this work was to enrich the research on the *M. oleifera* variety from Metro, Lampung through performing phytochemical screening and describing the effect of different parts (leaves, twigs, stem barks, and wood) on the antioxidant activity.

**EXPERIMENTAL**

**General**

*M. oleifera* samples (leaves, twigs, stem barks, and wood) were collected from Metro, Lampung, Indonesia during September-December 2019. Laboratory-grade Dragendorff’s reagent, Anthrone reagent, safrugeric acid, Ninhynidin reagent, Millon’s reagent, iron(III) chloride (FeCl₃), 3-indoleacetic acid, Folin-Ciocalteu reagents, sodium carbonate, gallic acid, catechin, aluminium(III) chloride (AlCl₃), sodium nitrite, and sodium hydroxide were purchased from Merck Sigma-Aldrich Reagent Pte, Singapore.

**Preparation of Aqueous Extract**

The extraction method refers to the research by previous literature [2,23] with some modifications. Fresh *M. oleifera* (leaves, twigs, stem barks, and wood) samples were washed using flowing water, dried under direct sunlight, and finally ground into a powder and stored at room temperature. Powder samples of 4 grams were mixed with 100 mL of distilled water. The mixture was then heated to 60 °C for 20 min. After heating, the solution was allowed to cool and filtered using filter paper (Whatman filter paper), and then the filtrate was collected.

**Phytochemical Analysis**

The extract *M. oleifera* was subjected to phytochemical analysis to detect the presence of alkaloids (Dragendorff’s test) [24], flavonoids (colorimetric AlCl₃ test) [25], saponins (Foam test) [25], carbohydrate (Anthrone test) [26], polyphenols (Puncal-D test) [27], proteins (Ninhydrin test) [27], amino acid (Millon’s test) [28], phenolics (Folin-Ciocalteu test) [2], triterpene (Salkowski test) [29], and anthraquinones (Bomtrager’s test) [29]. The extract solution was further analyzed to determine its total phenolic content with Folin-Ciocalteu reagent [30] in which the results were shown as the equivalent of a microgram for milligram (μg mg⁻¹) gallic acid (GAE). Catechin as a standard was employed in a colorimetric AlCl₃ determination of the total flavonoid content of the extract. The total flavonoid content of the extract was expressed in milligram (μg mg⁻¹) equivalent of catechin (CE) [31].

**FTIR Analysis**

Fourier transform infrared (FTIR) spectroscopic technique was used to identify the presence of functional groups in various parts of *M. oleifera* aqueous extract. FTIR spectrum of samples was recorded on FTIR spectrophotometer (Shimazdu IR Prestige 21). The spectrum was recorded in the wavelength range of 4000–500 cm⁻¹.

**Antioxidant Activity Test**

The extract sample's antioxidant activity was evaluated by DPPH radical testing following the procedure described by the previous report [2], using ascorbic acid as the positive control. DPPH 0.1 mM solution was prepared by dissolving in ethanol. As much as 1 mg of ascorbic acid was dissolved in 1 mL of methanol. Dilution was performed to produce a standard solution of ascorbic acid with varying concentrations (50-500 μg mL⁻¹). For each tube containing a stock solution of ascorbic acid (200 μL), 1 mL of 0.1 mM DPPH solution was added, and a further 800 μL 50 mM Tris-HCl buffer was added (pH 7.4). The final volume is adjusted to 4 mL with ethanol. Stock solutions for extract prepared by dissolving 1 mg of each sample in 1 mL of methanol.

Different aliquots of stock solution (50-500 μg) was added to the separate tube, and the final volume was adjusted to 2 mL using ethanol. A total of 1 mL of 0.1 mM DPPH solution and 800 μL 50 mM Tris-HCl buffer (pH 7.4) were added to each tube. The control was prepared by mixing 1 mL DPPH 0.1 mM, 800 μL 50 mM Tris-HCl buffer (pH7.4), and 2 mL of ethanol. Absorbance was recorded at room temperature after incubation for 30 minutes, measured at 517 nm by UV-Vis spectrophotometer (Analytic Jena Spectord 200 Plus). The percentage of antioxidant activity (inhibition percentage) was calculated using the following equation:

\[
\%\text{DPPH scavenging activity} = \left( \frac{Ac-As}{Ac} \right) \times 100 \%
\]

whereas *Ac* is the absorbance value of the control solution while *As* is the absorbance value of the sample. The mean and standard deviation were calculated based on triplicate measurement.
RESULTS AND DISCUSSION

Phytochemical Analysis of Natural Compounds from Different Part of *M. oleifera*

The extraction of natural compounds from different parts of *M. oleifera*, i.e. leaves, twigs, stem barks, and wood parts have been conducted. Each extract shall contain different natural compounds thus we performed the phytochemical analysis to prove it. The presence of alkaloids can be detected by Dragendorff’s reagent. Dragendorff’s reagent contains potassium iodide and bismuth (III) nitrate in acidic conditions. Since the alkaloids mainly contain tertiary amine group thus it can form a complex with tetraiodobismuthauide yielding an orange solid [24].

The presence of flavonoids was observed through a colorimetric AlCl₃ test. When the sample contains flavonoids thus the addition of AlCl₃ would give a yellow color [2]. The detection of saponins was conducted through a Foam test in which the presence of saponins can be observed by the stable foam formation on the surface of the sample solution [25].

The presence of carbohydrates was detected by using Anthrone test. Anthrone reagent will form bluish-green color when reacted with furfural form of carbohydrate in acidic condition [26]. The presence of polyphenols was observed through a Punctal-D test. The positive result was indicated by the formation of blue-colored complex. The detection of proteins was carried out through a Ninhydrin test. The free amino groups of proteins will form a blue-purplish complex with the ninhydrin reagent [27]. The presence of amino acids was evaluated by Millon’s test. Millon’s reagent was prepared by dissolving mercury in nitric acid. Hydroxyl aromatic groups of amino acids will form a complex with mercury ions yielding red precipitation [28].

The presence of phenolics was examined by the Follin-Ciocalteu test. Follin-Ciocalteu reagent contains a mixture of phosphomolybdate and phosphotungstate acid. Reduction-oxidation reaction with phenolic compounds would generate blue-colored solution [2]. On the other hand, the detection of triterpene was performed by the Salkowski test. The extract containing triterpenes will form a golden yellow color solution by the addition of concentrated sulfuric acid in chloroform media. Meanwhile, the presence of anthraquinones was identified by Borntrager’s test in which the positive result is indicated from the cherry-red color in the aqueous phase [29].

Table 1 shows the result of the phytochemical analysis of extracts from different parts of *M. oleifera*. It was found that all extracts contained flavonoid, carbohydrate and phenolic compounds. Either alkaloids or proteins or amino acids were found in all extracts except for the wood part of *M. oleifera* extract. Meanwhile, saponin and polyphenol were only detected in both leaf and twig extracts of *M. oleifera*. In contrast, all extracts did not contain triterpenes and anthraquinones. These qualitative results clearly show that different parts of *M. oleifera* plant would give different extracted natural compounds.

From Table 1, it can be noted that both leaf and twig parts of *M. oleifera* contains alkaloids, flavonoids, saponins, carbohydrates, polyphenols, proteins, amino acids, and phenolics. Meanwhile, the stem bark part of *M. oleifera* contained alkaloids, flavonoids, saponins, carbohydrates, and phenolics. On the other hand, only flavonoids, carbohydrates, and phenolics were found in the aqueous extract of the wood part of *M. oleifera*.

The phytochemical analysis revealed that all extracts contained flavonoid and phenolic compounds. Therefore, a further experiment was conducted to quantify the total phenolic content and total flavonoid content in each extract of different parts of *M. oleifera* plant. Extract sample was evaluated with the colorimetric AlCl₃ determination of the total flavonoid content of the extract. The total flavonoid content of the extract was expressed in milligram (µg · mg⁻¹) equivalent of catechin (CE) [31].

The results in total phenolic content and total flavonoid content in each extract of different parts of *M. oleifera* plant are listed in Table 2. The total phenolic content of the extracts of leaf, twig, stem bark, and wood part of *M. oleifera* plant was 684 ± 16.7, 628 ± 26.6, 112 ± 5.38, and 5.21 ± 1.20 µg GAE · mL⁻¹, respectively. It means that the aqueous extract of leaf part of *M. oleifera* plant contains a slightly higher total phenolic content than twig part, and much higher than either stem bark or wood part.

On the other hand, the total flavonoid content of the extracts of leaf, twig, stem bark, and wood part of *M. oleifera* plant was 514 ± 26.1, 79.0 ± 9.87, 38.4 ± 5.43, and 8.92 ± 3.98 µg CE · mL⁻¹, respectively. In agreement with the results of total phenolic content, the aqueous extract of leaf part of *M. oleifera* plant contains the highest total flavonoid content than other parts whereas the wood part contains the lowest total flavonoid content.

**Table 1.** Phytochemical analysis of *Moringa oleifera* extract

<table>
<thead>
<tr>
<th>Natural products</th>
<th><em>Moringa oleifera</em> aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
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<tr>
<td>Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>-</td>
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<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2.** Total phenolic and flavonoid contents as well as antioxidant activity of *Moringa oleifera* extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic content (µg GAE · mL⁻¹)</th>
<th>Total flavonoid content (µg CE · mL⁻¹)</th>
<th>DPPH scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>-</td>
<td>-</td>
<td>71.7 ± 0.56</td>
</tr>
<tr>
<td>Leaves</td>
<td>684 ± 16.7</td>
<td>514 ± 26.1</td>
<td>64.6 ± 0.69</td>
</tr>
<tr>
<td>Twigs</td>
<td>628 ± 26.6</td>
<td>79.0 ± 9.87</td>
<td>51.4 ± 1.24</td>
</tr>
<tr>
<td>Stem Barks</td>
<td>112 ± 5.38</td>
<td>38.4 ± 5.43</td>
<td>23.8 ± 2.31</td>
</tr>
<tr>
<td>Woods</td>
<td>5.21 ± 1.20</td>
<td>8.92 ± 3.98</td>
<td>10.0 ± 1.56</td>
</tr>
</tbody>
</table>

**FTIR Analysis**

Since the aqueous extract of different parts of *M. oleifera* plant yielded different extracted natural compounds, thus, the FTIR analysis of each extract was carried out. The FTIR analysis gave information on the identification of the functional groups of extracted natural compounds in various parts of *M. oleifera* plant. The FTIR spectrum of different parts of *M. oleifera* extract is shown in Figure 1. It was found that...
the extract from different part of M. oleifera plant gave different FTIR spectrum as different natural compounds exist.

From Figure 1, it was shown that the FTIR spectrum of leaf and twig extracts composed by a broad signal at ~3400 cm⁻¹, sharp signals at 1650-1750, 1400-1500, ~1250, and ~1100 cm⁻¹. This finding is in agreement with the result from the phytochemical analysis as leaf and twig extracts consist of similar natural products. Meanwhile, a simpler FTIR spectrum was found for stem bark extract with a broad signal at ~3400 cm⁻¹, sharp signals at 1650-1750 and ~1100 cm⁻¹. The simplest FTIR spectrum was recorded for wood extract with a broad signal at ~3400 cm⁻¹, and a sharp signal at 1650-1750 cm⁻¹ due to its lowest phytochemical content.

From the FTIR analysis, all extracts contained O-H functional group (broad signal at ~3400 cm⁻¹) as the usage of distilled water as the extraction media. Moreover, all extracts also contained C=O functional group which may come from the polar natural compounds as shown by a sharp signal at 1650-1750 cm⁻¹. The stem bark, twig and leaf extracts contained C-O functional groups as detected as a sharp signal at 1100 cm⁻¹. Meanwhile, the aliphatic and aromatic carbons were observed as sharp signals at 1400-1500 and ~1250 cm⁻¹, respectively.

The observed natural products in leaf and twigs extracts from the phytochemical analysis were alkaloids, flavonoids, saponins, carbohydrates, polyphenols, proteins, amino acids and phenolics. These compounds have O-H, C=O, C-O, aliphatic and aromatic carbons functional groups as displayed in the FTIR spectra of leaf and twigs extracts. On the other hand, the observed natural products in stem barks and woods extracts from the phytochemical analysis were flavonoids, carbohydrates, and phenolics thus only O-H, C=O, and C-O functional groups were observed in their FTIR spectra.

Figure 1. FTIR spectrum of different parts of Moringa oleifera extract.

Antioxidant Activity Test

A further experiment was conducted to evaluate the potential application of the different part of M. oleifera extract as the antioxidant agent through in vitro DPPH assay. The results from the antioxidant activity test are shown in Table 2. The DPPH scavenging activity of the extracts from leaf, twig, stem bark, and wood part of M. oleifera plant were 64.6 ± 0.69, 51.4 ± 1.24, 23.8 ± 2.31, and 10.0 ± 1.56%, respectively. This result is in agreement with the total phenolic content and total flavonoid content as reported in this work and other previous works. Higher total phenolic content and higher total flavonoid content gave a higher antioxidant activity indeed. Vongsak et al. reported that M. oleifera leaf extract showed a high total phenolic content as well as total flavonoid content thus consequently the extract gave a high antioxidant activity [5]. These results showed that both M. oleifera plants from Thailand and Lampung gave a similar trend on the antioxidant activity, i.e. leaves > twigs > stem barks > woods. As natural phenolic and flavonoid compounds contain free O-H groups thus all extracts showed antioxidant activity. The aqueous extract of leaf part of M. oleifera plant exhibited the highest antioxidant activity. Furthermore, it is worthy to note that the antioxidant activity of leaf extract is close to the antioxidant activity of ascorbic acid, which is remarkable.

CONCLUSION

The extraction of natural compounds from different parts of M. oleifera, i.e. leaves, twigs, stem barks, and wood parts have been carried out in this work. Phytochemical analysis revealed that all extracts contained flavonoid, carbohydrate and phenolic compounds with the absence of triterpenes and anthraquinones. From the quantitative analysis, it was found that aqueous extract from the leaf part of M. oleifera gave the highest total phenolic content (684 ± 16.7 µg GAE · mL⁻¹) and the highest flavonoid content (514 ± 26.1 µg CE · mL⁻¹) than other parts. The leaf extract contained natural compounds with O-H, C-O, C=C, and C=O functional groups as observed in the FTIR analysis. Consequently, the aqueous extract of leaf part of M. oleifera plant exhibited the highest antioxidant activity (64.6 ± 0.69%) which is close to the antioxidant activity of ascorbic acid (71.7 ± 0.56%) as the positive control, which is remarkable. Therefore, the leaf of M. oleifera plant can be utilized as the source of antioxidant agents in a further application.

Acknowledgement

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Abstrak
Pada penelitian ini, kami melaporkan studi tentang skrining fitokimia dan aktivitas antioksidan dari ekstrak berair tumbuhan Moringa oleifera yang diambil dari bagian-bagian yang berbeda seperti daun, ranting, kulit batang, dan kayu. Kandungan fitokimia dari tiap ekstrak dilakukan skrining melalui analisis kualitatif yang diikuti dengan analisis kuantitatif untuk penentuan kandungan fenol total dan kandungan flavonoid total. Hasil penelitian menunjukkan bahwa bagian tumbuhan Moringa oleifera yang berbeda memberikan perbedaan senyawa hasil alam yang berbeda pula. Baik bagian daun atau ranting dari tumbuhan Moringa oleifera mengandung alkaloid, flavonoid, saponin, karbohidrat, polifenol, protein, asam amino, dan senyawa fenolat. Sementara itu, hanya flavonoid, karbohidrat, dan senyawa fenolat yang ditemukan pada bagian ranting dan batang dari tumbuhan Moringa oleifera. Sepanada dengan hasil fitokimianya, ekstrak berair dari bagian daun Moringa oleifera memberikan kandungan fenol tertinggi (864 ± 16.7 μg GAE mL⁻¹) dan kandungan flavonoid tertinggi (514 ± 26.1 μg CE mL⁻¹) dibandingkan dengan bagian daun. Oleh karena itu, ekstrak daun membahayakan untuk dijadikan ekstrak obat. Pada penelitian ini, ekstrak daun Moringa oleifera diuji untuk mengetahui adanya aktivitas antioksidan, seperti antioksidan senyawa hidroksil, antioksidan senyawa hidroksil, dan antioksidan senyawa hidroksil. Pada dasar diuji, ekstrak daun Moringa oleifera memiliki potensi antioksidan yang baik, terutama pada bagian daun yang diuji. Dengan adanya aktivitas antioksidan senyawa hidroksil (71.7 ± 0.56%) sebagai kontrol positif. Penelitian ini penting untuk pemanfaatan daun Moringa oleifera sebagai sumber yang paling potensial dari agen antioksidan di masa depan.

Kata kunci: Moringa oleifera, fitokimia, kandan fenol, antioksidan