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Research paper

Phytochemical Analysis and Antioxidant Activity of Acetone Extract from *Moringa oleifera* Leaves

Analisis Fitokimia dan Uji Aktivitas Antioksidan Ekstrak Aseton Daun Kelor (*Moringa oleifera*)

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ABSTRACT

Indonesia's rich biodiversity includes *Moringa oleifera*, widely used in traditional medicine. However, environmental factors such as climate, soil, and agronomic practices can influence the phytochemical composition and bioactivity of its leaves. This study aimed to investigate the phytochemical profile and antioxidant activity of acetone extracts of M. oleifera leaves sourced from South Lampung. Leaves were air-dried, powdered, and extracted via maceration in acetone. Antioxidant capacity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, triterpenoids, and tannins, while saponins were not detected. In the DPPH assay, percentage inhibition increased with concentratio, yielding an IC_{50} of approximately 209.4 ppm. This relatively high IC_{50} indicates weak radical-scavenging activity under the present conditions. The findings provide the first detailed report of phytochemical constituents and DPPH-based antioxidant capacity for acetone extracts of M. oleifera leaves from South Lampung. Although secondary metabolites with known bioactivities were detected, the weak DPPH scavenging suggests that acetone may not optimally extract the most potent antioxidant compounds or that local growth conditions yield lower phenolic/flavonoid concentrations.

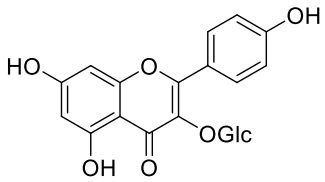
Keywords*: Moringa oleifera; acetone extract; phytochemical screening; antioxidant activity; South Lampung*

INTRODUCTION

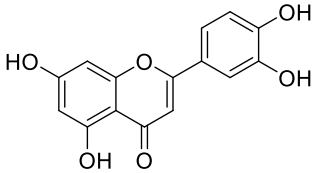
Indonesia hosts extensive biodiversity, including *Moringa oleifera*, which has long been employed in traditional medicine. Across different regions, local communities utilize various parts of this plant to treat skin disorders, reduce fever, alleviate diarrhea, and promote wound healing; it is also consumed as a vegetable or used as animal fodder (Dani et.al, 2018).

Nevertheless, the chemical composition and bioactive potential of *M. oleifera* leaves are

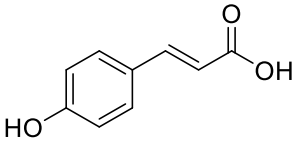
known to fluctuate in response to environmental parameters such as climate, soil characteristics, and agronomic practices (Pant et.al, 2021). The known bioactive chemical composition of *M. oleifera* leaves includes kaempferol glycosides, quercetin, coumaric acid, moringinine, niazicin and lupeol acetate (Chis, 2024). The chemical structures of the bioactive compounds are shown in Figure 1.



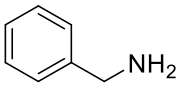
kaempferol glycosides (flavonoid)



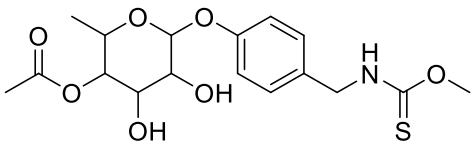
quercetin (flavonoid)



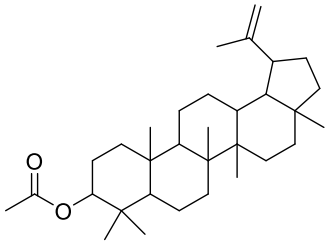
coumaric acid (phenolic)



moringinine (alkaloid)



niazicin (sulfur compounds)



lupeol acetate (terpenoid)

Figure 1. The chemical structures of the bioactive compounds *M. Oleifera* leaves

A critical factor in evaluating medicinal plants is their content of secondary metabolites—particularly flavonoids, alkaloids, tannins, steroids, and triterpenoids—which underlie key pharmacological activities, including antioxidant properties (Sabindo, 2024). Antioxidants are essential defenders against oxidative damage caused by free radicals, which, when in excess, can inactivate enzymes, oxidize lipids, and damage DNA, thereby increasing the risk of cellular mutations and degenerative diseases (Chaudhary *et.al*, 2023).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely adopted to quantify radical-scavenging capacity, relying on the measurable decrease in absorbance and visible color transition from purple to yellow upon reduction of the DPPH radical (Guicin, 2023). Previous investigations have reported widely varying IC_{50} values for DPPH scavenging by Moringa leaf extracts, depending on the choice of solvent and the geographic provenance of the plant material. For example, ethanol extracts have exhibited IC_{50} values ranging from tens to hundreds of µg/mL, whereas acetone extracts have yielded similarly heterogeneous results (Meigaria, 2016).

These discrepancies are attributed to differences in the profiles of phenolic and flavonoid constituents extracted, as well as to site-specific growth conditions. However, detailed data on the phytochemical profile and antioxidant activity of acetone extracts of M. oleifera leaves from South Lampung remain scarce.

This knowledge gap underscores the need to elucidate the influence of the South Lampung terroir on secondary metabolite content and antioxidant capacity. Considering that local environmental conditions can significantly modulate bioactive composition that may be influenced by environmental factors like soil condition, climate, agronomy, etc. So, it is imperative to perform standardized phytochemical screening to identify the principal classes of secondary metabolites in acetone extracts of *M. oleifera* leaves collected from South Lampung, alongside DPPH-based assays to determine representative IC_{50} values. Moreover, the *M. oleifera* leaves are very abundantly found in South Lampung, and they are not well explored yet in the field of herb

medicine. Therefore, in the present study, the antioxidant properties of *M. Oleifera* leaves are comprehended.

The objectives of the present study are: (1) to conduct phytochemical screening of acetone extracts of M. oleifera leaves from South Lampung in order to detect the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, and saponins; and (2) to evaluate antioxidant activity via the DPPH assay and calculate the corresponding IC_{50}. The resulting data are expected to provide a comprehensive overview of the local phytochemical characteristics and antioxidant potential of M. oleifera in South Lampung, thereby laying the groundwork for subsequent fractionation or compound isolation studies, optimization of extraction protocols, and development of functional formulations.

METHODS

Materials

The primary raw material, Moringa leaf samples, was obtained from Margomulyo Village, Jatiagung District, South Lampung Regency. The chemicals used included distilled organic solvents such as acetone, methanol, and ethanol. Other chemicals used were phytochemical reagents, including Dragendorff's reagent, Mayer's reagent, ferric chloride ($FeCl_3$), hydrochloric acid (HCl), and Liebermann-Burchard reagent, as well as the antioxidant reagent DPPH (1,1-diphenyl-2-picrylhydrazyl) Sigma Aldrich.

The equipment used in this study included Erlenmeyer flasks, test tubes, measuring cylinders, beakers, an analytical balance, round-bottom flasks, spatulas, stirring rods, a blender, aluminum foil, a Heidolph Rotary Evaporator Hei-VAP, filter paper, micropipettes, dropper pipettes, funnels, a distillation apparatus, and Thermo Scientific Genesys 50 UV Vis Spectrophotometer.

Procedure

Sample Preparation

Moringa leaf samples were washed, cut into small pieces, and dried by air-drying. The dried samples were then ground into powder and weighed

Extraction

In this study, sample extraction was carried out using acetone solvent that had gone through a distillation process. The extraction process was carried out using the maceration method, namely by soaking 100 grams of coarse sample powder in 350 ml of acetone in an Erlenmeyer flask. The container was then closed and left for three days in a condition protected from light, while stirring for at least 30 minutes each day. After the maceration process, the mixture was filtered using filter paper to obtain the filtrate. This step was repeated three times.

Phytochemical Test

About 1 g of extract was dissolved in 10 mL of acetone solvent until dissolved. Then the following test was carried out:

- Alkaloid Test: about 2 mL of the extract solution were added with one milliliter of HCl 2N, then divided into three test tubes. Tube 1 served as a control, Tube 2 was added with Dragendorff's reagent, and Tube 3 with Mayer's reagent. A positive result is indicated by the formation of a brick-red precipitate with Dragendorff's reagent and a cream or greenish precipitate with Mayer's reagent (Sriyanti, 2022).
- Steroid/Triterpenoid Test: about 2 mL of extract in a test tube was added with 2 mL of Liebermann-Burchard reagent. The appearance of a purple color indicates the presence of triterpenoid groups, while the appearance of a green-blue color indicates the presence of steroid compounds (Setyawati et.al, 2020)
- Saponin test: about 2 mL sample was dissolved in 10 mL of distilled water in a test tube. After cooling, the filtrate in the test tube was shaken vigorously for approximately 30 seconds. The reaction is considered complete or at rest when the reaction temperature equals room temperature. The formation of foam with a minimum height of 1 cm that persists for 10 minutes and does not disappear upon the addition of one drop of dilute hydrochloric acid indicates the presence of saponins (Setyawati et.al, 2020).
- Tannin test: A 2 mL extract solution was reacted with 10% ferric chloride solution. The formation of a dark blue, bluish-black, or greenish-black color indicates the presence of tannins (Setyawati et.al, 2020).
- Flavanoid test: about 2 mL of extract were added with magnesium powder, followed by the addition of 3–4 drops of concentrated hydrochloric acid. The appearance of a red

or orange color indicates the presence of flavonoids (Sriyanti, 2022).

Antioxidant Activity

DPPH at a concentration of 160 mg/L was prepared by weighing 4.0 mg of the compound and dissolving it in 25 mL of methanol in a volumetric flask. The resulting DPPH stock solution was stored under dark conditions, protected from light by wrapping the container with aluminum foil. An acetone extract sample of 10 mg was dissolved in methanol to a final volume of 10 mL, resulting in a stock solution with a concentration of 1000 ppm.

The design of the test sample concentrations was prepared by dilution as shown in Table 1. All test solutions were placed into test tubes and incubated at room temperature for 30 minutes, starting from the addition of the DPPH solution to the sample. The absorbance was then measured at the DPPH maximum wavelength of 517 nm. The IC_{50} value of the sample against the DPPH solution was determined using a linear regression equation obtained from the measurement of varying sample concentrations against the DPPH solution. The linear regression equation, $y=a + bx$, represents the relationship where the x-axis corresponds to the sample concentration and the y-axis represents the percentage of inhibition.

Tabel 1. Concentration design of sample .

Concentration (ppm)	Sample (µL)	Methanol (µL)	DPPH (µL)
0	0	4000	1000
100	500	3500	1000
130	650	3350	1000
160	800	3200	1000
190	950	3050	1000
210	1050	2950	1000

RESULT AND DISCUSSION

Phytochemical Test

Phytochemical screening is a qualitative test used to determine the classes of secondary metabolites present in the acetone extract of moringa leaves. The secondary metabolites to be identified include alkaloids, flavonoids, steroids, triterpenoids, tannins, and saponins. The result of phytochemical screening can be showed in the Table 2 and Figure 2.

Tabel 2. Phytochemical screening.

No	Secondary Metabolite	Reagent	Result	Indication
1	Alkaloid	Mayer	+	yellowish-green, precipitate
2	Alkaloid	Dragendroff	+	greenish-brown, precipitate
3	Steroid or Terpenoid	Lieberman-Burchard	+	(steroid) transition to bluish-green tones or (triterpenoid) change to reddish-purple
4	Tanin	$FeCl_3$	+	yellowish-brown
5	Flavanoid	Mg + HCl	+	green to brownish-green, distinct layer formation
6	Saponin	HCl	-	-

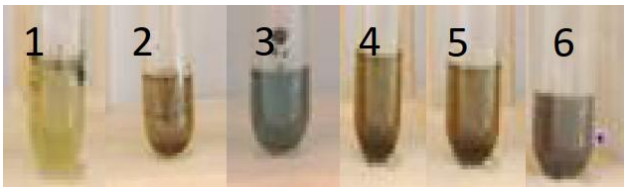


Figure 2. The result of phytochemical screening

The presence of alkaloids in the acetone extract of Moringa leaves was first confirmed using Mayer's and Dragendorff's reagents, yielding positive results as evidenced by yellowish-green and greenish-brown color changes accompanied by precipitate formation (Figure 2). These observations are consistent with Sriyanti et al.'s findings, indicating alkaloid constituents in the extract. The addition of HCl 2N to the sample aims to extract basic alkaloid compounds, leading to the formation of alkaloid salts. These alkaloid salts are then precipitated by the addition of reagents, namely Mayer's and Dragendorff's.

The precipitate formed results from a reaction between K^+ ions from the reagent and the nitrogen atom of the alkaloid, forming a potassium–alkaloid complex. The nitrogen atom in alkaloids possesses a lone pair of electrons that can form a coordinate covalent

bond with K^+ ions from potassium tetraiodomercurate (II) in Mayer's reagent, resulting in a yellowish-white precipitate (Figure 3a). The reddish-brown precipitate with Dragendorff's reagent forms due to the nitrogen in the alkaloid forming a covalent bond with K^+ ions from potassium tetraiodobismuthate, producing a reddish-brown potassium–alkaloid complex precipitate (Figure 3b) (Pardede et.al, 2013).

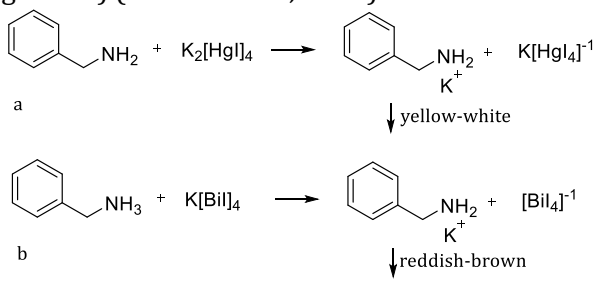


Figure 3. Alkaloid identification reaction (a) Mayer and (b) Dragendroff

Building on this, flavonoid screening was performed. A positive flavonoid test was indicated by a color change from green to brownish-green together with the formation of a distinct layer. A brownish green coloration observed during the Shinoda test (Mg and HCl) may suggest the presence of flavonoid compounds in low concentration or modified structural forms. Although not a classical positive result (typically red or orange), such color changes can still indicate flavonoid-related activity, especially in complex plant extracts. This occurs because flavonoid moieties react with magnesium and hydrochloric acid (HCl) to form flavylium salts as illustrated in Figure 4. The detection of flavonoids suggests antioxidant potential and contributes to the overall phytochemical profile of the extract

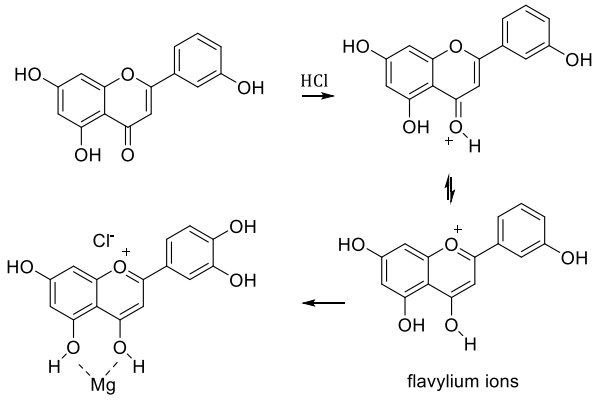


Figure 4. Flavonoid identification reaction (Lio et al, 2019)

Subsequently, the screening extended to steroid and triterpenoid compounds using Liebermann–Burchard reagent. A positive test for steroids was marked by a color transition to bluish-green tones, whereas a positive triterpenoid indication was observed as a change to reddish-purple. These color shifts arise from the reaction between steroidal or triterpenoid frameworks and the Liberman–Burchard reagent, which involves dehydration and complex formation leading to characteristic chromophores. Sulfuric acid initiates sulfonation of sterol molecules by eliminating water ($-H_2O$), resulting in sulfonated derivatives. Acetic anhydride, in conjunction with sulfuric acid, generates a conjugated pentenyl carbocation, which produces a blue coloration. Repeated desaturation and skeletal rearrangement lead to the formation of polyenes and subsequently aromatic compounds, causing a progressive color change from purple to blue to dark green (Lio et.al, 2019). The specific reaction sequence and color evolution are depicted in Figure 4. The presence of these compounds may correlate with a variety of bioactivities, such as anti-inflammatory or membrane-interacting effects.

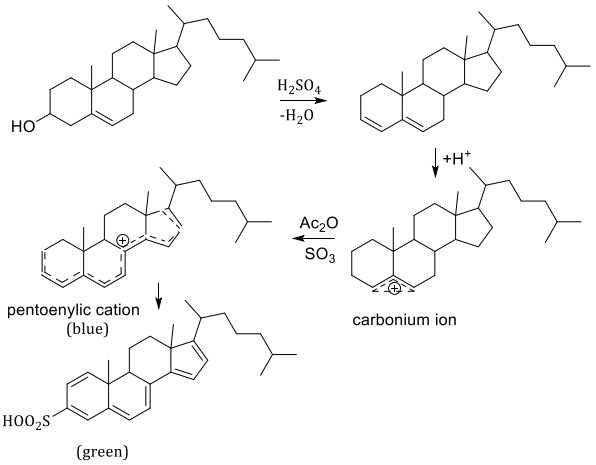


Figure 4. Terpenoid/steroid identification reaction (Liebermann-Burchard) (Lio et al, 2019)

The identification of tannin compounds showed a positive result, indicated by a yellowish-brown color change. This occurs due to the reaction between one of the hydroxyl groups of the tannin compound and $FeCl_3$, forming a complex with Fe^{3+} ions (Lestari et.al, 2021). The reaction between the tannin compound and $FeCl_3$ reagent is illustrated in Figure 5.

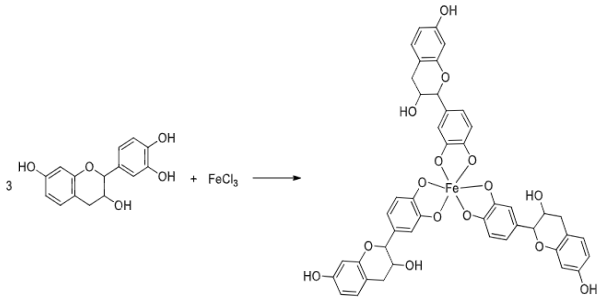


Figure 5. Tannin identification reaction

In contrast, the saponin test yielded a negative result, no stable foam was formed after vigorous mixing and addition of 2 N HCl. The absence of persistent frothing indicates that saponins are either absent or below detectable levels in this acetone extract. Since saponins often require aqueous or methanol extracts for more sensitive detection, their absence here may reflect the solvent choice (acetone) or genuinely low content. This negative result helps refine the phytochemical profile by excluding significant saponin contribution under the chosen extraction conditions.

Overall, the phytochemical screening of the acetone extract of Moringa leaves reveals the presence of alkaloids, flavonoids, steroids, and triterpenoids-each detected by characteristic reagent-induced color changes and/or precipitates-while saponins were not detected under the test conditions.

Antioxidant activity

This study utilized the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to assess antioxidant activity. In methanol as the organic solvent, mixing the DPPH solution with the sample initiated a reaction primarily governed by the hydrogen atom transfer (HAT) mechanism (Figure 6). This reaction reduces DPPH• to its non-radical form, DPPH-H (2,2-diphenyl-1-picrylhydrazine), resulting in a measurable decrease in absorbance at 517 nm, which correlates with increasing antioxidant concentration (Nakanishi et.al, 2021). The color shift from purple to yellow serves as an indicator of high antioxidant activity; a greater decrease in DPPH intensity denotes enhanced antioxidant efficacy of the sample (Carmona et.al, 2014)].

This method is chosen for its simple and straightforward procedure and employs a UV-Vis spectrophotometer for measurement. The results of the antioxidant activity test of the acetone extract are presented in Table 3.

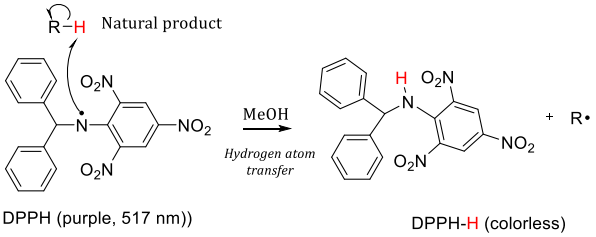


Figure 6. DPPH antioxidant mechanism.

Tabel 3. The antioxidant activity test of the acetone extract.

Concentration	% Inhibition	IC_{50}
0	0	
100	20.19	
130	26.76	209.42
160	34.21	
190	37.45	
210	53.13	

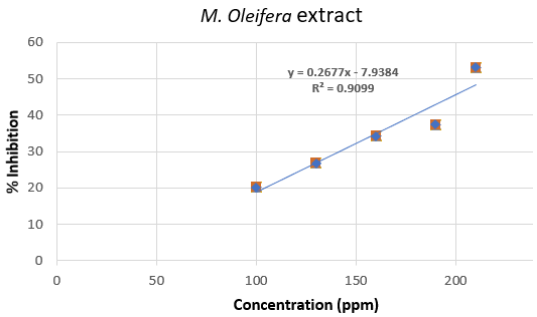


Figure 7. Inhibition curve of *M. Oleifera* antioxidant.

Tabel 4. The antioxidant activity test of the ascorbic acid.

Concentration	% Inhibition	IC_{50}
0	0	
2	25.1497	
4	28.64271	8.769151
6	33.333	
8	45.708	
10	58.782	

Antioxidant activity parameters are determined based on IC_{50} values. In determining IC_{50} for the acetone extract of Moringa leaves, the plot of extract concentration versus percentage inhibition was constructed based on the data in Table 3. The linear regression equation (y = 0.2677x - 7.9384) obtained from these data (was used to calculate an IC_{50} value of 209.46 ppm, indicating relatively weak DPPH scavenging capacity under the present assay conditions. As a positive control, the antioxidant activity of

ascorbic acid was also evaluated in this study (Table 4 and Figure 8).

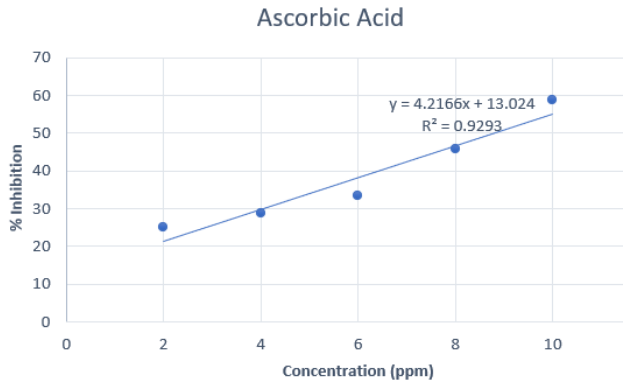


Figure 8. Inhibition curve of ascorbic acid antioxidant.

CONCLUSIONS

Phytochemical screening of acetone extracts of Moringa oleifera leaves from South Lampung confirmed the presence of alkaloids, flavonoids, steroids, triterpenoids, and tannins, while saponins were absent under the applied conditions. The DPPH assay demonstrated a concentration-dependent increase in radical-scavenging activity, with an IC_{50} of approximately 209.4 ppm, indicating relatively weak antioxidant potential. These results suggest that acetone extraction of local M. oleifera leaves yields detectable bioactive classes but may not efficiently recover potent antioxidant constituents. The study establishes a reference point for South Lampung samples, highlighting the influence of solvent choice and local environmental factors on extract composition and activity. Future research should explore alternative solvents or extraction techniques, quantitative profiling of phenolic/flavonoid contents, and isolation of key antioxidant molecules to better harness the bioactive potential of *M. oleifera* in this region.

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