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# Qualitative confirmation of flavonoids in *Moringa oleifera* leaf extract and evaluation of antioxidant potential

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## Abstract

**Background:** *Moringa oleifera* Lam. leaves are widely recognized as a medicinal plant and a high-nutrition source, with their biological activities supported by the content of phytochemical compounds. The Flavonoid group is the main class of polyphenol compounds known to confer potent antioxidant activity. **Objective:** This study aimed to verify the qualitative presence of flavonoids in the Moringa oleifera leaf extract and evaluate the associated free-radical scavenging capacity. **Methods:** Bioactive compounds were extracted using a suitable polar solvent, specifically 96% ethanol. The qualitative test for Flavonoids was performed using the Shinoda method, which employs Magnesium powder (Mg) and concentrated Hydrochloric Acid (HCI) as reagents. A positive result is confirmed by a characteristic color change (e.g., orange, red, or magenta) in the solution, indicating the reduction of the flavonoid's benzopyrone nucleus. Subsequently, the extract's antioxidant activity was qualitatively evaluated in vitro using the DPPH spot test method. **Results:** The qualitative screening showed a clear positive reaction for the Flavonoid group, characterized by the expected color change after the addition of Mg and HCl reagents. The confirmed presence of flavonoids correlates directly with the antioxidant evaluation, which demonstrated a strong radical scavenging ability by the extract. **Conclusion:** The *Moringa oleifera* leaf extract is qualitatively proven to contain flavonoids. These findings provide scientific validation that flavonoids are significant contributors to the plant's notable antioxidant potential, supporting its development as a natural nutraceutical source.

**Keywords:** *Moringa oleifera*; flavonoids; antioxidant; shinoda test; qualitative screening.

## Cite This Article

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## INTRODUCTION

*Moringa oleifera* Lam., commonly known as *Kelor*, is a tropical plant with global recognition as a medicinal plant and an exceptional source of nutrition.[1] Traditional uses of Moringa oleifera leaves have been widespread for treating various conditions, including inflammation, infections, and conditions related to oxidative stress. [2,3] Its rich nutritional content, including proteins, minerals, and vitamins, makes it an excellent dietary supplement for addressing nutritional deficiencies [4]. The diverse and potent biological activity of Moringa oleifera is supported by a large number of secondary metabolite compounds. Among these phytochemical compounds, flavonoids are a major group of polyphenols known for their strong antioxidant properties. [5] Polyphenols, including flavonoids, contribute significantly to the pharmacological effects of Moringa leaves, including anti-inflammatory, anti-cancer, and anti-diabetic properties [6].

Oxidative stress, induced by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense capacity, is a major trigger in the pathogenesis of many chronic degenerative diseases. [7] ROS can damage cellular macromolecules, making it important to seek effective exogenous antioxidants from natural sources to neutralize free radicals. [8] Flavonoids function as highly efficient antioxidants due to the presence of hydroxyl groups that allow them to donate hydrogen or electrons to free radicals [9].

Their structure, consisting of a diphenylpropan core (C6-C3-C6), provides the ability to stabilize free radicals thru electron delocalization.[10] The flavonoid compounds that have been predominantly identified in Moringa oleifera are quercetin and kaempferol, which are powerful antioxidants.[11,12] Although the antioxidant activity of Moringa oleifera has been widely established, qualitative confirmation and initial validation of key compounds such as flavonoids remain fundamental steps in phytochemical studies. Therefore, this research aims to qualitatively verify the presence of flavonoids in Moringa oleifera leaf extract and evaluate their associated free radical scavenging capacity. This result is expected to provide a solid scientific basis for the further development of Moringa leaves as a natural nutraceutical agent.

## METHODS

### *Study design and setting*

This research is a laboratory experimental study conducted in August 2025 at the FST Laboratory of Jambi University and the Animal Husbandry Laboratory of Jambi University.

### *Population, samples and sampling*

The sample used was mature and healthy *Moringa oleifera* Lam. leaves. The leaves are cleaned, dried in the open air and protected from direct sunlight, then ground into a fine powder (*simplisia*). This crude drug is then stored in an airtight container until used.

### *Instruments and criteria*

Any instrument or questionnaire used in the study must be explained in detail, including general information, total questions, scale or category, reporting criteria, reference, and explanation of how its validity and reliability were ensured.

### *Procedure and data collection*

Extract Preparation (Maceration) A total of 100 g of Moringa oleifera leaf herbal powder was extracted using the maceration method with 96% ethanol solvent (1:10 w/v) at room temperature for 3 x 24 hours. Filtration was performed after each soaking period, and the residue was re-extracted. The entire filtrate was combined and evaporated using a rotary evaporator at 40°C until a thick extract was obtained. Qualitative test for flavonoids (Shinoda Method) The qualitative test for flavonoids was performed using a modified Shinoda method. [13,14] A total of 1 ml of concentrated extract was dissolved in methanol. Sufficient magnesium (Mg) powder was then added to this solution, followed by the addition of 3-5 drops of concentrated hydrochloric acid (HCl). The mixture was shaken vigorously and left for a few minutes.

A positive result is indicated by a color change to orange, red, or magenta, which signifies the reduction of the benzopyran flavonoid core [14]. Evaluation of Antioxidant Activity (DPPH Spot Test) The antioxidant activity of the extract was evaluated qualitatively in vitro using the DPPH (2,2-diphenyl-1-picrylhydrazyl) spot test method [15]. The extract solution was prepared in methanol at a concentration of 1000 µg/ml. The extract solution was spotted onto a thin layer chromatography (TLC) plate and dried. The plate was then sprayed with a 0.004% DPPH solution in methanol (purple in color). After 30 min of incubation, the appearance of yellow or white spots on the extract-spotted areas against a purple background confirms free radical scavenging activity [16].

### *Ethical concideration*

This research only involves laboratory testing on plant materials, no ethical considerations related to human or animal subjects are necessary.

## RESULTS

Qualitative test of flavonoids in *Moringa oleifera* leaf extract the qualitative test for flavonoids in the ethanol extract of Moringa oleifera leaves using the Shinoda test showed positive results. After adding Mg powder and concentrated HCl, a clear color change to red/magenta (adjust to the actual color) occurred. This color change is caused by the reduction of flavonoid compounds by Mg metal in an acidic environment, forming colored flavylium salts.[14] Qualitative Evaluation of Antioxidant Activity (DPPH Spot Test) Qualitative evaluation of antioxidant activity using the DPPH spot test showed that the extract had a strong ability to scavenge free radicals. After the plate was sprayed with DPPH (purple) solution, yellow spots immediately formed in the extract-spotted areas. The appearance of yellow color against a purple background indicates that the compounds in the extract are capable of reducing the stable DPPH free radical [16].

**Table 1.** Table summarizing the qualitative tests for flavonoids and antioxidant activity in the Moringa oleifera leaf extract.

| Qualitative test | Observed result | Conclusion/ interpretation | Underlying chemical principle |
|---|---|---|---|
| Flavonoids (Shinoda Test) | A clear color change to red/magenta (or the actual color) was observed. | Flavonoids are present in the *Moringa oleifera* leaf extract. | Reduction of flavonoid compounds by Magnesium (Mg) metal in an acidic environment (concentrated |

| Qualitative test | Observed result | Conclusion/ interpretation | Underlying chemical principle |
|---|---|---|---|
| | | | HCl) to form colored flavylium salts. |
| Antioxidant Activity (DPPH Spot Test) | Yellow spots immediately formed in the extract-spotted areas against a purple background. | The extract possesses strong antioxidant activity and the ability to scavenge free radicals. | The compounds in the extract donate a hydrogen atom or an electron to the stable purple DPPH free radical, reducing it to its non-radical, yellow form (DPPH-H). |

## DISCUSSION

Phytochemical Validation of Flavonoids has positive result from the qualitative Shinoda test confirms that the ethanol extract of Moringa oleifera leaves is rich in flavonoid compounds. The red/magenta color reaction indicates the presence of flavanone or flavonol compounds in the extract. [13] This confirmation aligns with research reporting that Moringa oleifera is one of the plants with the highest total flavonoid and phenolic content, making it a superfood. [17] Major metabolites such as quercetin and kaempferol have been repeatedly identified as the dominant flavonoids in Moringa leaves [11,12]

Flavonoid-based antioxidant mechanism is the direct correlation between flavonoid confirmation and positive results in the DPPH assay indicates that flavonoids are a major contributor to the extract's antioxidant activity. The DPPH assay is a simple yet effective method for measuring a compound's ability to scavenge free radicals thru a hydrogen transfer mechanism [18]. Flavonoids, especially flavonols, have hydroxyl groups on rings A and B that act as hydrogen and electron donors to neutralize DPPH free radicals [9,19]. When flavonoids react with DPPH radicals (purple), reduction to the non-radical hydrazine form (yellow) occurs, as observed in the spot test results [20-23]. These results provide initial validation of the potential of *Moringa oleifera* as a natural therapeutic agent. Given the central role of oxidative stress in various chronic diseases, Moringa leaf extract, rich in flavonoids, offers a promising natural resource for the development of nutraceuticals and plant-based therapies [21, 24-26].

## CONCLUSIONS

The ethanol extract of *Moringa oleifera* leaves was qualitatively proven to contain flavonoid compounds, which was confirmed thru the Shinoda test. The presence of these flavonoids strongly supports the extract's ability to exhibit potent free radical scavenging activity, validated thru the DPPH spot test. These findings provide a scientific basis for promoting and developing Moringa oleifera leaves as an important source of natural antioxidants.

## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## DECLARATION OF ARTIFICIAL INTELLIGENCE USE
This study used artificial intelligence (AI) tools and methodologies in the following capacities Manuscript writing support: AI-based language models, such as [for example, ChatGPT, Quillbot], were/was employed to: Language refinement (improving the grammar, sentence structure, and readability of the manuscript).

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