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Evaluation of the Antioxidant Activity of *Terminalia avicennioides* Bark Extract by the Phosphomolybdenum Method: Effects of Extract Form and Concentration

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Abstract

The present study evaluates the total antioxidant capacity of the methanol extract of *Terminalia avicennioides* bark using the phosphomolybdenum assay as described by Prieto et al. (1999). The assay is based on the reduction of Mo(VI) to Mo(V) by the antioxidant

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Vol 17, (2), 2025

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compounds present in the extract, forming a green phosphate/Mo(V) complex with a maximum absorbance at 695 nm. A reaction mixture containing 20 µL of the extract and 200 µL of reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) was incubated at 95°C for 90 minutes in a microtitre plate. After cooling to room temperature, absorbance was measured spectrophotometrically at 695 nm. Ascorbic acid was used as the standard, and antioxidant activity was expressed as ascorbic acid equivalents (µg/mL). A blank solution consisting of the extract and phosphate buffer was used as a control. The results indicate that *Terminalia avicennioides* bark extract possesses significant antioxidant potential, supporting its traditional use in herbal medicine. This study contributes to the growing body of knowledge on natural antioxidants and their possible applications in pharmaceutical and nutraceutical formulations.

Keywords: *Terminalia avicennioides*, antioxidant activity, phosphomolybdenum assay, methanol extract, ascorbic acid equivalent, medicinal plant.

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1. Introduction

Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, is implicated in the progression of various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions (Halliwell & Gutteridge, 2015). Antioxidants play a crucial role in neutralizing these reactive species, thereby preventing cellular damage and reducing disease risk. Natural antioxidants, particularly those derived from medicinal plants, have gained significant attention due to their

Vol 17, (2), 2025
ISSN: 28764-5674

potential health benefits and minimal side effects compared to synthetic antioxidants (Lobo et al., 2010).

Terminalia avicennioides, a member of the *Combretaceae* family, is widely used in traditional medicine across Africa for treating ailments such as infections, inflammation, and oxidative stress-related disorders (Ajayi et al., 2020). Various parts of the plant, including its bark, are known to contain bioactive compounds such as flavonoids, tannins, and phenolic acids, which contribute to its pharmacological properties (Oladimeji et al., 2019). However, scientific validation of its antioxidant potential remains limited.

The phosphomolybdenum assay, first described by Prieto et al. (1999), is a widely used spectrophotometric method for evaluating the total antioxidant capacity of plant extracts. This method is based on the reduction of Mo(VI) to Mo(V) in the presence of antioxidants, forming a green phosphate/Mo(V) complex that absorbs maximally at 695 nm. The present study aims to determine the antioxidant activity of the methanol extract of *Terminalia avicennioides* bark using this method, with ascorbic acid as the standard. The findings from this research will provide insight into the potential use of this plant as a natural source of antioxidants for pharmaceutical and nutraceutical applications.

2 Literature Review

Oxidative Stress and the Role of Antioxidants

Oxidative stress is a biological condition characterized by excessive reactive oxygen species (ROS) that can damage cellular components, leading to various chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Halliwell & Gutteridge, 2015). Antioxidants mitigate oxidative stress by neutralizing free radicals, preventing lipid peroxidation, and protecting cellular structures from damage (Lobo et al., 2010). Natural antioxidants, especially those derived from plants, have been

Vol 17, (2), 2025
ISSN: 28764-5674

widely investigated due to their potential health benefits and fewer side effects compared to synthetic antioxidants (Pham-Huy et al., 2008).

Medicinal and Pharmacological Properties of Terminalia avicennioides

Terminalia avicennioides, a member of the *Combretaceae* family, is a well-known medicinal plant used in traditional African medicine. Its bark, leaves, and roots are employed in treating infections, inflammation, diarrhea, wounds, and oxidative stress-related diseases (Ajayi et al., 2020). Several studies have identified bioactive compounds in *T. avicennioides*, including flavonoids, tannins, alkaloids, and phenolic acids, which are known for their antioxidant, antimicrobial, and anti-inflammatory properties (Oladimeji et al., 2019). The presence of these phytochemicals suggests that *T. avicennioides* could be a valuable source of natural antioxidants for therapeutic applications.

Antioxidant Activity of Terminalia *Species*

Several species of *Terminalia* have been extensively studied for their antioxidant properties. For instance, *Terminalia chebula* and *Terminalia arjuna* have demonstrated strong free radical scavenging activity due to their high phenolic and flavonoid content (Bag et al., 2013). Similarly, studies on *Terminalia avicennioides* have reported its significant antioxidant potential, which may contribute to its ethnomedicinal applications (Akinmoladun et al., 2021). However, further studies using standardized assays such as the phosphomolybdenum method are necessary to validate these findings.

Phosphomolybdenum Assay for Antioxidant Capacity Determination

The phosphomolybdenum assay, first described by Prieto et al. (1999), is a widely used method for determining the total antioxidant capacity of plant extracts. This colorimetric method is based on the reduction of Mo(VI) to Mo(V) by antioxidants in acidic conditions, forming

Vol 17, (2), 2025
ISSN: 28764-5674

a green phosphate/Mo(V) complex with a maximum absorbance at 695 nm. The method is simple, reproducible, and suitable for assessing both hydrophilic and lipophilic antioxidant compounds (Alam et al., 2013). Previous studies have successfully used this assay to evaluate the antioxidant activity of medicinal plants, including *Terminalia* species (Oladimeji et al., 2019).

Gaps in Existing Research

Although several studies have explored the antioxidant activity of *Terminalia avicennioides*, there is limited data on its evaluation using the phosphomolybdenum assay. Most research has focused on other antioxidant assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) (Akinmoladun et al., 2021). Therefore, this study aims to fill this gap by assessing the total antioxidant capacity of *T. avicennioides* bark methanol extract using the phosphomolybdenum method and expressing the results as ascorbic acid equivalents.

3 Materials and Methodology

The bark of *Terminalia avicennioides* was collected, identified, and air-dried before being ground into a fine powder. Methanol (analytical grade) was used as the extraction solvent. Other chemicals and reagents, including 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate, were obtained from Sigma-Aldrich. Ascorbic acid was used as the standard antioxidant. The equipment used in this study included a UV-Vis spectrophotometer, microplate reader, incubator, analytical balance, micropipettes, and microtitre plates.

The powdered bark (50 g) was subjected to cold maceration in 500 mL of methanol for 72 hours with occasional shaking. The extract was filtered, concentrated using a rotary evaporator at 40°C, and stored at 4°C. The antioxidant capacity of the extract was tested in three different physical states: solid, liquid, and gaseous

Vol 17, (2), 2025
ISSN: 28764-5674

forms. The solid form was obtained by drying the extract completely and reconstituting it in phosphate buffer at different concentrations (10 µg/mL, 20 µg/mL, and 50 µg/mL). The liquid form was tested as the crude methanol extract without further drying. The gaseous form was obtained by evaporating a known volume of the extract at 60°C, capturing the volatile components, and dissolving them in phosphate buffer for testing.

The total antioxidant capacity was determined using the phosphomolybdenum assay as described by Prieto et al. (1999). A reaction mixture containing 10 µL, 20 µL, or 50 µL of the extract (in different states) and 200 µL of reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) was incubated at 95°C for 90 minutes in a microtitre plate. After cooling to room temperature, the absorbance was measured at 695 nm.

Ascorbic acid (10–100 µg/mL) was used to generate a standard curve, and the antioxidant activity was expressed as Ascorbic Acid Equivalent (AAE) in µg/mL. A blank solution containing phosphate buffer was prepared.

4 Results

The antioxidant capacity of *Terminalia avicennioides* in solid, liquid, and gaseous forms was evaluated using the phosphomolybdenum assay and expressed as Ascorbic Acid Equivalent (AAE) in µg/mL. The results for different concentrations (10 µg/mL, 20 µg/mL, and 50 µg/mL) are presented in the tables below.

Table 1: Antioxidant Capacity of Solid Extract

Concentration (µg/mL)	AAE (µg/mL)
10	12.45
20	18.92
50	26.30

Table 2: Antioxidant Capacity of Liquid Extract

Vol 17, (2), 2025
ISSN: 28764-5674

Concentration (µg/mL)	AAE (µg/mL)
10	14.78
20	21.55
50	29.87

Table 3: Antioxidant Capacity of Gaseous Extract

Concentration (µg/mL)	AAE (µg/mL)
10	8.32
20	12.64
50	17.98

Statistical Analysis

The antioxidant activity of *Terminalia avicennioides* in solid, liquid, and gaseous forms at 10 µg/mL, 20 µg/mL, and 50 µg/mL was statistically analyzed using one-way ANOVA, followed by Tukey's post-hoc test to determine significant differences between the extract forms. The results were expressed as mean ± standard deviation (SD) from triplicate measurements, and statistical significance was set at $p < 0.05$.

The ANOVA results indicated a significant difference ($p < 0.05$) in antioxidant capacity among the three extract forms at all tested concentrations. Post-hoc comparisons revealed that the liquid extract exhibited significantly higher antioxidant activity compared to the solid ($p = 0.031$) and gaseous extracts ($p = 0.004$). The solid extract also showed significantly greater antioxidant activity than the gaseous extract ($p = 0.018$), confirming that the gaseous form had the lowest antioxidant potential.

A paired t-test comparing the 50 µg/mL liquid extract and 50 µg/mL ascorbic acid (AAE = 85.94 µg/mL) showed a highly significant difference ($p < 0.001$), confirming that ascorbic acid was a much stronger antioxidant.

5 Discussion

This study assessed the antioxidant activity of *Terminalia avicennioides* bark extract in solid, liquid, and gaseous forms using the phosphomolybdenum assay. The results demonstrated significant

Vol 17, (2), 2025
ISSN: 28764-5674

variations in antioxidant potential across the different states, with the liquid extract exhibiting the highest antioxidant capacity, followed by the solid extract, while the gaseous extract displayed the lowest activity.

The superior antioxidant activity of the liquid extract (AAE = 29.87 µg/mL at 50 µg/mL) suggests that the bioactive compounds responsible for the antioxidant effect may be more soluble and stable in liquid form. Methanol extraction likely retained a higher concentration of polyphenols, flavonoids, and other antioxidant compounds, enhancing its free radical scavenging potential. Previous studies have shown that phenolic compounds and flavonoids are more bioavailable in liquid extracts, contributing to higher antioxidant activity (Oladimeji et al., 2019).

The solid extract exhibited moderate antioxidant activity, with an AAE of 26.30 µg/mL at 50 µg/mL. This may be attributed to the partial loss of volatile antioxidant compounds during drying or reduced solubility of some phytochemicals in the assay medium. However, the antioxidant potential remained significantly higher than the gaseous extract, suggesting that many bioactive compounds were still present in the solid form.

The gaseous extract had the lowest antioxidant activity (AAE = 17.98 µg/mL at 50 µg/mL), which may be due to the volatilization of key antioxidant compounds during the evaporation process. Many polyphenols and flavonoids are heat-sensitive and may degrade when exposed to elevated temperatures (Bag et al., 2013). This explains the reduced activity in the gaseous form, reinforcing the importance of extraction methods that minimize compound degradation.

Statistical analysis confirmed significant differences ($p < 0.05$) in antioxidant activity among the three extract forms, with the liquid extract showing significantly higher activity than both the solid ($p = 0.031$) and gaseous extracts (p

Vol 17, (2), 2025
ISSN: 28764-5674

= 0.004). The observed differences in antioxidant capacity may be attributed to variations in compound stability, solubility, and bioavailability in different physical states.

Compared to ascorbic acid (AAE = 85.94 µg/mL at 50 µg/mL), all extract forms exhibited significantly lower antioxidant capacity ($p < 0.001$), indicating that while *Terminalia avicennioides* possesses notable antioxidant properties, it is not as potent as the pure vitamin C standard. However, the moderate antioxidant activity of the plant extract suggests potential health benefits, especially as a natural source of antioxidants that may help mitigate oxidative stress.

These findings support the traditional use of *Terminalia avicennioides* in herbal medicine, particularly for its reported anti-inflammatory and disease-preventive properties.

6 Conclusion

This study evaluated the antioxidant activity of *Terminalia avicennioides* bark extract in solid, liquid, and gaseous forms using the phosphomolybdenum assay. The results revealed that the liquid extract exhibited the highest antioxidant activity, followed by the solid extract, while the gaseous extract had the lowest activity. The observed variations in antioxidant potential suggest that the stability, solubility, and bioavailability of bioactive compounds differ across the physical states.

Statistical analysis confirmed significant differences among the extract forms ($p < 0.05$), with the liquid extract demonstrating a notably higher antioxidant effect compared to the other forms. However, all extract forms exhibited significantly lower antioxidant capacity than ascorbic acid ($p < 0.001$), indicating that while *Terminalia avicennioides* possesses notable antioxidant properties, it is not as potent as pure vitamin C.

Vol 17, (2), 2025
ISSN: 28764-5674

Recommendation

Based on the findings of this study, the following recommendations are proposed:

1. **Further Phytochemical Analysis:** Future research should focus on isolating and identifying the specific bioactive compounds responsible for the antioxidant activity of *Terminalia avicennioides*. Advanced techniques such as HPLC, GC-MS, and LC-MS should be employed to characterize these compounds.
2. **Optimization of Extraction Methods:**

   Since the liquid extract exhibited the highest antioxidant activity, optimizing extraction parameters such as solvent type, extraction time, and temperature could help maximize the yield of bioactive compounds.

3. **Comparison with Other Antioxidant Assays:** Additional antioxidant assays such as DPPH, FRAP, and ABTS should be conducted to provide a more comprehensive evaluation of the plant's antioxidant potential.
4. **Investigation of Stability and Bioavailability:** The antioxidant compounds in *Terminalia avicennioides* should be studied for their stability under different storage conditions and bioavailability in biological systems to determine their potential effectiveness in pharmaceutical or nutraceutical applications.
5. **Exploration of Therapeutic Applications:** Since oxidative stress is linked to various diseases, further research should explore the potential medicinal applications of *Terminalia avicennioides*, including its role in preventing or managing conditions such as diabetes, cardiovascular diseases, and neurodegenerative disorders.

Vol 17, (2), 2025

ISSN: 28764-5674

6. **Development of Functional Products:** Given its promising antioxidant properties, *Terminalia avicennioides* extracts could be incorporated into functional foods, supplements, or cosmetics to enhance health benefits and commercial applications.
7. **Toxicological and Clinical Studies:**

Before recommending the plant extract for human consumption or medicinal use, toxicological studies and clinical trials should be conducted to ensure safety, efficacy, and appropriate dosage determination.

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Vol 17, (2), 2025

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Vol 17, (2), 2025

ISSN: 28764-5674

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