

*Evaluation of Antioxidant Activity of Methanol Extract of Neem Leaves (*Azadirachta indica*) Using DPPH, ABTS, and FRAP Methods*

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Abstract

Introduction: *Azadirachta indica* leaves are rich in bioactive compounds with antioxidant potential. **Aims:** To assess the antioxidant activity of methanolic neem leaf extract using three in vitro methods DPPH, ABTS, and FRAP and to identify its major secondary metabolites. **Method:** Extraction was performed using Ultrasound-Assisted Extraction (UAE) with methanol as the solvent. Qualitative phytochemical screening was conducted to determine the presence of key antioxidant-related compounds. Antioxidant activity was evaluated using a microplate reader, and IC_{50} values were calculated based on the inhibition percentage across several concentrations. **Results:** Phytochemical analysis confirmed the presence of flavonoids, phenolics, terpenoids, and alkaloids. The extract showed strong antioxidant activity in the DPPH assay, with an IC_{50} value of 54.91 ppm. However, the ABTS and FRAP assays did not yield measurable IC_{50} or RP_{50} values, even at concentrations up to 1000 ppm, suggesting limited electron transfer-based antioxidant capacity. These results indicate that the extract's primary antioxidant mechanism is likely through hydrogen atom transfer (HAT), rather than single electron transfer (SET). **Conclusion** Methanolic extract of *A. indica* leaves exhibits strong free radical scavenging activity through the HAT mechanism, as shown in the DPPH assay. This activity is presumably due to the presence of flavonoids and phenolics.

Keywords : *Azadirachta indica*, antioxidant, DPPH, ABTS, FRAP

Abstrak

Pendahuluan: Daun Mimba (*Azadirachta indica* A. Juss) dikenal mengandung berbagai senyawa bioaktif yang berpotensi sebagai antioksidan alami. **Tujuan penelitian:** untuk mengevaluasi aktivitas antioksidan ekstrak metanol daun Mimba secara simultan menggunakan tiga metode in vitro yang berbeda, yaitu DPPH, ABTS, dan FRAP, serta mengidentifikasi senyawa metabolit sekundernya. **Metode:** Ekstraksi dilakukan menggunakan metode *Ultrasound-Assisted Extraction* (UAE) dengan pelarut metanol dengan perbandingan 1:20 dan dipisahkan dengan rotary evaporator. Selanjutnya dilakukan skrining fitokimia. Uji potensi antioksidan dilakukan via DPPH, ABTS, dan FRAP dengan pengukuran absorbansi microplate reader. IC_{50} dihitung menggunakan regresi non linier berdasarkan data % inhibisi terhadap konsentrasi sampel. **Hasil:** Hasil skrining fitokimia menunjukkan bahwa ekstrak metanol daun Mimba mengandung flavonoid, fenolik, alkaloid dan terpenoid. Ekstrak metanol daun Mimba memiliki aktivitas antioksidan yang tergolong kuat pada metode DPPH, dengan nilai IC_{50} sebesar 54,91 ppm. Namun, pada metode ABTS dan FRAP, aktivitas antioksidan tidak mencapai ambang yang memadai untuk menghitung nilai IC_{50} atau RP_{50} , bahkan pada konsentrasi 1000 ppm. **Kesimpulan:** Ekstrak metanol daun Mimba memiliki potensi antioksidan yang signifikan, terutama melalui mekanisme HAT. Senyawa flavonoid dan fenolik yang terdeteksi diyakini merupakan kontributor utama terhadap aktivitas tersebut.

Kata kunci : *Azadirachta indica*, antioksidan, DPPH, ABTS, FRAP

I. INTRODUCTION

Oxidative stress is a pathological condition arising from an imbalance between the production of free radicals and the body's antioxidant system's ability to neutralize them.¹ Free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are known to contribute to the damage of essential biomolecules like DNA, proteins, and lipids. They are also strongly linked to the development of chronic degenerative diseases including cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders.² Given the high mortality rate from non-communicable diseases, which exceeds 41 million annually, preventive interventions through increased antioxidant intake have become crucial.^{3,4}

Antioxidants neutralize free radicals through various mechanisms, such as electron donation and oxidation inhibition, originating from both endogenous and exogenous sources. However, in pathological situations or under high stress exposure, the body requires additional antioxidants from external sources.⁵ Natural antioxidants from plants are now the primary choice due to concerns over the toxicity of synthetic antioxidants like BHA, BHT, and TBHQ, which pose carcinogenic risks and can disrupt lipid metabolism.⁶ Global trends indicate a significant increase in the use of plant-based antioxidants as a safer approach for health prevention and food preservation.⁷

Azadirachta indica A. Juss (Neem) is a tropical plant from the Meliaceae family widely recognized in traditional medicine for its diverse pharmacological properties, including antibacterial, antidiabetic, anticancer, and antioxidant effects.^{8,9} Nearly all parts of this plant are utilized, especially its leaves, which are rich in bioactive compounds such as flavonoids, phenolics, terpenoids, and alkaloids.^{8,10} Flavonoids like quercetin and kaempferol are known to possess phenolic groups that effectively

scavenge free radicals and stabilize ROS.¹¹ The presence of these bioactive compounds forms the scientific basis for various studies evaluating the antioxidant activity of Neem leaves, particularly through extraction using polar solvents like methanol.

Previous studies have reported significant antioxidant activity from Neem leaf extracts using various organic solvents. Among commonly used solvents, methanol is often chosen for its efficiency in extracting phenolic compounds and flavonoids.^{12,13} The flavonoid and other phenolic compounds in the methanol extract of Neem leaves are believed to play a significant role in free radical scavenging activity. Widodo et al. reported that methanol extracts of *Baccaurea racemosa* and *Macaranga subpeltata* leaves, also rich in phenolic compounds, showed high antioxidant activity, supporting a similar approach for the Neem plant.¹³ Furthermore, Sharma et al. demonstrated that methanol solvent yielded higher flavonoid content compared to ethanol and ethyl acetate in the extraction of medicinal plant leaves, implying a positive correlation between polar solvents and antioxidant activity.¹² Therefore, further evaluation with a more comprehensive and methodologically valid approach is essential, particularly through multi-method testing to obtain a complete overview of the extract's antioxidant capacity.

Considering the complexity of antioxidant mechanisms and the importance of comprehensive validation, this study proposes a simultaneous evaluation of the antioxidant potential of *Azadirachta indica* leaf methanol extract using three different *in vitro* methods: DPPH, FRAP, and ABTS. This multi-method approach is crucial for providing a more holistic and accurate picture of the extract's antioxidant capacity, encompassing its free radical scavenging ability, metal ion reducing power, and cationic radical scavenging capability. Additionally, comparison with standard

antioxidants like ascorbic acid and gallic acid will provide clear quantitative benchmarks regarding the extract's effectiveness. Thus, this research is expected to strengthen the scientific basis of Neem leaf's antioxidant potential and pave the way for the future development of natural raw materials in pharmaceutical and nutraceutical applications.

II. METHODS

Materials

Azadirachta indica simplicia powder was obtained from Balai Materia Medika Batu with identification number 000.9.3/2681/102.20/2025. Reagents for the ABTS antioxidant activity test included 2,2-azinobis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS), Potassium Persulfate ($K_2S_2O_8$), and Gallic Acid as a standard. For the DPPH test, the reagents used were 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ascorbic Acid as a standard. For the FRAP test, the reagents prepared were Ferric Chloride Hexahydrate ($Fe(III) 6H_2O$), 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ), 37% Hydrochloric Acid (HCl), Sodium Acetate, Glacial Acetic Acid, and Gallic Acid as a standard. Reagents for qualitative phytochemical screening included 2N Hydrochloric Acid (HCl), Wagner's reagent, Mayer's reagent, Ethanol, 5% and 10% Ferric Chloride ($FeCl_3$), N-Hexane, and concentrated Sulfuric Acid.

Preparation of Neem Leaf Extract

Neem leaf extraction was performed using the Ultrasound-Assisted Extraction (UAE) method with methanol as the solvent. A total of 200 g of dried neem leaf simplicia powder was used, with an optimal sample-to-solvent ratio of 1:20 methanol. After the extraction process, the mixture was filtered using Whatman filter paper and then evaporated using a rotary evaporator to obtain a viscous extract.

Phytochemical Screening

This method followed a validated and widely used standard protocol in phytochemical research.¹⁴

- **Saponins (Foam Test):** A small amount of extract was dissolved in hot water and shaken vigorously. The formation of persistent foam for at least 10 minutes indicated the presence of saponins.
- **Alkaloids (Wagner and Mayer Tests):** The extract sample was acidified with 1 mL of 2N HCl and 9 mL of water, heated, and then filtered. The filtrate was divided into two parts: one part was added 2 drops of Wagner's reagent (positive indication: reddish-brown precipitate), and the other part was added 2 drops of Mayer's reagent (positive indication: white precipitate).
- **Flavonoids (Shinoda Test/Mg-HCl Reduction):** The extract sample was dissolved in ethanol and homogenized. From the filtrate, 1 mL was taken and a few small pieces of magnesium ribbon were added, followed by the dropwise addition of concentrated hydrochloric acid. The formation of a pink to crimson-red color after a few minutes indicated the presence of flavonoids.
- **Phenolics (Ferric Chloride Test):** The extract sample was dissolved in ethanol and homogenized. From the filtrate, 1 mL was taken and 5% $FeCl_3$ solution was added. The formation of a dark green, bluish-black, or purple solution indicated the presence of phenolic compounds.
- **Tannins (Ferric Chloride Test):** The extract sample was dissolved in hot water and homogenized. From the filtrate, 1 mL was taken and 3 mL of distilled water and 3 drops of 10% $FeCl_3$ solution were added. The formation of a bluish-black or greenish-black solution indicated the presence of tannins.
- **Steroids & Terpenoids (Lieberman-Burchard Test):** The extract sample was dissolved in a 1:1 mixture of DMSO/distilled water and N-Hexane, homogenized, and left to stand until two layers formed. The filtrate from the upper

layer was taken and 3 drops of concentrated HCl and 1 drop of concentrated H₂SO₄ were added. A color change from violet to blue/green indicated the presence of steroids, while a reddish-brown color indicated terpenoids.

- **Triterpenoids (Salkowski's Test):** The extract sample was dissolved in a 1:1 mixture of DMSO/distilled water and N-Hexane, homogenized, and left to stand until two layers formed. The upper layer was taken and 5 drops of concentrated H₂SO₄ were added. The formation of a golden yellow layer indicated the presence of triterpenoids.

Antioxidant Activity Test

The DPPH, ABTS, and FRAP assays were selected to provide a comprehensive evaluation of antioxidant capacity, as each represents a distinct mechanism: DPPH reflects the Hydrogen Atom Transfer (HAT) mechanism, whereas ABTS and FRAP reflect the Single Electron Transfer (SET) mechanism. All assays were performed in triplicate to ensure reproducibility and reliability of the results.

DPPH Method

A 0.1 mM DPPH working solution was prepared from a 1 mM stock solution (39.4 mg/100 mL methanol) and stored in dark, cold conditions. Ascorbic acid standard solutions were prepared from a 1000 ppm concentration and serially diluted down to 0.39 ppm. Subsequently, the sample stock solution was diluted to several concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.63 ppm). Eighty µL of sample or standard solution was mixed with 80 µL of DPPH solution in a 96-well microplate and incubated for 30 minutes at 25°C in the dark. Absorbance was read at 492 nm on a microplate reader. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = ((\text{Absorbance of blank} - \text{Absorbance of sample}) / \text{Absorbance of blank}) \times 100$$

The IC₅₀ value was obtained from the non-linear regression curve between concentration and % inhibition. All tests were performed in triplicate to ensure data reliability.

FRAP Method

The FRAP assay was performed by mixing a freshly prepared and light-protected FRAP DYE working solution (300 mM sodium acetate, TPTZ, and FeCl₃·6H₂O; 10:1:1). Gallic acid standard (1000 ppm) was serially diluted in methanol. The sample stock solution was diluted to several concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.63 ppm). Twenty µL of sample or standard solution was added to a 96-well plate, followed by the addition of 280 µL of FRAP DYE solution, and incubated for 10 minutes in the dark at room temperature. Absorbance was measured at 492 nm using a microplate reader. All tests were performed in triplicate. A standard regression curve was generated using a non-linear regression model. Reducing Power (%) was calculated using the following formula:

$$\% \text{ Reducing Power} = ((\text{Absorbance of sample} - \text{Absorbance of blank}) / \text{Maximum standard absorbance}) \times 100$$

The RP₅₀ value (concentration providing 50% reduction activity) was obtained by interpolation from the sample regression curve.

ABTS Method

The ABTS⁺ solution was prepared by reacting ABTS (19.2 mg/5 mL distilled water) with 88 µL of potassium persulfate (37.8 mg/mL), incubated in the dark for 18–22 hours at room temperature. The working solution was diluted with ethanol until the absorbance was 0.7 ± 0.02 (at 630 nm). Gallic acid (1000 ppm) and sample extract (100,000 ppm in DMSO) were diluted to several concentrations. Ten µL of sample or standard was added to a 96-well plate, then 290 µL of ABTS working solution was

added. Incubation was performed for 6 minutes in the dark at room temperature. Absorbance was measured at 630 nm using a microplate reader, and all tests were performed in triplicate. If the sample was highly colored, a sample blank correction was applied. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = ((\text{Absorbance of blank} - \text{Absorbance of sample}) / \text{Absorbance of blank}) \times 100$$

A regression curve was constructed from standard and sample data, with an R^2 of at least 0.95. The IC_{50} value was obtained from the regression equation representing the sample concentration required to inhibit 50% of ABTS radicals.

Statistical Analysis

Antioxidant activity values for each sample were reported as the mean of all measurements accompanied by standard deviation (SD) using GraphPad Prism. The free radical inhibition results by the extract were then analyzed using non-linear regression to determine IC_{50} and RP_{50} values.

III. RESULTS

Figure 1 displays the calibration curves for the three antioxidant potential measurement methods: DPPH (a), FRAP (b), and ABTS (c). Each curve shows a linear relationship between the concentration of the standard antioxidant solution and the obtained results, expressed as percentage inhibition for DPPH and ABTS, and reducing power for FRAP. All calibration curves exhibited a coefficient of determination (R^2) value of ≥ 0.9996 . This indicates that the three methods employed for measuring the antioxidant potential of the neem leaf extract possess high precision.

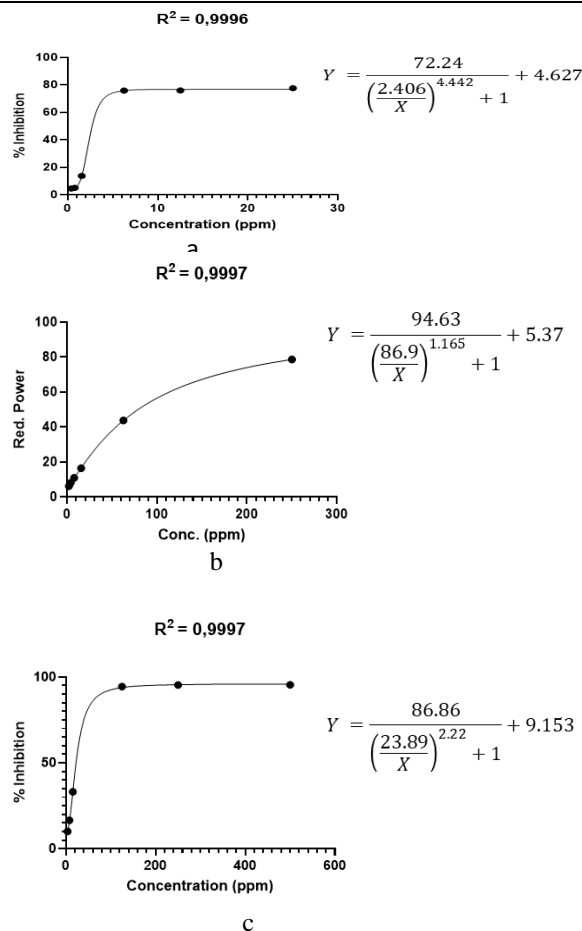


FIGURE 1. CALIBRATION CURVES FOR ANTIOXIDANT ASSAYS USING VARIOUS METHODS: A. DPPH, B. FRAP, C. ABTS.

TABLE 1. ANTIOXIDANT ASSAY OF AZADIRACHTA INDICA LEAF METHANOL EXTRACT BY DPPH METHOD.

Sampl	Concentrat ion (ppm)	% inhibition	IC_{50} (ppm)
Ascorbic Acid	25,00	77,64 ± 0,39	2,71
	12,50	75,99 ± 0,62	
	6,25	75,90 ± 0,37	
	1,56	13,82 ± 0,52	
	0,78	5,12 ± 0,86	
	0,39	4,64 ± 0,41	
Azadirachta indica Leaves extract	1.000,00	90,86 ± 0,71	54,91
	500,00	87,58 ± 0,76	
	250,00	86,80 ± 1,26	
	125,00	74,03 ± 0,65	
	62,50	52,06 ± 0,78	
	31,25	39,14 ± 0,49	
	15,63	27,87 ± 0,79	

Table 1 presents the results of the antioxidant activity testing of the methanol extract from neem leaves using the DPPH method, with ascorbic acid as a positive

control. The percentage inhibition value of the neem leaf methanol extract increased with increasing concentration. Although the maximum percentage inhibition value of the extract approached that of ascorbic acid at high concentrations, a significantly larger dose was required to achieve a similar effect.

TABLE 2. ANTIOXIDANT ASSAY OF AZADIRACHTA INDICA LEAF METHANOL EXTRACT BY FRAP METHOD.

Sampel	Concentration (ppm)	% Reducing Power	RP ₅₀ (ppm)
Gallic Acid	250,00	78,60 ± 0,11	78,83
	62,50	43,75 ± 0,50	
	15,63	16,43 ± 0,96	
	7,81	10,99 ± 0,18	
	3,91	8,10 ± 0,16	
	1,95	6,21 ± 0,10	
<i>Azadirachta indica</i> Leaves extract	1.000,00	10,96 ± 0,13	-
	500,00	7,14 ± 0,11	
	250,00	5,37 ± 0,07	
	125,00	4,49 ± 0,14	
	62,50	4,07 ± 0,04	
	31,25	3,66 ± 0,05	
	15,63	3,56 ± 0,02	

TABLE 3. ANTIOXIDANT ASSAY OF AZADIRACHTA INDICA LEAF METHANOL EXTRACT BY ABTS METHOD.

Sampel	Concentration (ppm)	% Reducing Power	RP ₅₀ (ppm)
Gallic Acid	500,00	95,50 ± 0,25	22,64
	250,00	95,41 ± 0,20	
	125,00	94,46 ± 0,18	
	15,63	33,21 ± 0,87	
	7,81	16,66 ± 0,57	
	3,91	10,14 ± 0,78	
<i>Azadirachta indica</i> Leaves extract	1.000,00	25,34 ± 2,00	-
	500,00	15,87 ± 0,09	
	250,00	13,36 ± 0,27	
	125,00	9,87 ± 0,24	
	62,50	8,70 ± 0,15	
	31,25	9,47 ± 0,16	
	15,63	6,75 ± 0,21	

Tables 2 and 3 present the antioxidant activity data of the methanol extract from neem leaves using the FRAP and ABTS methods, with gallic acid as a positive control. Gallic acid demonstrated very high reducing activity and radical scavenging ability, evidenced by RP₅₀ values of 78.83 ppm (FRAP) and 22.64 ppm (ABTS), respectively, and a maximum inhibition

percentage exceeding 95% within the concentration range of 250–500 ppm. On the other hand, the methanol extract of neem leaves showed relatively limited reducing and inhibitory activities, with maximum percentage values of 10.96 ± 0.13% (FRAP) and 25.34 ± 2.00% (ABTS) at a concentration of 1000 ppm, without reaching a statistically measurable RP₅₀ threshold.

TABLE 4. PHYTOCHEMICAL SCREENING OF AZADIRACHTA INDICA LEAF METHANOL EXTRACT

Test Parameter	Positive Indication	Result
Saponins	Persistent foam formation	Negative
Alkaloids	Reddish-brown precipitate (Wagner) / White precipitate (Mayer)	Positive
Flavonoids	Pink / crimson-red color	Positive
Phenolics	Dark green / bluish-black / purple color	Positive
Tannins	Bluish-black / greenish-black color	Negative
Steroids	Greenish-purple / brown color	Negative
Terpenoids	Reddish-brown color	Positive
Triterpenoids	Golden yellow layer	Negative

The qualitative phytochemical screening results of the methanol extract of Neem leaves showed positive indications for alkaloids, flavonoids, phenolics, and terpenoids, as presented in Table 4. This profile provides an initial overview of the extract's chemical composition that potentially contributes to its biological activity.

IV. DISCUSSION

In vitro antioxidant activity testing aims to evaluate the extract's ability to neutralize free radicals through two main mechanisms: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET).¹⁵ The HAT mechanism involves the donation of a hydrogen atom from an antioxidant compound to inhibit free radical chain reactions, while the SET mechanism works

by transferring a single electron from an antioxidant compound to a free radical, thereby converting it into a more stable and non-reactive form.^{16,17} One common approach to evaluate antioxidant activity based on these mechanisms is the DPPH method, which operates on the HAT principle.¹⁸ The DPPH method measures the ability of antioxidant compounds to donate hydrogen atoms to DPPH radicals, characterized by a color change from purple to yellow.¹⁹

Subsequently, to obtain a quantitative overview of the observed antioxidant activity, data from the assays were analyzed by constructing calibration curves for each testing method, as shown in **Figure 1**. After obtaining the calibration curves, the next step was to calculate the IC₅₀ values to determine the extract's effectiveness in neutralizing free radicals. The IC₅₀ value is a crucial quantitative indicator for assessing the strength of the antioxidant activity of the tested extract. **Table 1** shows that the methanol extract of Neem leaves has an IC₅₀ value of 54.91 ppm, which is categorized as a strong antioxidant.²⁰ For comparison, the ascorbic acid antioxidant standard showed an IC₅₀ of 2.71 ppm, indicating significantly higher antioxidant activity. Generally, a lower IC₅₀ value signifies higher antioxidant potential of a compound, as a smaller amount is capable of neutralizing 50% of free radicals.²¹ Based on this, the DPPH activity of the Neem leaf extract indicates that its active compounds primarily act through the HAT (Hydrogen Atom Transfer) mechanism.

To complement the DPPH assay, which is based on the HAT mechanism, the FRAP (Ferric Reducing Antioxidant Power) method, representing the SET mechanism, was also performed. This method measures the antioxidant's ability to reduce the Fe³⁺-TPTZ complex to Fe²⁺-TPTZ, indicated by the formation of an intense blue color.^{22,23} Based on Table 2, the methanol extract of

Neem leaves showed relatively low reducing activity, with the RP₅₀ value not measurable up to a concentration of 1000 ppm, meaning the extract's ability to reduce iron ions is very limited. Conversely, the gallic acid standard showed an RP₅₀ value of 78.83 ppm, reflecting a much higher reductive potential. These findings indicate that the antioxidant activity of Neem leaf extract is more dominant through the HAT mechanism than SET, suggesting that the active compounds likely act through hydrogen atom donation rather than electron transfer.

To further strengthen these results, the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) method, which also evaluates the SET mechanism, was conducted. In this method, antioxidant activity is measured based on its ability to reduce the ABTS⁺ radical, indicated by a decrease in absorbance at a specific wavelength.²⁴ Based on the results in **Table 3**, the methanol extract of Neem leaves showed a maximum reducing activity of 25.34%, but the RP₅₀ value was not achieved even at a concentration of 1000 ppm. Conversely, the gallic acid standard showed an RP₅₀ value of 22.64 ppm, indicating high efficiency in the SET mechanism. The consistency between the FRAP and ABTS assay results further reinforces that the primary antioxidant mechanism of Neem leaf extract is HAT, not SET. If an extract works via HAT, it indicates protective ability against lipid peroxidation, a major cause of cell membrane damage in oxidative stress. HAT reactions are direct and fast because they do not require the formation of radical ion intermediates. This is highly beneficial in situations of acute oxidative stress, such as post-stroke, sepsis, or other diseases like cardiovascular, neurodegenerative, and cancer, where cell membrane damage is the primary pathophysiology.²⁵

The positively identified presence of flavonoids and phenolics in the methanol extract of Neem leaves strongly supports the

observed antioxidant activity, especially in the DPPH assay. These compounds have chemical structures with abundant hydroxyl groups, allowing them to effectively donate hydrogen atoms to stabilize free radicals, which is a key mechanism in the DPPH assay.^{26,27} Flavonoids have been extensively studied and are known as potent antioxidants with various mechanisms of action, such as direct radical scavenging, inhibition of reactive oxygen species (ROS) formation, and enhancement of endogenous antioxidant enzyme activity.^{28,29}

In addition to flavonoids, other phenolic compound groups also make significant contributions to antioxidant activity. These compounds can donate hydrogen atoms, stabilize free radicals through electron delocalization in resonance structures, act as reducing agents, radical scavengers, and chelators of pro-oxidant metals.^{27,30} Although alkaloids and terpenoids were also detected, their specific contribution to the total antioxidant activity may vary depending on their specific structure and concentration in the extract. Some studies indeed report antioxidant activity from alkaloids and terpenoids, but their contribution is often lower compared to phenolics and flavonoids.^{31,33} Therefore, it can be concluded that flavonoids and phenolics are the main contributors to the antioxidant activity of this extract.

V. CONCLUSION

The present study demonstrated that the methanolic extract of Neem (*Azadirachta indica*) leaves possesses strong antioxidant potential, predominantly acting through the Hydrogen Atom Transfer (HAT) mechanism, as indicated by an IC_{50} value of 54.91 ppm in the DPPH assay. The antioxidant activity is primarily attributed to the presence of flavonoid and phenolic compounds identified in the extract. These results highlight the extract's potential for development as a natural antioxidant source in pharmaceutical

or nutraceutical applications. To further substantiate its pharmacological relevance, future studies should aim to isolate and characterize the major active constituents and evaluate their efficacy and safety in *in vivo* models

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