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## Evaluation of In-vitro antioxidant activities of *Phyllanthus reticulatus* extract

Deepinderjeet Kaur<sup>1</sup>, Rajkumar Mathur<sup>2</sup><sup>1</sup>Research scholar, Maulana Azad University, Jodhpur, India<sup>2</sup>Research Supervisor, Maulana Azad University, Jodhpur, India

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Corresponding author: Deepinderjeet Kaur

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### Abstract:

Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, plays a crucial role in the pathogenesis of various chronic diseases including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions. Medicinal plants are rich sources of natural antioxidants that can neutralize free radicals and reduce oxidative damage. *Phyllanthus reticulatus*, a traditionally used medicinal plant, has been reported to possess several pharmacological properties. The present study aims to evaluate the **in-vitro antioxidant activity** of *Phyllanthus reticulatus* extract using standard antioxidant assays. The plant material was extracted using suitable solvents, and the antioxidant potential was assessed through free radical scavenging methods such as DPPH and hydrogen peroxide scavenging. The results demonstrated a concentration-dependent antioxidant activity of the extract, indicating its significant free radical scavenging potential. The findings of this study support the traditional use of *Phyllanthus reticulatus* and suggest that it may serve as a promising source of natural antioxidants for therapeutic applications.

**Keywords:** *Phyllanthus reticulatus*, Antioxidant activity, Free radicals, In-vitro evaluation, Medicinal plants

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### Introduction

Free radicals are unstable molecules generated during normal metabolic processes and environmental exposures such as pollution, radiation, and chemicals. Excessive production of reactive oxygen species (ROS) leads to oxidative stress, which can damage cellular components including lipids, proteins, and nucleic acids. Oxidative stress has been implicated in the development of various pathological conditions such as aging, inflammation, cancer, cardiovascular diseases, and neurodegenerative disorders. Antioxidants play a vital role in neutralizing free radicals

and protecting the body from oxidative damage.

Although synthetic antioxidants are widely used, their long-term use has raised safety concerns. Therefore, there is growing interest in identifying natural antioxidants from plant sources due to their effectiveness, safety, and availability. Medicinal plants have been traditionally used as remedies for numerous ailments and are known to contain bioactive compounds such as flavonoids, phenolics, tannins, and alkaloids, which exhibit potent antioxidant properties.

*Phyllanthus reticulatus* Poir., belonging to the family Euphorbiaceae, is a well-known medicinal plant distributed in tropical and subtropical regions of India. Traditionally, different parts of the plant have been used for the treatment of inflammation, liver disorders, diabetes, and microbial infections. Phytochemical investigations have reported the presence of polyphenolic compounds in *Phyllanthus reticulatus*, suggesting its potential antioxidant activity.

In this context, the present research work is designed to evaluate the in-vitro antioxidant activity of *Phyllanthus reticulatus* extract using various standard antioxidant assays. The study aims to provide scientific evidence supporting the antioxidant potential of this plant and to explore its possible application as a natural source of antioxidants in pharmaceutical formulations.

#### **Materials and method:**

The aerial parts of *Phyllanthus reticulatus* were selected for the proposed study. The plant material was collected from local area. After collection, the plant parts were thoroughly cleaned to remove dirt and other foreign matter. The cleaned plant material was then dried under shade to prevent degradation of phytoconstituents and preserved for further use.

After drying in sunlight, the plant material was ground into a coarse powder and initially macerated with petroleum ether for defatting. The extraction process was continued until the material was completely free from fatty substances. Subsequently, an exhaustive extraction was carried out on 30 g of the dried aerial parts of *Phyllanthus reticulatus* using the maceration method with a hydroalcoholic solvent (ethanol: water, 70:30).

#### **In-vitro antioxidant activity**

##### **DPPH radical scavenging assay**

Guchu et al. described a method for estimating the DPPH radical scavenging activity, which was slightly modified for the present study. Extract samples of varying concentrations (50, 100, 150, 200, and 250 µg/mL) were prepared for the assay. Methanol was used as the solvent for the preparation of all extract samples. A standard solution of ascorbic acid at concentrations similar to those of the test samples was also prepared.

In a clean test tube, 1 mL of the test sample was mixed with 0.5 mL of 0.3 mM methanolic DPPH solution and stirred thoroughly to ensure complete mixing. The prepared solutions were then kept at room temperature in the dark for 15 minutes to allow the reaction to occur.

The control solution consisted of 1 mL methanol mixed with 2.5 mL DPPH solution. The absorbance of the samples was measured at 517 nm using a UV-visible spectrophotometer. The percentage of DPPH radical scavenging (inhibitory) activity was calculated using the following

$$\% \text{Radical inhibiting activity} = \frac{C - S}{C} \times 100$$

C-Amount of absorbance by the control solution

S-Amount of absorbance by the extract/standard solution

The IC<sub>50</sub> value of the extract sample and the standard solution was measured. Three replicates of each experiment were performed and the mean values of the results were calculated formula (Guchu et al., 2020; Adebiyi et al., 2017).

#### **Inhibitory Effects of the Extract on Hydroxyl Radical**

The inhibitory activity of the hydroxyl radical was measured using a modified

method described by Gupta et al. The test samples were prepared by adding 1 mL of each extract concentration (50, 100, 150, 200, and 250 µg/mL) to a reaction mixture containing 0.1 mL EDTA (1 mM), 0.36 mL deoxyribose, 0.01 mL FeCl<sub>3</sub> (10 mM), 0.1 mL H<sub>2</sub>O<sub>2</sub> (10 mM), and 0.33 mL phosphate buffer (50 mM, pH 7.9). A standard solution of ascorbic acid with concentrations similar to those of the test samples was also prepared.

The reaction mixtures were incubated at 37 °C for 1 hour. After incubation, 1 mL of the reaction mixture was treated with 1 mL of 10% trichloroacetic acid (TCA) and 1 mL of 0.5% thiobarbituric acid (TBA). The resulting mixtures were then heated, cooled, and the absorbance was measured at 532 nm using a UV–visible spectrophotometer.

The percentage of hydroxyl radical scavenging activity was calculated using the following equation (Gupta et al., 2007; Sivagamasundari et al., 2021):

$$\text{Percentage inhibition} = \frac{C - SC}{C} \times 100$$

Where:

C = Absorbance of the control solution

S = Absorbance of the extract or standard solution

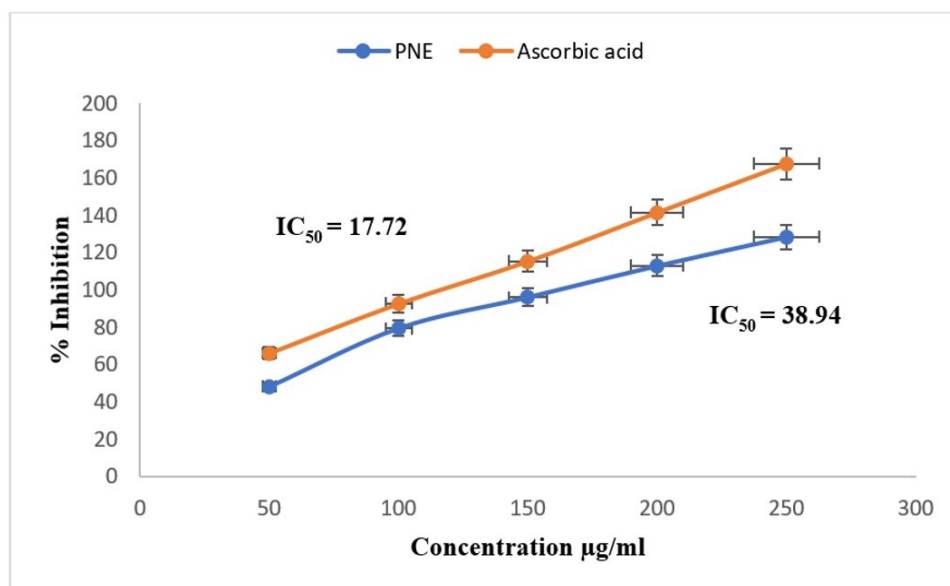
The percentage of hydroxyl radical scavenging activity of the extract or standard represents the inhibition of deoxyribose degradation. The IC<sub>50</sub> value of the extract and the standard solution was determined. All experiments were performed in triplicate, and the results were expressed as mean values.

## Results and discussion

### DPPH Radical scavenging activity

Results shows the DPPH radical scavenging activity of *Phyllanthus reticulatus* extract (PE) compared with ascorbic acid. The extract exhibited a concentration-dependent increase in DPPH scavenging activity. Although ascorbic acid showed higher antioxidant activity at all tested concentrations, PNE demonstrated significant free radical scavenging potential. The IC<sub>50</sub> value of PNE (38.94 µg/mL) indicates good antioxidant activity, supporting its potential as a natural antioxidant source.

Concentration (µg/ml)	DPPH Scavenging%	
	PNE	Ascorbic acid
50	48.2±0.24	66.1±0.63
100	79.5±0.57	92.7±0.81
150	96.1±0.14	115.4±0.26
200	112.9±0.51	141.6±0.18
250	128.3±0.47	167.7±0.59
IC <sub>50</sub>	38.94	17.72



Scavenging effect of Phyllanthus reticulatus on DPPH radicals

### Hydroxyl Ion scavenging activity

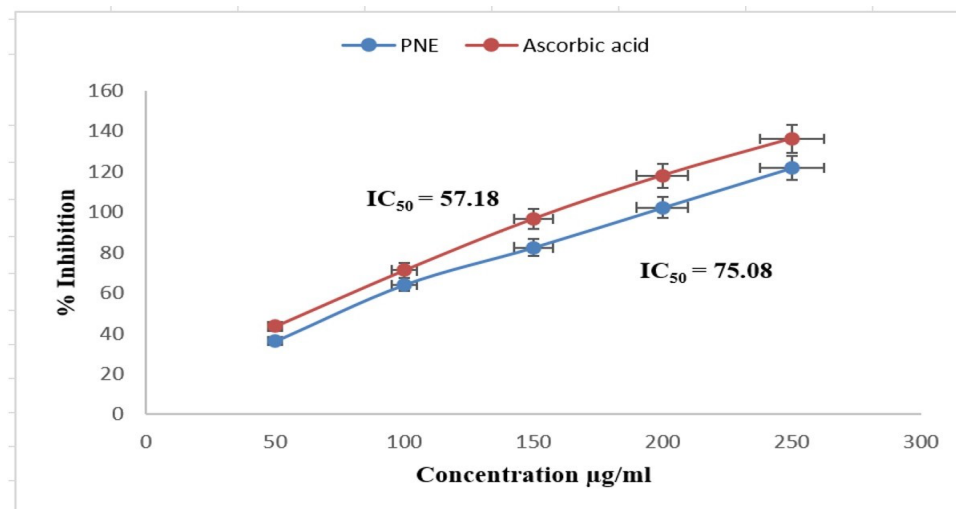
The hydroxyl radical scavenging activity of Phyllanthus reticulatus extract (PNE) increased in a dose-dependent manner. Although ascorbic acid exhibited higher

scavenging activity, PNE showed appreciable hydroxyl radical inhibition with an  $IC_{50}$  value of 75.08  $\mu\text{g/mL}$ , indicating its potential antioxidant effectiveness.

Concentration ( $\mu\text{g/ml}$ )	Hydroxyl Scavenging %	
	PNE	Ascorbic acid
50	36.2±0.73	43.5±0.96
100	64.1±0.64	71.2±0.49
150	82.5±0.32	96.7±0.53
200	102.2±0.48	118.1±0.17
250	121.8±0.91	136.3±0.28
$IC_{50}$	75.08	57.18

Values are reported as the mean±SEM from three repetitions of each experiment.

PNE: Phyllanthus reticulatus extract



Scavenging effect of *Phyllanthus reticulatus* on hydroxyl radicals.

### Conclusion

The present study concludes that the hydroalcoholic extract of the aerial parts of *Phyllanthus reticulatus* exhibits significant in-vitro antioxidant activity, as evidenced by DPPH and hydroxyl radical scavenging assays. The extract showed a clear concentration-dependent free radical scavenging effect, although its activity was comparatively lower than the standard ascorbic acid. The observed antioxidant potential may be attributed to the presence of polyphenolic and other bioactive constituents in the plant. These findings scientifically support the traditional use of *Phyllanthus reticulatus* and suggest that it may serve as a promising natural source of antioxidants for pharmaceutical and therapeutic applications.

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