International Journal of Dental and Clinical Studies 2024; 5(2) 01-05 ISSN: 2767-8245

Review Article

In-Vitro Antioxidant Activities of Herbal Plant Extract

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Received: 11-03-2024 / Revised: 15-04-2024 / Accepted: 20-05-2024
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Conflict of interest: Nil

Abstract

Several chronic diseases, including as cancer, cardiovascular disorders, diabetes, and neurodegenerative ailments, are influenced by oxidative stress, which occurs when the body's generation of reactive oxygen species (ROS) is out of sync with its antioxidant defense mechanism. Natural antioxidants found in medicinal plants can help neutralize free radicals and lessen the harm caused by oxidative stress. The medicinal plant Phyllanthus reticulatus has a history of use and is thought to have several pharmacological effects. The purpose of this research is to use established antioxidant assays to determine the phytolanthus reticulatus extract's in vitro antioxidant activity. We used appropriate solvents to extract the plant material, and then we used free radical scavenging methods like DPPH and hydrogen peroxide scavenging to evaluate its antioxidant capability. The results showed that the extract's antioxidant activity increased with increasing concentration, suggesting that it has great potential to scavenge free radicals. This study's results provide credence to Phyllanthus reticulatus's long history of medicinal usage and raise the possibility that it could be a useful source of natural antioxidants in the future.

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

Introduction

Metabolic reactions and exposure to pollutants, radiation, and chemicals in the environment both produce free radicals, which are unstable molecules. Overproduction of reactive oxygen species (ROS) causes oxidative stress, which in turn damages lipids, proteins, and nucleic acids, among other biological components. Numerous clinical problems, including aging, inflammation, cancer, cardiovascular disease, and neurological illnesses, have been linked to oxidative stress. The significance of antioxidants in protecting the organism from oxidative damage and neutralizing free radicals is crucial.

The widespread usage of synthetic antioxidants has, however, brought up safety concerns over their use over the long term. Since natural antioxidants derived from plants are effective, safe, and readily available, there

is a rising interest in their discovery. Traditional medicine practitioners have long relied on medicinal plants for their healing abilities, and for good reason: these plants contain bioactive chemicals with powerful antioxidant capabilities, including alkaloids, tannins, flavonoids, and phenolics.

A popular medicinal herb native to India's tropical and subtropical zones, Phyllanthus reticulatus is a member of the Euphorbiaceae family. Inflammation, liver diseases, diabetes, and microbiological infections have all traditionally been treated with various portions of the plant. Research into phytochemicals has shown that Phyllanthus reticulatus contains polyphenolic substances, which may indicate that it has antioxidant properties. The purpose of this study is to assess the in vitro antioxidant activity of Phyllanthus reticulatus extract by employing a battery of well-established antioxidant assays. This study intends to substantiate the plant's antioxidant properties and investigate its potential use as an antioxidant source in medicinal formulations.

Materials and method:

For this planned research, we used the aerial portions of Phyllanthus reticulatus. The plant parts were gathered from the surrounding region. The plant portions were carefully washed after harvest to eliminate any debris or dirt. After cleaning, the plant material was dried in a shaded area to maintain its phytoconstituents and avoid their deterioration.

The plant material was sun-dried, then pounded into a coarse powder. It was first macerated with petroleum ether to remove any fat. The extraction process was maintained until all lipids were removed from the sample. Then, 30 g of dried Phyllanthus reticulatus aerial portions were completely extracted using the maceration process and a hydroalcoholic solvent (ethanol: water, 70:30).

In-vitro antioxidant activity

DPPH radical scavenging assay

The present study somewhat modified a method for evaluating the DPPH radical scavenging activity that was described by Guchu et al. Different concentrations of extract samples (50, 100, 150, 200, and 250 µg/mL) were made for the test. For the production of all the extract samples, methanol was utilized as the solvent. Along with the test samples, a standard ascorbic acid solution was also made at amounts comparable to them.

To make sure everything was combined, 1 mL of the test sample and 0.5 mL of a 0.3 mM methanolic DPPH solution were placed in a clean test tube and given a good stir. To facilitate the reaction, the solutions were left at room temperature and maintained in darkness for 15 minutes.

A mixture of 1 milliliter of methanol and 2.5 milliliters of DPPH solution was used as the control solution. The samples were tested for absorbance at 517 nm using a UV-visible spectrophotometer. This is how the percentage of DPPH radical scavenging activity was determined:

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

C-Amount of absorbance by the control solution

S-Amount of absorbance by the extract/standard solution

The IC50value 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 $FeCl_3$ (10 mM), 0.1 mL H_2O_2 (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):

Percentage inhibition=C−SC×100

Where:
C = Absorbance of the control solution
S = Absorbance of the extract or standard solution

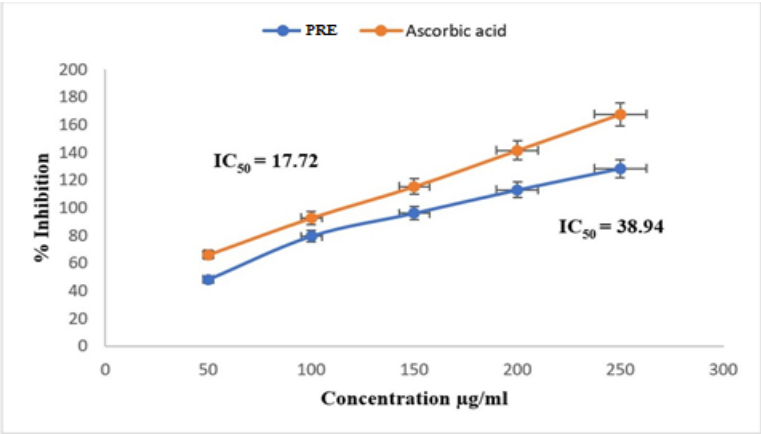
An indicator of the extract's or standard's ability to block deoxyribose breakdown is its percentage of hydroxyl radical scavenging activity. The standard solution and extract both had their IC_{50} values calculated. The results were presented as the mean of three independent experiments.

Results and discussion

DPPH Radical scavenging activity

Phyllanthus reticulatus extract (PE) outperformed ascorbic acid in scavenging DPPH radicals, according to the results. The extract's ability to scavenge DPPH increased as the concentration increased. At all concentrations studied, ascorbic acid exhibited superior antioxidant activity, but PRE showed promising free radical scavenging capabilities. With an IC_{50} value of 38.94 µg/mL, PRE shows promising antioxidant activity, lending credence to its promise as an all-natural antioxidant source.

Concentration (µg/ml)	DPPH Scavenging%	
	PRE	Ascorbicacid
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
IC50	38.94	17.72



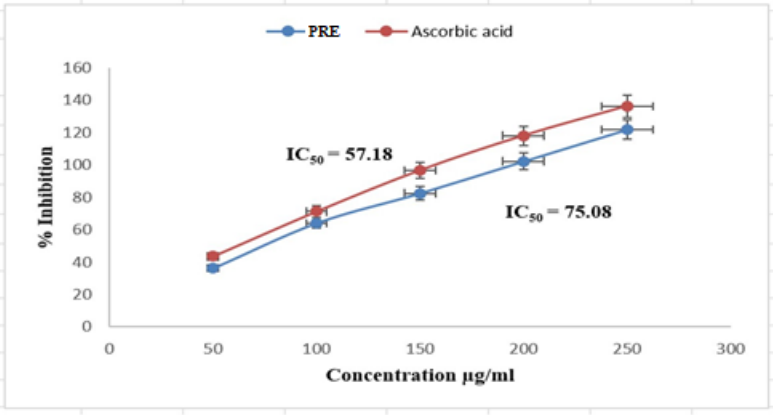
Scavenging effect of Phyllanthus reticulates on DPPH radicals

Hydroxyl Ion scavenging activity

There was a dose-dependent increase in the hydroxyl radical scavenging activity of the Phyllanthus reticulatus extract (PRE). With an IC_{50} value of 75.08 µg/mL, PRE demonstrated a respectable level of hydroxyl radical inhibition, suggesting its potential antioxidant efficiency, even though ascorbic acid shown stronger scavenging activity.

Concentration (µg/ml)	Hydroxyl Scavenging %	
	PRE	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
IC50	75.08	57.18

Values are reported as the mean±SEM from three repetitions of each experiment. PRE: Phyllanthus reticulates extract



Scavenging effect of Phyllanthus reticulates on hydroxyl radicals.

Conclusion

The current research found that DPPH and hydroxyl radical scavenging experiments demonstrated that the hydroalcoholic extract of Phyllanthus reticulatus aerial parts exhibited strong in-vitro antioxidant activity. The extract's free radical scavenging efficacy was concentration dependant, however it was less active than the gold standard, ascorbic acid. The presence of polyphenolic and other bioactive components in the plant could be responsible for the reported antioxidant activity. These results provide credence to Phyllanthus reticulatus's long history of medicinal usage and raise the possibility that it could be a useful pharmacological and therapeutic antioxidant.

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