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Original Article

Phytochemical Profiling and Antioxidant Activity Assessment of Ethanolic Extract of *Phyllanthus niruri*

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Abstract

Introduction: *Phyllanthus niruri*, commonly known as Stonebreaker due to its medicinal properties, has been utilised in traditional medicine for centuries. This plant demonstrates significant antioxidant activity, attributed to various bioactive components such as alkaloids, phenols, and flavonoids. **Aim:** This study aimed to screen for bioactive components and investigate its antioxidant activity. **Methods:** The aerial parts of the plant were harvested, dried, and ground into a fine powder. Ten grams of the powdered plant material was soaked in 100 ml of ethanol to prepare an ethanolic extract. A qualitative examination was carried out to identify the phytochemicals in the sample. DPPH and ABTS assays were performed to determine antioxidant activity. Absorbance was recorded at 517 nm for the DPPH assay and 734 nm for the ABTS assay. Gallic acid was used as the reference standard in both assays. **Results:** Several bioactive compounds, including flavonoids, alkaloids, phenols, triterpenoids, tannins, saponins, steroids, glycosides, and carbohydrates, were identified in the plant extract. The plant extract demonstrated notable antioxidant activity, showing an IC_{50} value of 3.12 ± 0.26 µg/ml in the DPPH assay, which was lower than that of gallic acid (IC_{50} 1.51 ± 0.02 µg/ml). In the ABTS assay, the IC_{50} value of the plant extract was 19.98 ± 0.09 µg/ml, which was higher than that of gallic acid (5.69 ± 0.05 µg/ml). **Conclusion:** Our results revealed the potential antioxidant activity of *Phyllanthus niruri*, which could be beneficial in treating several clinical conditions, such as inflammation and cellular damage caused by oxidative stress.

Keywords: *Antioxidants; Free Radical Scavenging; Oxidative Stress; Phyllanthus niruri; Phytochemicals*

Introduction

Therapeutic herbs have been widely used in Indian healthcare systems, such as Unani and Ayurveda. These plants are often considered safer alternatives to synthetic medications, as they originate from natural sources and are believed to have fewer side effects. Many herbs possess natural antioxidant and anti-inflammatory properties, promoting healing in a comprehensive and natural manner. In recent decades, increasing scientific research has validated the therapeutic properties of numerous herbs. Plant-based traditional medicine remains a crucial component of healthcare, with approximately 80% of the global population relying primarily on traditional medicines for their basic healthcare needs (Singh *et al*., 2016).

Oxidative stress is defined as a condition that arises due to instability in the balance between free radicals and antioxidants within the body, leading to mitochondrial dysfunction (Skibska *et al*., 2023), and chronic diseases such as cardiovascular diseases and neoplasms (Markiewicz-Górka *et al*., 2019). Antioxidants are substances that help defend the body against oxidative stress by donating electrons to stabilise free radicals, thereby preventing further cellular damage.

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Phyllanthus niruri is extensively used across South America and Asia as a remedy for various health issues. This plant is particularly recognised for its effectiveness in managing hepatitis, diabetes, jaundice, asthma, flu, malaria, haemorrhages, diarrhoea, dysentery, cough, and anaemia (Muhammad *et al*., 2020). Several studies have reported that this plant possesses diverse therapeutic properties, with effective anti-inflammatory (Mostofa *et al*., 2017), antidiabetic (Beidokhti *et al*., 2017), hepatoprotective (Ezzat *et al*., 2020; Singh *et al*., 2024), and anticancer effects (Abdel-Sattar *et al*., 2023).

Phyllanthus niruri is rich in several bioactive compounds, which are believed to contribute to the plant's medicinal properties and therapeutic potential (Lee *et al*., 2016). Plant secondary metabolites like polysaccharides, alkaloids, phenols, and flavonoids (Trivadila *et al*., 2025) are unique in their health benefits, possessing antibacterial, antiviral, antioxidant, and anticarcinogenic effects, and playing a key role in the prevention and management of diseases (Roy *et al*., 2022; Hilal *et al*., 2024). This research aimed to identify the bioactive compounds in *Phyllanthus niruri* through qualitative analysis and evaluate its antioxidant properties using DPPH and ABTS assays.

Materials and Methods

Chemicals:

Gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were sourced from Sigma-Aldrich, while the remaining reagents were obtained from HI Media.

Collection of Plant Material:

Phyllanthus niruri was collected from local fields and authenticated. Plants were washed thoroughly, dried, powdered and stored in a glass container for further use.

Preparation of Ethanolic Extract:

The ethanolic extract of *Phyllanthus niruri* was prepared by soaking 10g of plant material in 100ml of ethanol with manual shaking for a duration of 48 hours at room temperature. After 48 hours, the ethanolic extract was filtered using Whatman No. 1 filter paper and placed in a rotary evaporator to evaporate the solvent. The resulting viscous ethanolic extract was collected and stored in a sealed container.

Preliminary Phytochemical Screening:

Qualitative analysis was carried out to identify entity of bioactive compounds in the sample (Shaikh & Patil, 2020).

1)Tests for alkaloids

a) Dragendroff's test

A small amount of dilute HCL,1000µL of Dragendroff's reagent was mixed with 500 µL of sample. Presence of alkaloids in the sample is indicated by the appearance of an orange red coloured precipitate.

b) Mayer's test

Few drops of dilute HCL,1000µL of Mayer's reagent was added to 500 µL of sample. Presence of alkaloids in the sample is indicated by the development of white precipitate.

2)Test for phenols

Ferric chloride Test

To a volume of 500 µL from the sample, 2 ml of 5% Ferric chloride solution was added. Occurrence of dark green colour indicates the presence of phenols in a sample.

3)Tests for flavonoids

Shinoda's test

Few pieces of magnesium ribbon and a few drops of concentrated hydrochloric acid were added to 500 μL of sample and boiled in a water bath for 5 minutes. Pink colour formation is often used as an indication of entity of flavonoids in the extract.

4)Test for glycosides

Aqueous NaOH test

500 μL of sample was dissolved in one millilitre of distilled water. To these few drops of aqueous sodium hydroxide (NaOH) were incorporated. Appearance of yellow colouring suggests that glycosides are present in the sample.

5)Test for tannins

1ml of extract was taken into test tube, followed by adding few drops of 10% ferric chloride. Formation of a bluish green colorization confirms the presence of tannins.

6)Test for terpenoids:

1ml of sample was extracted with few drops of chloroform. To this solution, 2ml of concentrated H_2SO_4 was added boiled in water bath. Formation of grey colour indicates terpenoids presence.

7)Test for saponin

Foam Test

0.5 ml of sample along with 3 ml water was shaken well, profuse lather formation reveals presence of saponins.

8) Test for steroids:

To 2ml of extract few drops of concentrated H_2SO_4 were added. Appearance of brown colouration indicates steroids in sample.

9) Test for carbohydrates:

1ml of Barfoed's reagent is to 1ml of sample and mixed well and heated in a water bath for 4 minutes. Brick red colorization confirms entity of carbohydrates in the sample.

Free Radical Scavenging Activity:

DPPH Scavenging Assay:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was conducted (Noreen *et al*., 2017) with minor modifications. A 3 ml solution of 150μM DPPH radical was prepared and mixed with 500μL of the sample, with concentrations ranging from 0.1 to 250μg/mL. The mixture was incubated for 30 minutes at 37 ± 2°C in a dark room. A 500μL aliquot of ethanol was used as the control. The decrease in absorbance was recorded at 517 nm using a Systronics UV-Spectrophotometer TS119, India. Gallic acid was used as the antioxidant standard.

%DPPH quenched = (control-sample/control) X 100

ABTS Scavenging Assay:

The ABTS (Azino-bis diammonium salt) assay was conducted (Noreen *et al*., 2017) with slight modifications. The ABTS radical reaction was initiated by mixing 7.0 μM ABTS solution with 2.45 μM potassium persulfate. The reagent was placed in the dark at room temperature for 16 hours to complete the reaction. The solution was then diluted with water to obtain the ABTS working solution, which had an absorbance of 0.70. Three millilitres of the ABTS radical solution were combined with 500 μL of the sample, with concentrations ranging from 0.1 to 250 μg/mL and incubated at 37 ± 2°C for 6 minutes in the dark. A 500 µL aliquot of ethanol was used as the control. The decrease in absorbance was measured at 734 nm using a Systronics UV-Spectrophotometer.

%ABTS quenched = (control-sample/control) X 100

Results

This study was conducted to identify the bioactive compounds in the plant extract through qualitative analysis and to assess its antioxidant properties using DPPH and ABTS assays. The findings indicated the presence of alkaloids, phenols, flavonoids, saponins, triterpenoids, and tannins at high concentrations, along with moderate levels of glycosides, steroids, and carbohydrates (Table 1).

Table 1: Preliminary Qualitative Phytochemical Analysis

Sl.No	Constituents	Results
1	Alkaloids	Positive
2	Phenols	Positive
3	Flavonoids	Positive
4	Glycosides	Positive
5	Steroids	Positive
6	Triterpenoids	Positive
7	Saponins	Positive
8	Tannins	Positive
9	Carbohydrates	Positive

Antioxidant Activity:

Antioxidant potential of the *Phyllanthus niruri* was assessed using DPPH and ABTS assays, which demonstrated strong antioxidant effects with low IC_{50} values.

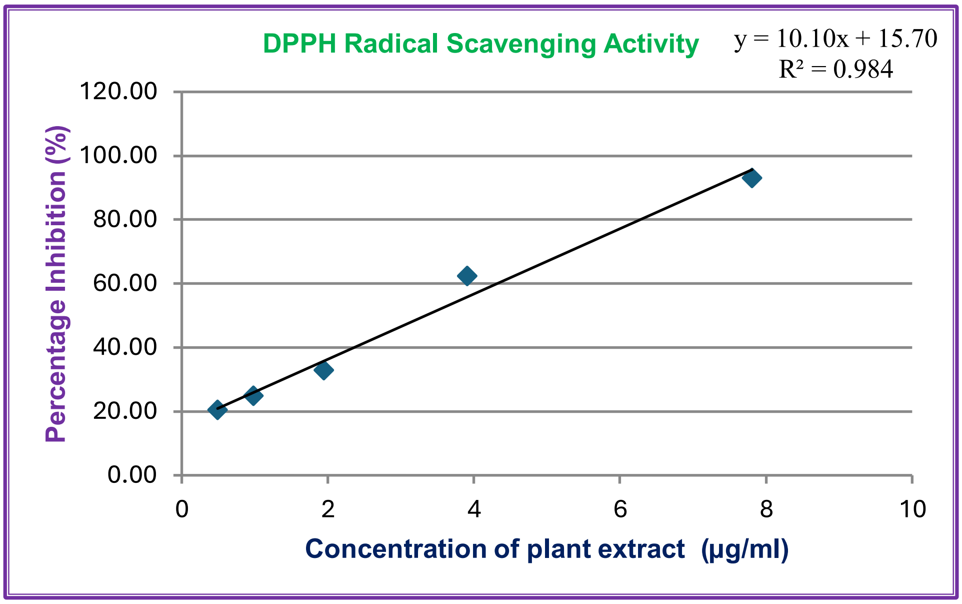
DPPH Scavenging Assay

Exposure to various toxic compounds results in the production of reactive oxygen species (ROS), which are highly unstable due to unpaired electrons in their outermost orbit. To counter this, antioxidants donate electrons to stabilise them. In the present study, the antioxidants present in the plant extract effectively reduced DPPH to DPPH-H, leading to a colour change from purple to colourless.

Table 2: Different Concentrations (Mg/Ml) Of Phyllanthus niruri and Their Percentage Inhibitions

S.NO	Concentration (µg/ml)	Percentage Inhibition (%)
1	0.49	20.45
2	0.98	25.00
3	1.95	32.95
4	3.91	62.50
5	7.81	93.18

Concentration dependent antioxidant activity of *Phyllanthus niruri* ranging from 0.49-7.81 µg/ml, which exhibited strong antioxidant activity (93.18%) at concentration 7.81 µg/ml (Table 2).



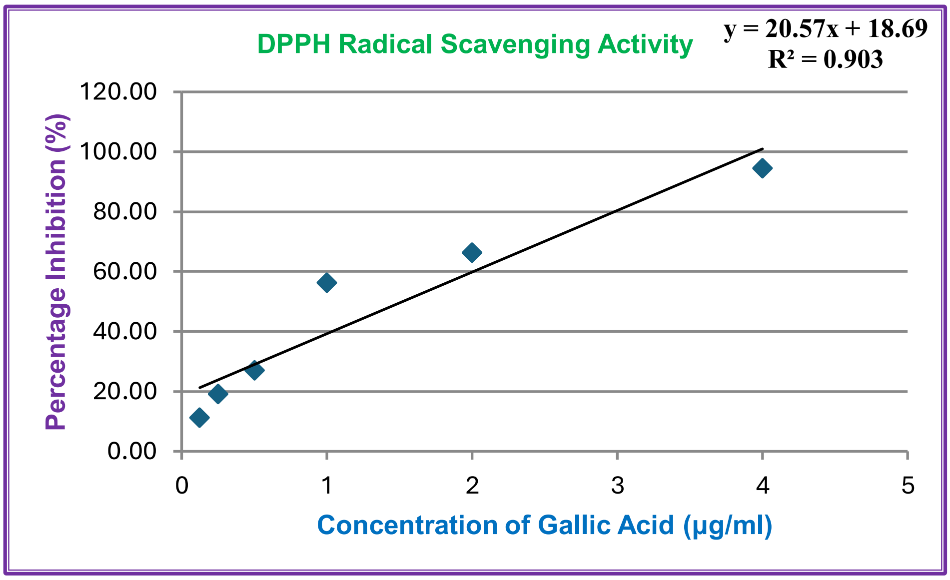
Data presented as mean ± SD

Figure 1: *Graph using Concentrations vs Percentage Inhibitions (%) of Phyllanthus niruri*

Table 3: Different Concentrations (Mg/Ml) of Gallic Acid and their Percentage Inhibitions

S.NO	Concentration (µg/ml)	Percentage Inhibition (%)
1	0.125	11.24
2	0.25	19.10
3	0.5	26.97
4	1	56.18
5	2	66.29
6	4	94.38

Concentration dependent antioxidant activity of gallic acid ranging from 0.125-4µg/ml, which exhibited strong activity (94.38%) at concentration 4 µg/ml (Table 3). Notably, gallic acid achieves comparable high-level inhibition (94.38%) at 4 µg/ml, while *Phyllanthus niruri* requires nearly double the concentration (7.81 µg/ml) to reach similar levels (93.18%).



Data presented as mean ± SD

Figure 2: *Graph using Concentrations vs Percentage Inhibitions (%) of Gallic Acid*

Table 4: *IC50 Value of Gallic Acid and Phyllanthus niruri*

S.NO	Compounds	IC50 (µg/ml)
1	Gallic Acid	1.51±0.02
2	Plant extract	3.12 ± 0.26

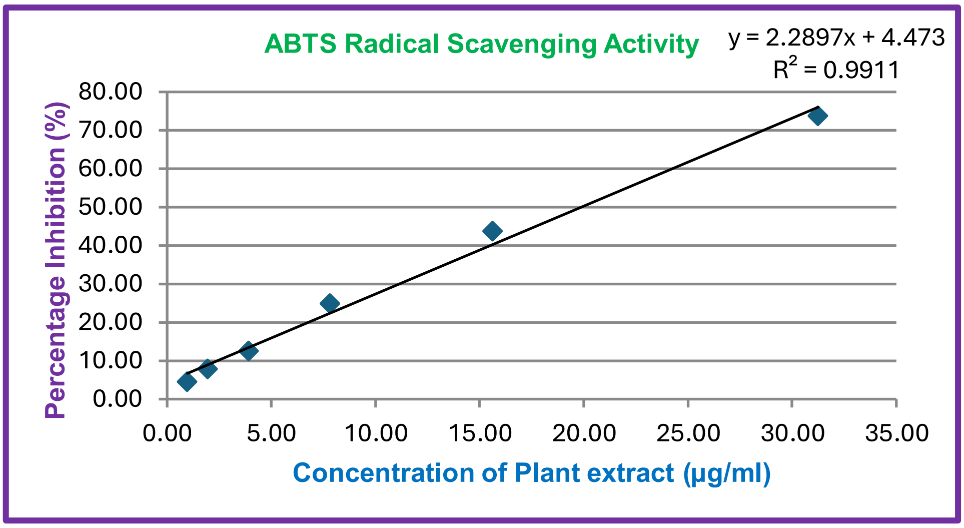
The IC_{50} for plant extract was found to be 3.12 ± 0.26µg/ml while that of gallic acid was at 1.51±0.02 µg/ml (Table 4). This indicates *Phyllanthus niruri* possesses potential antioxidant effect.

ABTS Scavenging Assay

Table 5: Different concentrations (µg/ml) of Phyllanthus niruri and their Percentage inhibitions

S. No	Concentration (µg/ml)	Percentage Inhibition (%)
1	0.98	4.57
2	1.95	7.92
3	3.91	12.57
4	7.81	24.97
5	15.63	43.89
6	31.25	73.79

Table 5 showing dose dependant antioxidant activity of *Phyllanthus niruri* at 6 different concentrations 0.98-31.25 µg/ml. These findings revealing that antioxidant activity of plant extract progressively increases with higher concentrations, reaching 73.79% inhibition at the highest concentration of 31.25 µg/ml.



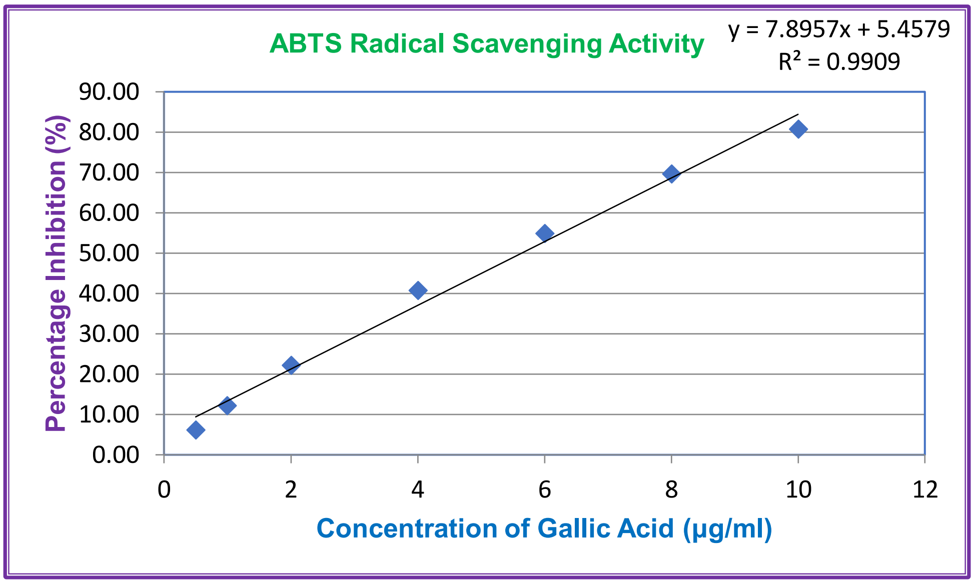
Data presented as mean ± SD.

Figure 3: *Graph Using Concentrations vs Percentage Inhibitions (%) of Phyllanthus niruri.*

Table 6: Different Concentrations (Mg/Ml) of Gallic Acid and Their Percentage Inhibitions

S. NO	Concentration (µg/ml)	Percentage Inhibition (%)
1	0.5	6.18
2	1	12.21
3	2	22.17
4	4	40.84
5	6	54.97
6	8	69.70
7	10	80.84

Table 6 demonstrates the antioxidant activity of gallic acid with concentrations ranging from 0.5 to 10 µg/ml. Even at the lowest tested concentration (0.5 µg/ml), gallic acid exhibited notable activity, with 6.18% radical inhibition, reflecting its strong antioxidant capacity. At the highest concentration (10 µg/ml), gallic acid reached 80.84% inhibition



Data presented as mean ± SD

Figure 4: *Graph using Concentrations vs Percentage Inhibitions (%) of Gallic Acid.*

Table 7: IC50 Value of Gallic Acid and Phyllanthus niruri

Sl.No	Compounds	IC50 (µg/ml)
1	Gallic Acid	5.69±0.05
2	Plant extract	19.98 ±0.09

Gallic acid exhibited high antioxidant effectiveness, as indicated by a low IC50 value of 5.69 ± 0.05 µg/ml. Meanwhile, the ethanolic extract of *Phyllanthus niruri* showed a moderate but significant antioxidant effect, with an IC50 of 19.98 ± 0.09 µg/ml (Table 7).

The ethanolic extract of *Phyllanthus niruri* exhibited significant antioxidant activity, with lower IC50 values in the DPPH assay compared to the ABTS assay, although gallic acid is a stronger antioxidant. This activity is likely attributed to bioactive compounds such as alkaloids, flavonoids, and polyphenols.

Discussion

Oxidative stress (OS) is a major cause in the manifestation of various diseases such as neurological disorders, cancers, and inflammatory diseases (Krishnamurthy *et al*., 2024). Excessive generation of free radicals leads to oxidative stress, which induces cellular damage and contributes to various health problems like cancer and diabetes (Zonooz *et al*., 2021). Antioxidants can prevent or repair cellular damage caused by free radicals, thereby protecting against diseases linked to oxidative stress. Herbal medicine has been an integral part of traditional healing systems across the world for thousands of years. Natural compounds found in plants can support and enhance the body's healing processes. Phytochemicals such as phenols, flavonoids, and alkaloids are recognised for their ability to neutralise ROS. Most of these herbs contain high levels of secondary metabolites, such as alkaloids, flavonoids, and polyphenols, which possess antioxidant, antibacterial (Tang *et al*., 2024; Tiwana *et al*., 2024), antimicrobial, and anti-cancer (Ilhami *et al*., 2025) properties.

Phyllanthus niruri, often referred to as stone breaker, is a well-known plant with reported benefits such as antitumour, anti-carcinogenic, hypolipidemic, hepatoprotective, antidiuretic (Nawfetrias *et al*., 2023), and antiviral effects. It is commonly used in treating urolithiasis and respiratory problems (Vishal *et al*., 2025). Several studies have reported that *Phyllanthus niruri* contains antidiabetic (Abdel-Sattar *et al*., 2023) and anti-inflammatory (Mohammed *et al*., 2014; Wulandari *et al*., 2024) properties, which help prevent cellular damage, infections, and chronic diseases. The entire plant has been traditionally used in medicine to treat conditions such as hepatitis, hyperlipidaemia, and renal stones. It also demonstrates strong antioxidant activity, effectively neutralising superoxide and inhibiting lipid peroxidation (Giribabu *et al*., 2014). *Phyllanthus niruri* contains high levels of flavonoids and phenolic compounds, which contribute to its powerful antioxidative potential (Al Zarzour *et al*., 2017).

Secondary metabolites are special compounds synthesized by plants to protect themselves from environmental stress, animals, and pathogens. These compounds possess various pharmacological properties, such as antimicrobial, anti-cancer, and antioxidant effects (Kumar *et al*., 2025). Flavonoids, which are plant secondary metabolites belonging to the polyphenol group, naturally occur in fruits and vegetables and possess potential antioxidant activity. They neutralise free radicals by donating electrons (Himawati *et al*., 2024). Alkaloids, nitrogen-containing compounds, possess anti-cancer, anti-diabetic, anti-inflammatory, and anti-malarial properties (Lin *et al*., 2022; Rajput *et al*., 2022).

In the present study, the ethanolic extract of *Phyllanthus niruri* exhibited various phytochemicals, such as alkaloids, flavonoids, phenols, saponins, and tannins, in high concentrations. These phytochemicals are known for the plant's diverse therapeutic properties. Our findings are consistent with previous studies that also reported the presence of these phytochemicals, including alkaloids, flavonoids, and phenols (Singh & Ahmad, 2020; Sirajudeen *et al*., 2017).

The DPPH assay was performed to assess the scavenging ability of antioxidants present in *Phyllanthus niruri* to neutralise free radicals present in the DPPH solution by donating electrons. DPPH is a dark purple-coloured stable free radical. Different concentrations of the plant extract were mixed with the DPPH solution and incubated for 30 minutes. The decrease in absorbance was measured using a Systronics UV-Spectrophotometer TS119 at 517 nm. A visible decrease in colour from dark purple to colourless was observed, indicating the scavenging activity of *Phyllanthus niruri*. In the present study, a lower IC50 value (3.12 ± 0.26µg/ml) was observed, which is almost similar to the standard, i.e., gallic acid, indicating strong antioxidant potential. Similar findings were reported by Pineda *et al*. (2025), who stated that the ethanolic extract of *Phyllanthus niruri* exhibited strong free radical scavenging activity in the DPPH assay.

ABTS is stable free radicle with bluish green in colour. Different concentrations of plant extract were mixed with ABTS solutions and incubated for 6 min. Decrease in absorbance was noted at 734nm using Systronics UV-Spectrophotometer. After incubation period, change in colour from greenish blue to colour less was observed. In this study a lower IC50 value (19.98 ±0.09 µg/ml) was observed. However, *Phyllanthus niruri* exhibited strong antioxidant activity by DPPH assay than ABTS. Our findings are in accordance with study by Arif *et al*. (2024), who reported that *Phyllanthus niruri* exhibited strong antioxidant activity by ABTS assay.

A key limitation of this study is the use of ethanol for extraction. Incorporating solvents of varying polarities, such as methanol, water, and chloroform, could enhance the efficiency of extraction and help isolate both polar and non-polar bioactive compounds. Additional in vivo studies are required to explore the antioxidant potential, underlying molecular mechanisms, and assess the possible toxicity of the plant extract.

Conclusion

The current research demonstrated therapeutic potential and antioxidant activity of *Phyllanthus niruri* through phytochemical and antioxidant analysis. The observed results could be due to presence of wide range of phytochemicals such as alkaloids, phenols, flavonoids, and other bioactive compounds. These findings suggest that *Phyllanthus niruri* may serve as abundant source of antioxidants, offering promising therapeutic benefits for a range of health conditions and also in managing oxidative stress related diseases. Additional in vivo studies are required to explore the antioxidant potential, underlying molecular mechanisms, and assess the possible toxicity of the plant extract.

Conflict of Interest

The authors declare no conflicts of interest related to this work.

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