



Tropical Journal of Natural Product Research

Available online at <https://www.tjnpr.org>

Original Research Article

Phytochemical and Antioxidant Profile of the Ethanol Extracts of *Phyllanthus niruri* and *Bergenia ligulata*

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ARTICLE INFO

ABSTRACT

Article history:

Received 18 November 2025

Revised 29 November 2025

Accepted 18 December 2025

Published online 01 January 2026

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Cells produce free radicals that act as signaling molecules but also cause cellular damage, leading to oxidative stress and disease progression. Endogenous antioxidants offer protection, yet their limited levels are often inadequate against reactive oxygen species (ROS). In contrast, natural antioxidants provide more effective defense with better biocompatibility and fewer side effects than synthetic ones. Among medicinal plants, *Phyllanthus niruri* (PN) and *Bergenia ligulata* (BL) are traditionally valued for their therapeutic properties in managing diverse health disorders. The present study aimed to evaluate the phytochemical composition and antioxidant capacity of ethanolic extracts derived from PN leaves and BL rhizomes. Extracts were prepared by Soxhlet extraction method, and subsequently analyzed for total phenolic and flavonoid contents, along with antioxidant activity. Quantitative phytochemical analysis revealed that PN and BL extracts contained substantial phenolic contents (290 ± 0.03 mg GAE/g and 240 ± 0.04 mg GAE/g, respectively) and flavonoid contents (56.09 ± 0.48 mg RUT/g and 58.80 ± 0.84 mg RUT/g, respectively). Antioxidant assays demonstrated marked activity, with PN and BL exhibiting ferric reducing antioxidant power (FRAP) values of $65.96 \pm 0.48\%$ and $62.04 \pm 0.07\%$, respectively, and DPPH radical scavenging activities of $71.57 \pm 0.06\%$ and $75.60 \pm 0.50\%$, respectively. No significant differences ($P < 0.05$) were observed between the antioxidant activities of both extracts and that of ascorbic acid ($77.49 \pm 0.13\%$ for DPPH and $64.35 \pm 0.07\%$ for FRAP). These findings strongly support the potential application of PN leaves and BL rhizomes in mitigating oxidative stress-related disorders.

Keywords: *Phyllanthus niruri*, *Bergenia ligulata*, Polyphenol, Flavonoid, Antioxidant assay.

Introduction

Oxidative stress is a condition where the cells exhibit high level of free radicals that overwhelm the endogenous antioxidants.¹ Accumulation of free radicals lead to distinctive structural damage to cellular components, such as DNA, lipids, and proteins. All human cells have antioxidant defenses against oxidative damage, and failure of these defense mechanisms causes free radical-induced oxidative damage, which eventually reinforces multiple disease progression such as cancer, neurodegenerative disorders, cardiovascular diseases, respiratory disorders, and kidney diseases.² Antioxidants obtained from dietary products or supplements and/or synthesized by the cells can scavenge accumulated free radicals in the cells and tissues. Researchers have been evaluating medicinal plants for their antioxidant properties, and this has led to a growing trend in the use of natural antioxidants over synthetic ones. Natural antioxidants have greater efficacy and biocompatibility, and lack substantial adverse effects compared to synthetic antioxidants.¹

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Citation: Majidinia L, Alabdali AYM, Panneerselvam J, Ranganathan V, Chinnappan S, Mani RR. Phytochemical and Antioxidant Profile of the Ethanol Extracts of *Phyllanthus niruri* and *Bergenia ligulata*. Trop J Nat Prod Res. 2025; 9(12): 6003 – 6007 <https://doi.org/10.26538/tjnpr/v9i12.13>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Herbal remedies like *Phyllanthus niruri* (PN) and *Bergenia ligulata*

(BL) contain a variety of phytochemicals, especially polyphenols and flavonoids, which are recognized for their antioxidant capabilities, to scavenge free radicals and promote protection against oxidative stress.^{3,4} Thus, the consumption of these herbal remedies has become necessary as a means of preventing, treating, or mitigating a variety of oxidative stress-induced diseases. *Phyllanthus niruri* (PN) commonly known as stone broker is a perennial tropical shrub from the *Phyllanthaceae* family. The plant is widely distributed in the south and south-east Asian countries. From historical time, the plant had been widely used as medicines to treat renal stones, diarrhea, asthma, bronchitis, and tuberculosis.⁵ The phytochemical investigation of PN leaves has revealed abundant phytochemicals including alkaloids, flavonoids, tannins, terpenes, coumarins, lignans, and phenylpropanoids.⁶ PN, also contains a considerable amount of polyphenol compounds and flavonoids, including nirurin, gallic acid, catechin, rutin, quercitrin, and niruriflavone,⁵ which are primarily responsible for the herb's therapeutic qualities. The ethanolic extract of PN has been shown to exhibit high antioxidant activity in streptozotocin-induced diabetic rats. The extract reduced glutathione (GSH) levels and increased synthesis of endogenous antioxidants such as superoxide dismutase (SOD) and catalase (CAT).⁷ The crude aqueous extract of *Phyllanthus niruri* leaves has also been shown to inhibit Fe (II)-induced lipid peroxidation, and exhibit hepatoprotective effect in diabetic rats.^{7,8} The neuroprotective effect of PN through iron-chelation has also been demonstrated in Alzheimer's disease rat model.⁹ *Bergenia ligulata* (BL) (family: Saxifragaceae) is an herbal plant grown in the Himalayas. The herb is also known by the approved name *Bergenia pacumbis*. The plant's roots and rhizomes are used by Himalayan cultures to treat wounds, infections, colds and coughs, heart conditions, asthma, inflammation, gastrointestinal disorders, and

various urinary tract disorders.¹⁰ *Bergenia ligulata* contains a considerable amount of polyphenols and flavonoids, including bergenin, catechin, syringic acid, and ferulic acid, which are primarily responsible for the herb's therapeutic qualities. In addition to polyphenols and flavonoids, steroids, alkaloids, and terpenoids are among other phytochemicals found in BL. These compounds have several important biological activities, such as free radical scavenging, anti-inflammatory, and antimicrobial activities.¹¹ Bergenin which is the major bioactive compound of BL has been shown to be effective in neutralizing superoxide anion radicals, and its reducing ability has been linked to electron-transfer mechanism. The benzoyl moiety of Bergenin 11-*O*-galloylbergenin has three hydroxyl groups—two meta and one para—which gives it a unique structural orientation that makes it easy to interact with and scavenge free radicals. Moreover, bergenin combines with Fe (II) and blocks the synthesis of hydroxyl radicals, thereby exhibiting scavenging activity against hydroxyl radicals.^{10,12} In addition to its antioxidant activity, the methanolic extract of BL showed anti-inflammatory effect in oxalate-induced urolithiasis rats by reducing the expression of inflammatory mediators such as MAPK, OPN, and NF- κ B in rat kidney epithelial cells.¹³ Due to their remarkable pharmacological activities, especially antioxidant and anti-inflammatory activities, PN and BL have gained significant attention by scientists in the food and pharmaceutical industries for their potential application as functional foods, and herbal nutraceuticals.^{2,10} This study therefore aim to comparatively evaluate the ethanolic extracts of PN and BL for their phytoconstituents and antioxidant properties.

Materials and Methods

Chemicals and Equipment

Aluminum chloride (Merck, Germany), sodium hydroxide (Merck, Germany), sodium nitrite (Merck, Germany), folin-ciocalteu reagent (sigma-Aldrich, Germany), sodium carbonate (Merck, Germany), phosphate buffer (Merck, Germany), hydrogen peroxide (Merck, Germany), ascorbic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl) (sigma-Aldrich, Germany), ethanol (Merck, Germany). The equipment used comprised a spectrophotometer (UV-Visible U2900 twin beam Thomas Edison) and an analytical balance (Mettler toledoab204-s).

Plant collection

Phyllanthus niruri (PN) leaf powder was purchased from sierra India organic and *Bergenia ligulata* (BL) rhizome powder was purchased from Jeyam herbal India.

Extraction of plant materials

PN and BL (50 g each) were extracted by Soxhlet extraction method at 65°C using ethanol as solvent. Subsequently, the extracts were concentrated using a vacuum rotary evaporator, then air-dried at room temperature for 2 weeks. The dried extracts were preserved in an airtight container until needed for analysis.¹⁴

Determination of total phenolic content

Stock solutions of the extracts were prepared by dissolving 20 mg extract in 20 mL distilled water. Different dilutions (0.05, 0.1, 0.15, 0.20, 0.25) of the extracts were prepared from the stock solution using methanol (0.1% HCl) as diluent. This solution at different concentrations (0.05, 0.1, 0.15, 0.20, 0.25 mg/mL) was mixed with 0.5 mL of Folin-Ciocalteu (FC) reagent, followed by the addition of 10 mL of 7.5% Na₂CO₃ and 25 mL of distilled water. Blanks were treated in a similar manner, except that 0.5 mL of methanol (0.1% HCl) was used in place of the extracts. The mixture was allowed to stand in the dark at room temperature for 1 h, after which the absorbance was measured at 750 nm using a UV-Vis spectrophotometer. A calibration curve of gallic acid was prepared, and the total phenolic content was derived from the equation of the curve using the below formula. Results were expressed as gallic acid equivalents (GAE) per gram of extract.¹⁵

$$\text{Total phenolic content} \left(\text{mg} \frac{\text{GAE}}{\text{g}} \right) = \frac{C \times V}{M}$$

Where;

C = Concentration of phenolic compounds from the calibration curve (mg/mL)

V = Volume of the extract (mL)

M = Mass of the herbal sample (g)

Determination of total flavonoid content

The total flavonoid content of the plant extracts was determined using aluminum chloride colorimetric assay. Standard solution of rutin was prepared by dissolving 20 mg rutin in 20 mL distilled water. From the stock solution were prepared dilutions of different concentrations (0.05; 0.1; 0.15; 0.20; 0.25; 0.30). For the sample extract solution, 20 mg of each ethanolic extract was dissolved in 20 mL distilled water, and mixed with 0.3 mL of 5% sodium nitrite solution. To 1 mL of the sample extract, 0.3 mL of 10% aluminum chloride solution was added. After 6 minutes of incubation at room temperature (37°C), 2 mL of 1 M sodium acetate solution was added and the total volume was made up to 10 mL with distilled water. The mixture was vortexed thoroughly and incubated at room temperature for 30 min. After incubation, the absorbance was measured at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content was estimated from the standard curve of rutin, and the result expressed as mg rutin equivalents per gram of sample (mg RUT/g).¹⁵

$$\text{Total flavonoid content} \left(\text{mg} \frac{\text{RE}}{\text{g}} \right) = \frac{C \times V}{M}$$

Where;

C = Concentration of flavonoid compounds from the calibration curve (mg/mL)

V = Volume of the extract (mL)

M = Mass of the herbal sample (g)

Determination of antioxidant activity

DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.25 mM) in methanol was prepared. The extract solutions of PN and BL (1 mL each) at different concentrations (50 μ g/mL, 100 μ g/mL, 150 μ g/mL, 200 μ g/mL, 250 μ g/mL, 300 μ g/mL) were combined with 0.5 mL of the DPPH solution. The mixtures were incubated in the dark at 25°C for 30 min. Thereafter, the absorbance of the solution was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid at the same concentrations was used as the reference standard. The percentage of inhibition and antioxidant activity was calculated using the formula below.¹⁵

$$\text{Percentage inhibition of DPPH (\%)} = \frac{A \text{ Control} - A \text{ Sample}}{A \text{ Control}} \times 100$$

Where;

Acontrol is the absorbance of the DPPH solution without the sample

Asample is absorbance of the DPPH solution with the sample

Ferric reducing antioxidant power (FRAP) assay

Different concentrations (50 μ g/mL, 100 μ g/mL, 150 μ g/mL, 200 μ g/mL, 250 μ g/mL, 300 μ g/mL) of the ethanolic extracts of PN and BL were mixed with 1 mL of potassium ferricyanide solution and the mixtures were incubated at 50°C for 20 min. After incubation, 1 mL of 0.1 M ferric chloride solution and 1 mL of 1% sodium carbonate solution was added and incubated at room temperature for 10 min. The absorbance of the reaction mixture was measured at 700 nm using a UV-Visible spectrophotometer. Ascorbic acid at the same concentrations as the samples was used as the reference standard.¹⁵ The percentage inhibition was estimated using the below formula.

$$\text{Percentage inhibition (\%)} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

Where;

A control is the absorbance of the FRAP solution without the sample

Asample is the absorbance of the FRAP solution with the sample

Statistical analysis

All the tests were conducted in triplicate and data were presented as mean \pm standard deviation (SD). Differences between mean values were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's post hoc test using SPSS Statistics software version 17.0. P-value < 0.05 was considered statistically significant.

Results and Discussion

Total phenolic content of the extracts

The total phenolic content (TPC) of *Phyllanthus niruri* (PN) and *Bergenia ligulata* (BL) was quantified using the Folin-Ciocalteu assay and results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g). The calibration curve of gallic acid generated for quantification of TPC had the regression equation: $y = 1.6221x + 0.413$, $R^2 = 0.9987$ (Figure 1). Based on this assay, the phenolic content was determined to be 290 ± 0.03 mg GAE/g for BL and 240 ± 0.04 mg GAE/g for PN (Table 1).

Table 1: Total Phenolic and Flavonoid contents of *Phyllanthus niruri* and *Bergenia ligulata* extracts

Extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RUT/g)
<i>Phyllanthus niruri</i>	240 ± 0.04	58.80 ± 0.84
<i>Bergenia ligulata</i>	290 ± 0.03	56.09 ± 0.48

Values are expressed as Mean \pm SEM, n = 3; mg GAE/g: milligram Gallic Acid Equivalent; mg RUT/g: milligram Rutin Equivalent

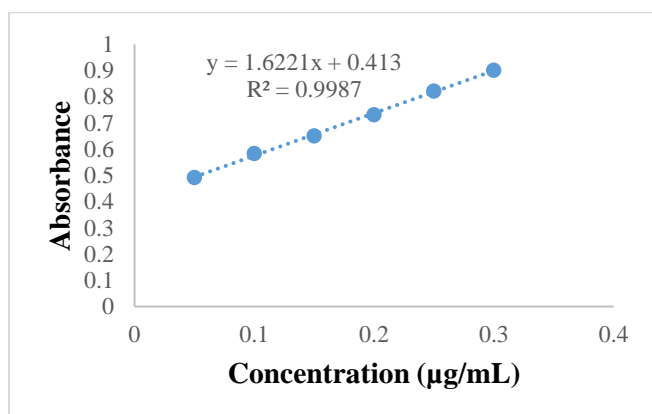


Figure 1: Gallic acid standard curve

These values are in agreement with previous findings, where *Phyllanthus* infusions were reported to contain approximately 242 mg GAE/g of phenolic content, while *Bergenia* species extracts typically exhibited phenolic contents ranging from 290 to 500 mg GAE/g, depending on the solvent for extraction and the plant part analyzed. Such variations across studies are often influenced by factors including solvent polarity, extraction method, plant tissue specificity, geographical origin, post-harvest drying conditions, and differences in assay protocols.¹⁶

Phenolic compounds, as major bioactive constituents of plants, have been extensively studied for their diverse biological properties. Their importance lies in their pronounced antioxidant and free radical scavenging potential, which has been consistently demonstrated across numerous investigations. The hydroxyl groups present in phenolic compounds' structures play a critical role in these activities by serving as hydrogen donors, acting as reducing agents, and functioning as quenchers of singlet oxygen. Thus, the structural features of plant phenolics directly influence their antioxidant efficacy and their capacity to neutralize reactive oxygen species.¹⁷

Total flavonoid contents of the extracts

Flavonoids, a major group of secondary plant metabolites, represent a broad class of polyphenolic compounds that play crucial roles in the biological activities of many plants. In this study, the total flavonoid

content (TFC) of *Phyllanthus niruri* (PN) and *Bergenia ligulata* (BL) was quantified using the aluminum chloride colorimetric method. Quantification was carried out with reference to a calibration curve of rutin defined by the regression equation $y = 0.112x + 0.0015$ ($R^2 = 0.9893$), and results were expressed as milligrams of rutin equivalents per gram of sample (mg RUT/g) (Figure 2). The TFC was calculated to be 58.80 ± 0.84 mg RUT/g for PN and 56.09 ± 0.48 mg RUT/g for BL (Table 1). These values are consistent with previously reported ranges for these genera. For instance, optimized *Phyllanthus* extracts have been documented to yield flavonoid contents in the tens of mg/g, reaching up to ~76 mg QE/g under ultrasonic extraction, while *Bergenia* species have shown rutin-equivalent values within the tens to high-tens mg/g range. Such comparisons indicate that the present findings are both plausible and well-aligned with published literature.^{15,18}

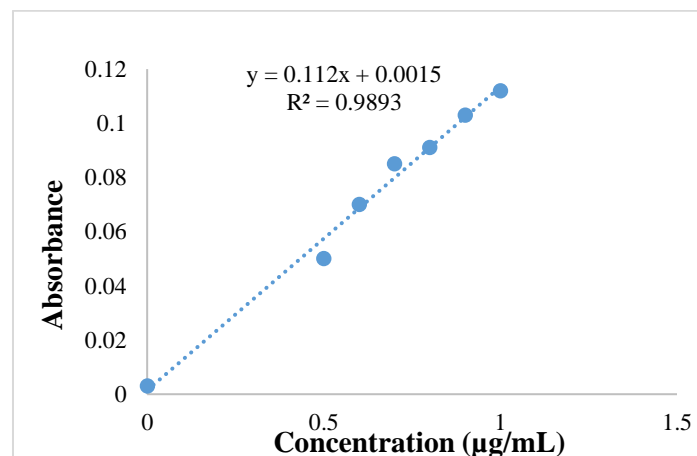


Figure 2: Rutin standard curve

The results further suggest that ethanol serves as an efficient solvent for extracting flavonoid glycosides and aglycones, as previously demonstrated. This can be attributed to the intermediate polarity of ethanol, which effectively dissolves flavonoids that are less polar than many phenolic acids. Beyond their extractability, flavonoids are widely distributed in plant-derived products, including fruits, vegetables, flowers, and seeds, where they contribute significantly to biological functions. Their antioxidant potential is well-documented, encompassing free radical scavenging, metal chelation, and the inhibition of lipid peroxidation. Additionally, flavonoids exert anti-inflammatory effects and modulate cellular signaling by enhancing enzymatic defense pathways such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In recent years, considerable research attention has been directed toward the identification of phenolic compounds and flavonoids from edible plants, underscoring their nutritional, therapeutic, and preventive relevance.^{10,19}

Antioxidant activity of the extracts

The antioxidant activity of *Phyllanthus niruri* (PN) leaf and *Bergenia ligulata* (BL) rhizome ethanolic extracts was evaluated using the conventional DPPH radical scavenging assay as well as the ferric reducing antioxidant power (FRAP) assay. The DPPH method relies on the reduction of the stable, purple-colored DPPH radical, which exhibits a characteristic absorbance at 517 nm. Upon interaction with antioxidant compounds, the radical is reduced, resulting in a measurable decrease in absorbance. Ascorbic acid was employed as the reference standard. The results demonstrated a concentration-dependent increase in antioxidant activity for both extracts. At 500 µg/mL, *P. niruri* exhibited $75.6 \pm 0.50\%$ scavenging activity and *B. ligulata* showed $71.57 \pm 0.06\%$ scavenging activity, values that were not significantly different ($p < 0.05$) from that of ascorbic acid ($77.49 \pm 0.13\%$) (Figure 3).

The high antioxidant activity of both plant extracts, approaching that of

ascorbic acid, suggests that they are rich in bioactive phenolic constituents. These findings are consistent with earlier reports indicating that ethanol is an effective solvent for extracting phenolic compounds, as its intermediate polarity enhances the solubility of flavonoid glycosides and aglycones. Furthermore, ethanol–water mixtures have been shown to broaden the spectrum of extracted phenolics, particularly those of low to medium polarity, supporting the notion that these plants contain diverse and abundant phenolic and flavonoid constituents. The positive correlation observed between total phenolic and flavonoid contents and DPPH radical scavenging further strengthens this interpretation.

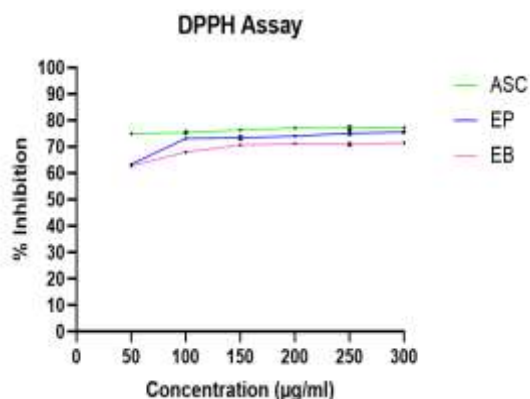


Figure 3: DPPH radical scavenging activity of *Phyllanthus niruri* and *Bergenia ligulata* extracts

Key: ASC: Ascorbic acid, EP - ethanol extract of *Phyllanthus niruri*, EB - ethanol extract of *Bergenia ligulata*

The present results are corroborated by results from previous studies. For example, Okoduwa *et al.* (2024)²⁰ demonstrated that the ethanolic extract of *P. niruri* possessed strong antioxidant capacity, coupled with substantial phenolic and flavonoid contents. Their study also reported weak antibacterial activity against both Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*).²⁰

Similarly, Ezzat *et al.* (2020)² highlighted the potent antioxidant activity of *P. niruri* ethanolic extracts using both DPPH and FRAP assays, linking these effects to hepatoprotective and renoprotective properties. Their work demonstrated that phytochemicals in *P. niruri* alleviated CCl₄-induced hepatotoxicity in HepG2 cells, thereby confirming the therapeutic relevance of its antioxidant constituents.²

On the other hand, the FRAP assay is based on the reduction of the Fe³⁺–ligand complex to its Fe²⁺ form, which subsequently develops a blue coloration in the presence of antioxidants under acidic conditions. This reduction leads to an increase in absorbance measured at 700 nm. The results demonstrated that FRAP activity was concentration-dependent, with antioxidant activity rising proportionally with increasing extract concentration. As illustrated in Figure 4, the FRAP activity of *Phyllanthus niruri* (62.04 ± 0.07%), *Bergenia ligulata* (65.96 ± 0.48%), and ascorbic acid (64.35 ± 0.07%) at 500 µg/mL showed no significant difference ($p < 0.05$) compared to the standard antioxidant. These findings support the principle that compounds with similar polarity and structural characteristics tend to dissolve in solvents of corresponding polarity. The comparable antioxidant activities observed in both the DPPH and FRAP assays reinforce the notion that ethanolic extracts of PN and BL contain similar proportions of phytochemicals with similar polarity, consistent with the results obtained from total polyphenol and flavonoid assays. The comparable performance of these extracts to ascorbic acid highlights their robust antioxidant potential.^{21–23}

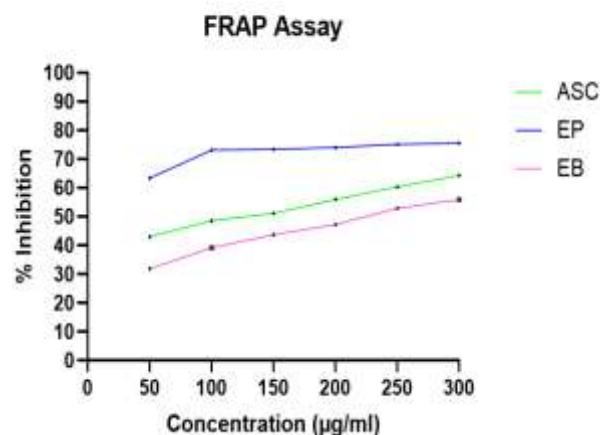


Figure 4: Ferric reducing antioxidant power (FRAP) of *Phyllanthus niruri* and *Bergenia ligulata* extracts

Key: ASC: Ascorbic acid, EP - ethanol extract of *Phyllanthus niruri*, EB - ethanol extract of *Bergenia ligulata*

The results further indicate that the antioxidant capacity of PN and BL increases with concentration, likely due to the presence of “reductones” - potent reducing agents capable of neutralizing free radicals through hydrogen donation. Such compounds are well recognized for their role in mitigating oxidative stress and are frequently implicated in therapeutic strategies against diseases associated with free radical damage. Consequently, PN and BL may contribute to disease prevention by strengthening the body’s antioxidant defense system and could serve as valuable natural supplements.

These outcomes align with previous studies investigating the antioxidant potential of PN and BL through both FRAP and DPPH assays, consistently demonstrating their strong free radical–scavenging activity.^{2,20} The antioxidant efficacy of BL has likewise been validated in multiple studies. For example, Singh *et al.* (2022)¹³ reported that methanol extracts of BL effectively reduced oxidative stress and inhibited calcium oxalate kidney stone formation, underscoring its therapeutic relevance in urolithiasis. They also demonstrated that methanol-based rhizome extracts of BL possessed high antioxidant potential in DPPH assays, with phenolic and flavonoid levels significantly correlated with both antioxidant and antibacterial activities.¹³

Conclusion

Ethanol extracts of the leaves of *Phyllanthus niruri* and rhizomes of *Bergenia ligulata* are rich in antioxidant polyphenols and flavonoids, and they are able to prevent oxidative stress, which could be beneficial in the treatment of a variety of metabolic diseases, such as cancer, Parkinson’s disease, Alzheimer’s disease, cardiovascular disorders, respiratory diseases, urolithiasis, and renal failure. This study provides significant scientific support for the antioxidant properties of the ethanolic extracts of *Phyllanthus niruri* and *Bergenia ligulata*, which could be harnessed as a functional foods or herbal remedies for the treatment or prevention of a variety of ailments.

Conflict of Interest

The authors declare no conflict of interest.

Authors’ Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This work was funded by Research Excellence and Innovation grant (REIG), UCSI University with grant number REIG-FPS-024/003.

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