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Phytochemical Profiling and Free-Radical Scavenging Potential of *Phyllanthus niruri* Whole-Plant Extract: A Laboratory Study

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ABSTRACT:

Background: Oxidative stress arising from an imbalance between reactive oxygen species and endogenous antioxidant defenses contributes to the pathogenesis of a wide spectrum of chronic non-communicable diseases. Medicinal plants used in the Ayurvedic and Siddha systems of South India represent an abundant and inexpensive reservoir of natural antioxidants. *Phyllanthus niruri* L. (locally "keezhanelli" or bhumyamalaki) is a widely used ethnomedicinal herb whose whole-plant preparations enjoy a long-standing reputation for hepatoprotective and antioxidant activity.

Objective: To characterise the phytochemical composition of a *P. niruri* whole-plant hydroalcoholic extract and to quantify it's in vitro free-radical scavenging and ferric-reducing potential relative to ascorbic acid.

Methods: A laboratory-based phytochemical and in vitro antioxidant study was performed. The whole plant was subjected to hydro alcoholic extraction, and percentage yield was recorded across six batches. Qualitative phytochemical screening was undertaken using standard generic tests. Total phenolic content (TPC) was measured by the Folin–Ciocalteu method (mg Gallic acid equivalents [GAE]/g) and total flavonoid

content (TFC) by the aluminum chloride method (mg quercetin equivalents [QE]/g). Antioxidant activity was evaluated by DPPH, ABTS and FRAP assays across 10–100 μg/mL in triplicate, with ascorbic acid as reference standard. Half-maximal inhibitory concentrations (IC50) were derived by four-parameter logistic regression. Data are expressed as mean ± standard deviation, with $p<0.05$ considered significant.

Results: The mean extraction yield was 12.30 ± 1.12% (dark green-brown, semi-solid extract). Screening revealed abundant flavonoids, phenolics, tannins, terpenoids, glycosides and carbohydrates; steroids were absent. TPC was 119.38 ± 2.00 mg GAE/g and TFC 62.88 ± 5.96 mg QE/g. Radical scavenging was concentration-dependent, reaching 55.33% (DPPH) and 60.75% (ABTS) at 100 μg/mL. Extract IC50 values were 52 μg/mL (DPPH) and 46 μg/mL (ABTS) versus 28 μg/mL for ascorbic acid, and ferric-reducing power rose to 217.13 μmol Fe(II)/g. The extract was consistently less potent than ascorbic acid but showed substantial, reproducible activity.

Conclusion: The *P. niruri* whole-plant extract is rich in phenolic and flavonoids and exhibits moderate-to-high, concentration-dependent free-radical scavenging and ferric-reducing capacity. These findings provide a plausible mechanistic basis for, and low-cost laboratory support of, its traditional South Indian medicinal use.

1. INTRODUCTION

Reactive oxygen species (ROS) and other free radicals are continuously generated as by-products of aerobic metabolism, and at physiological concentrations they participate in cell signaling and host defense. When their production outpaces the capacity of endogenous enzymatic and non-enzymatic antioxidant defenses, the resulting state of oxidative stress damages lipids, proteins and nucleic acids. This cumulative oxidative injury is now recognized as a common upstream mechanism in the pathogenesis of numerous chronic non-communicable diseases, including atherosclerosis and cardiovascular disease, diabetes mellitus and its complications, neurodegenerative disorders, chronic liver disease and cancer [1,2]. Dietary and plant-derived antioxidants, which can scavenge free radicals, chelate transition metals and reinforce endogenous defenses, are therefore of considerable therapeutic and preventive interest.

Medicinal plants have long served as a principal source of antioxidant phytochemicals, particularly polyphenols and flavonoids, whose conjugated aromatic structures and hydroxyl substituents enable them to donate hydrogen atoms or electrons to reactive species and to stabilize the resulting radicals through resonance [3,4]. India possesses one of the richest traditions of plant-based medicine in the world, codified in the Ayurvedic and Siddha systems that remain widely practiced across South India. Within this tradition, hundreds of species are employed for conditions now understood to have an oxidative-stress component, yet only a fraction have been systematically characterized for their phytochemical constituents and antioxidant activity using modern in vitro assays. Bridging this gap is important both for validating traditional practice and for identifying candidate species for further pharmacological development.

Phyllanthus niruri L. (family Phyllanthaceae) is a small, glabrous annual herb that grows abundantly as a weed across the plains and cultivated fields of South India. It is known in Tamil as "keezhanelli" and in Sanskrit/Ayurveda as bhumyamalaki, and preparations of the whole plant have been used for generations in the management of jaundice, liver disorders, urinary complaints, diabetes and inflammatory conditions. The pharmacology and phytochemistry of the genus have attracted growing scientific attention, and *P. niruri* in particular has been reported to contain a diverse array of bioactive constituents—lignans, flavonoids, tannins, phenolic acids and terpenoids—associated with hepatoprotective, antihypertensive, antidiabetic, antimicrobial, anti-inflammatory and wound-healing activities [5,6]. The extraction solvent and drying method strongly influence the recovered metabolite profile and, consequently, the measured bioactivity [7], underscoring the value of standardised extraction and characterization.

A single antioxidant assay is rarely sufficient to characterize a complex plant matrix, because different assays interrogate distinct chemistries—hydrogen-atom transfer, single-electron transfer and metal reduction—and a given constituent may perform differently in each. A panel comprising the DPPH radical, the ABTS radical cation and the ferric-reducing FRAP reaction therefore offers a more balanced and internally cross-validating assessment than any test in isolation, while remaining inexpensive and instrumentally undemanding [8,9]. Coupling such a panel with quantitative estimation of total phenolic and flavonoids allows the measured activity to be interpreted against the underlying chemistry rather than reported in isolation.

Despite this substantial body of work, locally contextualized laboratory characterization that couples phytochemical screening with quantitative phenolic/flavonoid estimation and a panel of complementary antioxidant assays remains valuable, particularly as a low-cost approach suited to resource-limited academic settings in South India. The present study was therefore undertaken to profile the phytochemical composition of a *P. niruri* whole-plant hydro alcoholic extract and to quantify its free-radical scavenging (DPPH, ABTS) and ferric-reducing (FRAP) capacity in comparison with the reference antioxidant ascorbic acid [10]. We hypothesized that the extract would contain abundant phenolic and flavonoids and would display concentration-dependent antioxidant activity providing a mechanistic rationale for its traditional use.

2. MATERIALS AND METHODS

2.1 Plant collection and authentication

Whole plants of *Phyllanthus niruri* L. were collected during [v.no 4231] from the region surrounding South India. The material was taxonomically authenticated at the Department of Pharmacognosy/Botany, and a voucher specimen [v.no 4231] was deposited in the departmental herbarium for future reference. Collected plants were washed, shade-dried, and coarsely powdered prior to extraction.

2.2 Preparation of the hydroalcoholic extract

The dried whole-plant powder was subjected to hydro alcoholic extraction using an ethanol–water solvent system. The extract was concentrated under reduced pressure to yield a dark green-brown, semi-solid mass. The percentage extraction yield was calculated relative to the weight of the starting dry powder for each of six independent batches, and the mean ± standard deviation was recorded. The concentrated extract was stored protected from light and moisture until analysis, and working solutions were freshly prepared in the appropriate solvent for each assay.

2.3 Qualitative phytochemical screening

The extract was screened for major secondary-metabolite classes using standard qualitative reactions described generically as follows: alkaloids (Mayer's and Wagner's reagent tests), flavonoids (alkaline reagent/Shinoda tests), phenolic and tannins (ferric chloride and gelatin tests), saponins (persistent-froth test), terpenoids and steroids (Salkowski/Liebermann–Burchard tests), glycosides (Keller–Killiani test), proteins (biuret test) and carbohydrates (Molisch and Fehling tests). Each class was recorded as present or absent, with the qualifier "trace" applied where a positive reaction was observed inconsistently across replicates.

2.4 Total phenolic and total flavonoid content

Total phenolic content (TPC) was determined by the Folin–Ciocalteu colorimetric method using gallic acid as the calibration standard, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). Total flavonoid content (TFC) was measured by the aluminum chloride colorimetric method using quercetin as the calibration standard, with results expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). All determinations were carried out in triplicate.

2.5 In vitro antioxidant assays

Three complementary assays were performed across a concentration range of 10–100 μg/mL, each in triplicate, with ascorbic acid as the reference standard.

DPPH radical scavenging assay. The capacity of the extract to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the decrease in absorbance at 517 nm and expressed as percentage inhibition relative to a reagent control.

ABTS radical cation scavenging assay. Decolourisation of the pre-formed 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was measured at 734 nm and expressed as percentage inhibition.

FRAP assay. The ferric reducing antioxidant power was determined by the reduction of the ferric-tripyridyltriazine complex, with results expressed as micromoles of Fe(II) equivalents per gram of extract (μmol Fe(II)/g).

2.6 Statistical analysis

All assays were performed in triplicate and results are expressed as mean ± standard deviation (SD). Concentration–response relationships for the DPPH and ABTS assays were modelled by four-parameter logistic (4PL) regression, from which the half-maximal inhibitory concentration (IC50) and its 95% confidence interval (CI) were derived for the extract and for ascorbic acid. A p-value <0.05 was considered statistically significant.

3. RESULTS

3.1 Extraction yield, total phenolic and total flavonoid content

Hydro alcoholic extraction of the *P. niruri* whole plant produced a dark green-brown, semi-solid extract with a mean yield of 12.30 ± 1.12% across six batches (range 10.67–13.97%). The extract was rich in phenolic and flavonoid constituents, with a TPC of 119.38 ± 2.00 mg GAE/g and a TFC of 62.88 ± 5.96 mg QE/g (Table 1). The high phenolic and flavonoid loading is consistent with the qualitative screening findings and provides a plausible chemical basis for the antioxidant activity described below.

Table 1. Extraction yield and total phenolic/flavonoid content of *Phyllanthus niruri* whole-plant hydro alcoholic extract.

Parameter	Value (mean ± SD)	Notes
Extraction yield (%)	12.30 ± 1.12	n = 6 batches; range 10.67–13.97%
Extract appearance	Dark green-brown, semi-solid	—
Total phenolic content (mg GAE/g)	119.38 ± 2.00	Folin–Ciocalteu; gallic acid standard
Total flavonoid content (mg QE/g)	62.88 ± 5.96	Aluminium chloride; quercetin standard

3.2 Qualitative phytochemical profile

Qualitative screening confirmed a metabolite-rich extract (Table 2). Flavonoids, phenolics, tannins, terpenoids, glycosides and carbohydrates were consistently present. Alkaloids and sapiens gave positive reactions in trace amounts (detected in a single replicate each), and proteins were present only in trace amounts. Steroids were not detected in any replicate. This profile, dominated by phenolic and flavonoid classes, is in keeping with previous phytochemical characterizations of the species [1,2,3].

Table 2. Qualitative phytochemical screening of *Phyllanthus niruri* whole-plant extract.

Phytoconstituent	Result
Flavonoids	Present (+)
Phenolics	Present (+)
Tannins	Present (+)
Terpenoids	Present (+)
Glycosides	Present (+)
Carbohydrates	Present (+)
Alkaloids	Trace (present in one replicate)
Saponins	Trace (present in one replicate)
Proteins	Trace
Steroids	Absent (−)

3.3 Free-radical scavenging activity: DPPH and ABTS

Both the extract and ascorbic acid displayed clear concentration-dependent radical scavenging across 10–100 μg/mL (Table 3, Figure 1). In the DPPH assay, extract inhibition rose from 12.60% at 10 μg/mL to 55.33% at 100 μg/mL, while ascorbic acid

rose from 23.86% to 73.13% over the same range. The ABTS assay showed a similar pattern, with extract inhibition increasing from 15.04% to 60.75% and ascorbic acid from 25.18% to 71.01%. Across every concentration and in both assays the extract was less potent than the reference standard but retained substantial activity, surpassing 50% inhibition at the higher concentrations.

The IC50 values derived by four-parameter logistic regression quantified this relationship. For the extract, the DPPH IC50 was 52 µg/mL (95% CI 47.0–57.5) and the ABTS IC50 was 46 µg/mL (95% CI 41.5–50.8), compared with 28 µg/mL (95% CI 25.0–31.0) for ascorbic acid in both assays. The slightly lower ABTS IC50 indicates marginally greater efficiency of the extract against the ABTS radical cation than against the DPPH radical.

Table 3. DPPH and ABTS radical scavenging activity (% inhibition) of *Phyllanthus niruri* extract versus ascorbic acid standard, with IC50 values.

Concentration (µg/mL)	DPPH – Extract	DPPH – Standard	ABTS – Extract	ABTS – Standard
10	12.60	23.86	15.04	25.18
20	23.25	36.22	26.23	37.85
40	35.93	53.62	42.02	53.11
60	44.23	60.33	49.87	60.96
80	50.20	69.47	56.89	66.62
100	55.33	73.13	60.75	71.01
IC50 (µg/mL)	**52 (47.0–57.5)**	**28 (25.0–31.0)**	**46 (41.5–50.8)**	**28 (25.0–31.0)**

Values are mean of triplicate determinations; IC50 by four-parameter logistic regression (95% CI in parentheses).

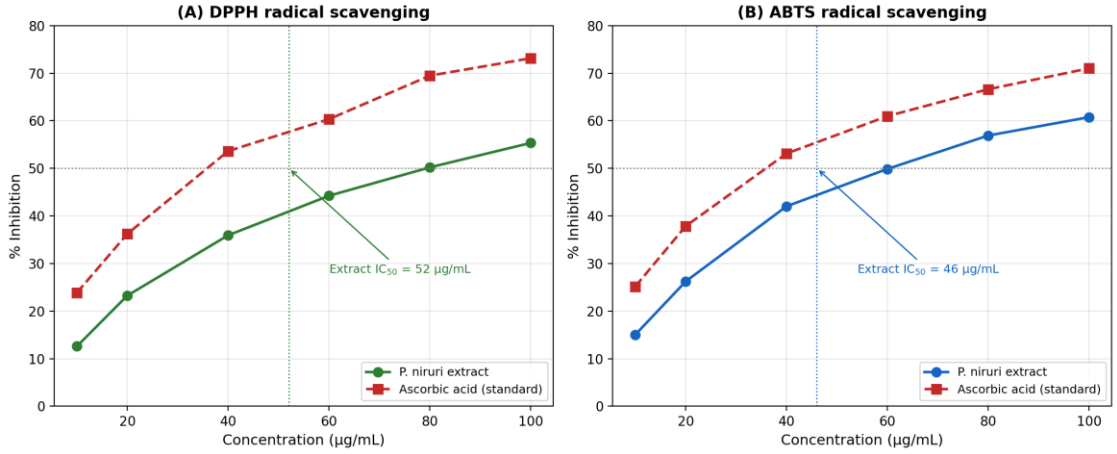

Figure 1. Concentration-dependent DPPH (A) and ABTS (B) radical scavenging (% inhibition) by *Phyllanthus niruri* whole-plant extract and ascorbic acid standard over 10–100 µg/mL. Dotted markers indicate the extract IC50 values (DPPH 52 µg/mL; ABTS 46 µg/mL). Ascorbic acid was consistently more potent (IC50 28 µg/mL in both assays).

3.4 Ferric-reducing antioxidant power (FRAP)

The FRAP assay corroborated the radical-scavenging findings, demonstrating a strong, concentration-dependent ferric-reducing capacity (Table 4, Figure 2). Extract FRAP values increased steeply from 15.79 µmol Fe(II)/g at 10 µg/mL to 217.13 µmol Fe(II)/g at 100 µg/mL, following the same rank order as ascorbic acid, which rose from 35.17 to 309.78 µmol Fe(II)/g. The parallel, monotonic increase across all three assays indicates internally concordant antioxidant behavior and a reducing capacity that, while lower than that of ascorbic acid, is substantial in absolute terms.

Table 4. Ferric reducing antioxidant power (FRAP, µmol Fe(II)/g) of *Phyllanthus niruri* extract versus ascorbic acid standard.

Concentration (µg/mL)	FRAP – Extract	FRAP – Standard
10	15.79	35.17
20	39.80	66.47
40	94.96	121.37
60	129.65	182.02
80	170.31	253.10
100	217.13	309.78

Values are mean of triplicate determinations.

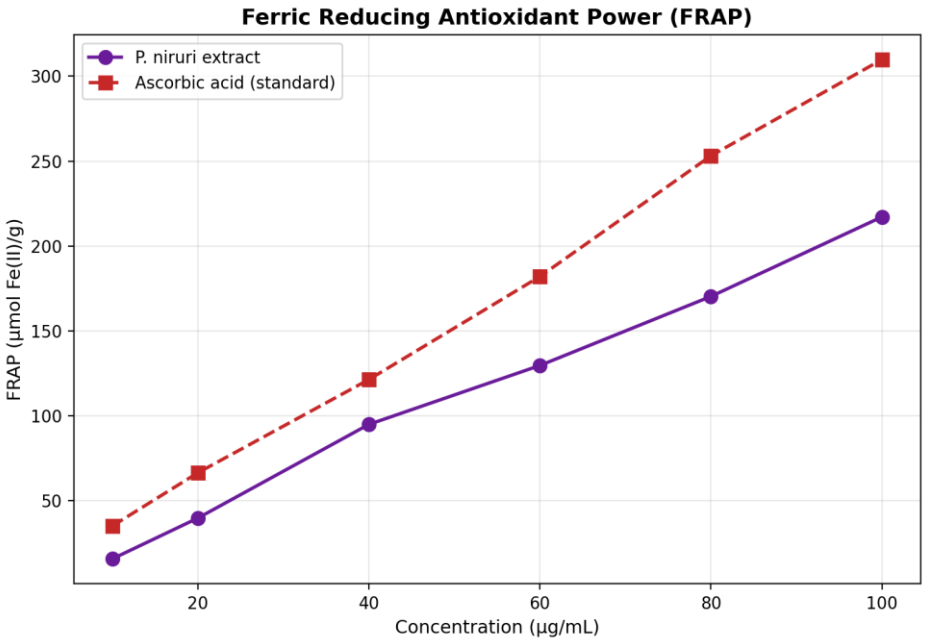

Figure 2. Ferric reducing antioxidant power (FRAP, µmol Fe(II)/g) of *Phyllanthus niruri* whole-plant extract and ascorbic acid standard across 10–100 µg/mL. Both preparations show concentration-dependent reducing capacity, with the extract consistently below the reference standard.

4. DISCUSSION

The present laboratory study characterized a *Phyllanthus niruri* whole-plant hydroalcoholic extract and demonstrated that it is rich in phenolic and flavonoid constituents and possesses moderate-to-high, concentration-dependent free-radical scavenging and ferric-reducing activity. The high total phenolic content (119.38 mg GAE/g) and total flavonoid content (62.88 mg QE/g) are the most likely chemical determinants of the observed activity, and the internal concordance of three mechanistically distinct assays—two based on radical scavenging (DPPH, ABTS) and one on single-electron reduction (FRAP)—strengthens confidence in this interpretation.

Phenolic–antioxidant relationship. Polyphenols and flavonoids are widely regarded as the principal antioxidant constituents of plant extracts, acting through hydrogen-atom transfer, single-electron transfer and metal-chelation mechanisms. The prominence of flavonoids, phenolic and tannins in the qualitative screen, together with the high quantitative TPC and TFC values, provides a coherent mechanistic basis for the measured radical-scavenging and reducing activity. This aligns with reports

linking the phenolic content of *P. niruri* and related medicinal plant extracts to their antioxidant and anti-inflammatory effects [9], and with metabolomics analyses showing that the recoverable bioactive profile—and hence activity—depends strongly on solvent and drying method [11-14].

Concordance across DPPH, ABTS and FRAP. The three assays produced consistent rank ordering, with the extract reproducibly less potent than ascorbic acid yet retaining substantial activity. The extract IC50 values (DPPH 52 µg/mL; ABTS 46 µg/mL) fall within the moderate-to-high range typically reported for crude hydro alcoholic plant extracts, and the marginally lower ABTS IC50 is a common observation attributable to the ABTS radical cation's accessibility to a broader range of both hydrophilic and lipophilic antioxidants. The steep, monotonic FRAP response reinforces that the extract's activity reflects genuine electron-donating capacity rather than assay-specific artefact. Comparable antioxidant behavior has been described for *P. niruri*-derived systems, including endophyte-associated metabolites and extracts evaluated for anticancer, antidiabetic and antimicrobial endpoints [9,15].

Relation to reported pharmacology and traditional use. The antioxidant profile observed here is mechanistically relevant to the traditional applications of *P. niruri* in South Indian Ayurveda and Siddha, where the whole plant ("keezhanelli"/bhumyamalaki) is valued particularly for liver disorders. Oxidative stress is central to many forms of hepatic injury, and experimental studies have repeatedly linked the phytochemical richness of *P. niruri* to hepatoprotection—including protection against carbon tetrachloride-induced liver injury—and to modulation of oxidative stress and inflammation in models of vascular function and dermal wound healing [11,16]. A robust capacity to scavenge free radicals and reduce ferric ions, as demonstrated here, offers a plausible upstream explanation for these organ-level benefits and supports the rationale for the plant's continued ethno medicinal use across South India [14,17]. It is notable that the extract achieved roughly two-thirds to four-fifths of the reference standard's activity at the top of the tested range despite being a crude, unfractionated preparation containing non-antioxidant ballast such as carbohydrates and proteins; the true specific activity of the responsible phenolic and flavonoid constituents is therefore likely to be higher than the crude-extract figures suggest. This observation is consistent with the widely reported dependence of *P. niruri* bioactivity on the recovered metabolite fraction and reinforces the value of solvent-optimized hydro alcoholic extraction for maximizing antioxidant yield [18,19,3].

Implications. The combination of qualitative screening, quantitative TPC/TFC estimation and a multi-assay antioxidant panel provides a reasonably comprehensive first-pass antioxidant fingerprint that can guide subsequent bioassay-guided fractionation and mechanistic work [20-23].

5. CONCLUSION

The *Phyllanthus niruri* whole-plant hydro alcoholic extract examined in this laboratory study was rich in phenolic and flavonoids (TPC 119.38 mg GAE/g; TFC 62.88 mg QE/g) and exhibited concentration-dependent, moderate-to-high free-radical scavenging (DPPH IC50 52 µg/mL; ABTS IC50 46 µg/mL) and ferric-reducing power. Although consistently less potent than ascorbic acid, the extract showed substantial and internally concordant antioxidant activity, for which its high phenolic and flavonoid content provides a plausible mechanistic basis. These findings offer low-cost laboratory support for the traditional South Indian medicinal use of *P. niruri* and justify further phytochemical and pharmacological investigation.

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