

Research Article

Phytochemical Profiling and Antioxidant Potential of *Phyllanthus acidus* and *Phyllanthus maderaspatensis* from South Odisha, India

Arpita Panda¹ , Aparna Yerra² , Subhashree Aparajita^{3,*} , Manoj Das² 

¹Department of Life Science, Gandhi Institute of Engineering and Technology University, Gunupur, India

²Department of Biotechnology, Gandhi Institute of Engineering and Technology University, Gunupur, India

³Department of Botany, Binayak Acharya College, Berhampur, India

Abstract

The genus *Phyllanthus* comprises several medicinally important plant species recognized for their diverse phytochemical constituents and wide- ranging therapeutic applications. *Phyllanthus acidus* (star gooseberry) and *Phyllanthus maderaspatensis* (Madras leaf-flower) are naturally distributed in the biodiversity-rich regions of South Odisha, India, but are relatively underexplored and traditionally used by indigenous communities only. Antioxidants, especially plant-derived bioactive molecules neutralize these radicals and are considered safer alternatives to synthetic antioxidants. Scientific evidence on the phytochemical composition and radical scavenging potential of the root extracts of these two species remains limited. So, the primary objective of this study was to screen the phytochemical constituents and evaluate the *in-vitro* antioxidant activities of methanolic root extracts of both *P. acidus* and *P. maderaspatensis* collected from South Odisha, India. Qualitative phytochemical screening revealed the presence of diverse secondary metabolites, including flavonoids, tannins, alkaloids, saponins, phenols, terpenoids, glycosides and steroids in both of the *Phyllanthus* species, with notable variation in their abundance. *In-vitro* antioxidant assay evaluated using the DPPH and FRAP assay indicated strong antioxidant activity for *P. acidus* over *P. maderaspatensis*.

Keywords

Phyllanthus acidus, *Phyllanthus maderaspatensis*, Phytochemical Screening, DPPH Assay, FRAP Assay

1. Introduction

Over the years, humans have been encountering many diseases, discomfort and health challenges and have always struggled to overcome them with various approaches. Among the various factors contributing to disease, one main cause is oxidative stress, which is produced when reactive oxygen species

(ROS) exceed antioxidant levels in the body. ROS can be scavenged by both natural (plant-derived) and synthetic (chemical) antioxidants [1]. Plants are an abundant source of secondary metabolites such as alkaloids, phenols, polyphenols, flavonoids,

*Correspondence: Subhashree Aparajita (subha.aparajita@gmail.com)

Received: 31 December 2025; Accepted: 19 January 2026; Published: 17 October 2026



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and many others that demonstrate antioxidant potential. However, due to concerns about potential toxicity, adverse side effects, and high cost, natural antioxidants derived from plants are preferred over synthetic antioxidants. The genus *Phyllanthus* L. (family: Phyllanthaceae) represents one of the most important groups of medicinal plants, comprising more than 800 species, with 53 species reported from India, out of which 23 are endemic. Species of this genus have long been a part of traditional medicinal systems [2, 3]. Two species of this genus, *Phyllanthus acidus* and *Phyllanthus maderaspatensis*, are widely distributed in the biodiversity-rich regions of South Odisha, India. However, they remain underexplored and scientific evidence on the phytochemical composition and antioxidant potential of these two species remains limited. To address this knowledge gap, the present study focuses on the qualitative and quantitative phytochemical analysis of root extracts of *P. acidus* and *P. maderaspatensis* and the evaluation of their antioxidant

potential, intending to scientifically validate and harness their ethnomedicinal uses for future applications.

2. Materials and Methods

2.1. Collection and Preparation of Root Extract

Roots of *Phyllanthus acidus* and *Phyllanthus maderaspatensis* were identified, authenticated and collected from different natural locations across southern Odisha, India (Figure 1). 50 gm of dried root powder were subjected to extraction using 300 ml of methanol, ethyl acetate and n-hexane using a Soxhlet apparatus for the recovery of both polar and non-polar phytochemicals. The root extracts were concentrated under reduced pressure using a rotary evaporator at 45 °C and the dried extracts were preserved at 4 °C for further use.



Figure 1. (a) Morphological representation of *P. acidus*. (b) Morphological representation of *P. maderaspatensis*.

2.2. Phytochemical Analysis

Qualitative phytochemical analysis showing the presence or absence of bioactive compounds in the root extract of *P. acidus* and *P. maderaspatensis* was carried out by the standard method with slight modification [4]. Total Alkaloid Content (TAC) was quantified by the spectrophotometric method [5] based on the reaction between alkaloid and bromocresol green (BCG). The absorbance of the complex in chloroform was measured at 470 nm. A standard curve of caffeine was plotted for quantification, taking distilled water as a blank. Folin–Ciocalteu method [6], taking Gallic acid as standard, was used to quantify Total phenolic content (TPC) with absorbance recorded at 750 nm. Results were expressed as mg of gallic acid equivalents (GAE)/g dry weight. Total flavonoid content (TFC) was determined using Quercetin as a standard and taking the absorbance at 510 nm [7]. Results were expressed as mg quercetin equivalents (QE)/g dry extract. The Folin–Ciocalteu method [8] was used to quantify Total Tannin content (TTC) using Tannic acid standards (20–100 µg/ml) for a calibration curve with absorb-

ance measured at 700 nm against a blank. Results were expressed as mg tannic acid equivalents/g dry sample.

2.3. Antioxidant Activity

The antioxidant potential of *Phyllanthus acidus* and *Phyllanthus maderaspatensis* was measured by DPPH [9] and FRAP [10] assay. Both the assay was performed in quadruplicate and a microplate reader (iMark, Bio-Rad) was used to measure absorbance, and GraphPad Prism 6 software was used to determine IC₅₀ values. Ascorbic acid was taken as the reference standard for the DPPH assay and absorbance was measured at 517 nm after 30 minutes of incubation in the dark. For FRAP activity, Trolox was used as the standard and Absorbance was measured at 630 nm. Antioxidant activity by FRAP was expressed as µg/ml of Trolox equivalent.

2.4. Statistical Analysis

All experiments were arranged in a completely randomised design (CRD) as described by [11]. Differences were consid-

ered statistically significant at $p < 0.005$. Statistical computations were performed using MS Excel, and results were expressed as mean $\pm$ standard deviation.

3. Results

3.1. Phytochemical Screening and Quantification

Our study revealed the presence and variability of phytoconstituents in the root extracts of both *P. acidus* and *P.*

maderaspatensis using three different extraction solvents: a polar solvent (methanol), a medium-polar solvent (ethyl acetate), and a non-polar solvent (n-hexane). Qualitative analysis of the different solvent extracts revealed the presence of several bioactive constituents, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, carbohydrates, glycosides, and steroids, with variation in their abundance between the two species. The results of the qualitative phytochemical analysis are listed in Table 1.

Table 1. Qualitative phytochemical analysis of root extracts of *P. acidus* and *P. maderaspatensis* using different solvent systems.

Sl. no	Name of Metabolite	Name of test	Methanol		Ethyl Acetate		n-Hexane	
			PA	PM	PA	PM	PA	PM
1	Alkaloids	Mayers test	+++	+++	-	-	-	+
		Wagners test	+++	+++	+	+	+	-
2	Flavonoids	Ammonia Test	++	++	-	+	+	+
3	Tannins	Ferric chloride test	+++	+	-	+	-	-
4	Saponins	Foam test	+	+	-	-	+	+
5	Phenols	Ferric chloride test	+++	++	+	+	-	-
6	Terpenoids	Salkowski test	+	+	+	+	-	-
7	Phytosterols	Salkowski Test	+	-	-	-	-	-
8	Carbohydrates	Molish's test	+	+	+	+	+	+
9	Proteins	Biuret test	-	-	-	-	+	+
10	Glycosides	Sulphuric acid test	+	+	+	-	+	-
11	Steroids	Liebermann–Burchard test	+	+	+	-	+	-

Note: +++= strongly present, ++= moderately present, += present, -= absence; PA= *Phyllanthus acidus* and PM = *Phyllanthus maderaspatensis*

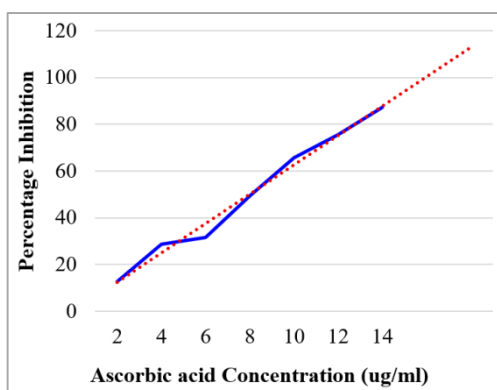
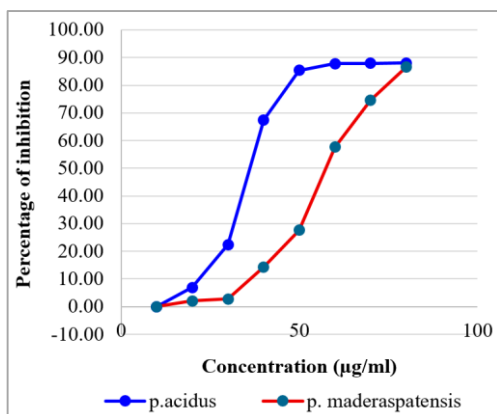
Among the extracts tested, the methanolic root extract of both species exhibited the greatest diversity of phytochemicals as compared to the ethyl acetate and n-hexane extracts. Polysterol were detected only in *P. acidus*, while proteins were absent in the root extracts of both species. Quantitative phytochemical analysis of Plant extract using a spectrophotometer provides the concentration of bioactive molecules, which is essential for determining the potency and dosage of the extract. The atropine sulphate standard calibration curve displayed a linear regression of $Y = 0.0066x + 0.053$ and $R^2 = 0.993$. The unit of measurement for the

total alkaloids was mg/g Atropine sulphate equivalent. The linear regression of $Y = 0.0303x + 0.273$ and $R^2 = 0.968$ was displayed by the standard calibration curve of quercetin. The total flavonoid content was represented as mg/g quercetin equivalent. The linear regression of $Y = 0.098 + 0.049$ and $R^2 = 0.956$ was displayed by the standard calibration curve of gallic acid. Similarly, linear regression of $Y = 0.164 - 0.127$ and $R^2 = 0.979$ was displayed by the standard calibration curve of Tannic acid. The quantities of TPC, TFC, TAC and TC are presented as mean $\pm$ SD of triplicates, as shown in Table 2.

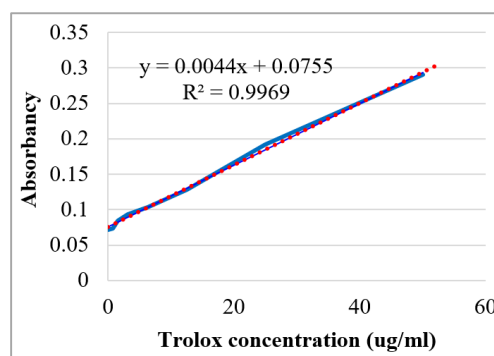
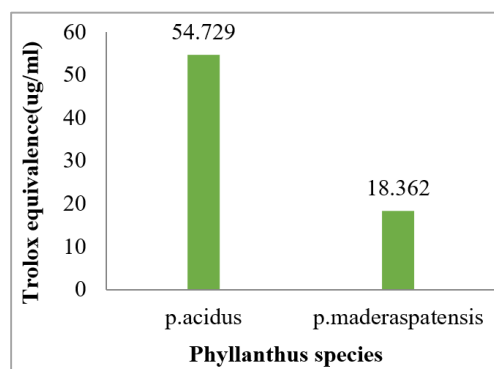
Table 2. Quantitative phytochemical contents of methanolic root extracts of *p.acidus* and *P. maderaspatensis*.

Sl. No.	Name of plant species	Part used	Extraction solvent	Total phenolic content mg (GAE)/g PE	Total flavonoid content mg QE/g PE	Total alkaloid content mg/g of mg AE/g PE	Total tannin content Mg TAE/g PE
1	<i>P.acidus</i>	Root	Methanol	181.36±3.7	81.0±0.32	78±0.55	2.87±1.24
2	<i>P.maderaspatensis</i>	Root	Methanol	28.42±0.65	36±0.62	54±0.32	0.956±0.39

3.2. Antioxidant Activity

**Figure 2.** Ascorbic acid standard calibration curve used for DPPH assay.**Figure 3.** Comparative DPPH radical scavenging activity of *Phyllanthus acidus* and *Phyllanthus maderaspatensis*.

DPPH radical scavenging activity of the root extracts was evaluated using ascorbic acid as the reference standard (Figure 2). Ascorbic acid exhibited a clear concentration-dependent increase in DPPH radical scavenging activity with $IC_{50} = 8.962 \pm 0.023$ $\mu\text{g/ml}$. Among the test species, *Phyllanthus acidus* showed markedly higher antioxidant activity ($IC_{50} = 26.78$ $\mu\text{g/mL}$) than *Phyllanthus maderaspatensis* ($IC_{50} = 175.2 \pm 0.039$ $\mu\text{g/mL}$) (Figure 3).

**Figure 4.** Trolox standard calibration curve used for FRAP assay.**Figure 5.** Ferric reducing antioxidant power (FRAP) of methanolic root extracts of *Phyllanthus acidus* and *Phyllanthus maderaspatensis* expressed as Trolox equivalents ($\mu\text{g/ml}$).

Trolox standard calibration curve (Figure 4) was used for the FRAP assay. A progressive discolouration from purple to yellow indicates increasing antioxidant activity with rising extract concentration. At a concentration of 1 $\mu\text{g/mL}$, the root extract of *Phyllanthus acidus* exhibited a ferric reducing antioxidant power of 54.73 $\mu\text{g/mL}$ Trolox equivalents, whereas *Phyllanthus maderaspatensis* showed a lower FRAP value of 18.362 $\mu\text{g/mL}$ Trolox equivalents (Figure 5).

4. Discussion

Phyllanthus genus, having essential phytochemicals, plays a

great role in preventing and curing numerous human ailments [12]. Environmental conditions, soil type and plant parts greatly affect phytochemical profile [13]. Qualitative and quantitative analyses allow quick and correct screening of different germplasm to find the most bioactive-rich plants before committing to expensive quantitative extraction or clinical trials. The composition and yield of natural compounds in plant extracts are greatly influenced by the extraction solvent [14]. Variability in extract yield depends on the nature, quantity of secondary metabolites present and the polarity of the extraction solvent [15]. As per recent studies, the *Phyllanthus* genus has emerged as a natural alternative to synthetic antioxidants. DPPH and FRAP assays are generally used, which measure different chemical mechanisms of free radical neutralization [16]. The DPPH assay measures the ability of a substance to donate a hydrogen atom or electron to neutralize a stable free radical, whereas the FRAP assay measures the "reducing power" of an extract. A lower IC₅₀ (higher potency) of 41.23 µg/ml is shown by *Phyllanthusemblica* seed extract than the synthetic antioxidants. High DPPH and FRAP inhibition activity of *P. niruri* and *P. acidus* correlates with their higher content of total flavonoid and phenolic content [17]. Antioxidant activity of *P. emblica* seed extracts is retained after baking at 180 °C for 2 hours, which makes them superior to synthetic antioxidants for food preservation [18]. Reports show that antioxidants obtained from *Phyllanthus* species prevent oxidative damage of DNA caused by different mutagens [19, 20].

5. Conclusion

The results show that the methanolic root extracts of *Phyllanthus acidus* and *Phyllanthus maderaspatensis* are a rich source of bioactive compounds. Higher free radical scavenging and reducing power is shown by *Phyllanthus acidus* over *Phyllanthus maderaspatensis*. The richer phytochemical profile, particularly the higher abundance of phenolic compounds and alkaloids, as well as the presence of polysterol corelates with enhanced antioxidant activity of *P. acidus*. Future research is necessary to isolate, identify, characterize, and clarify the structure of biologically active substances that will lead to the development of new medications for the treatment of various ailments.

Abbreviations

ROS	Reactive Oxygen Species
TAC	Total Alkaloid Content
BCG	Bromocresol Green
TPC	Total Phenolic Content
GAE	Gallic Acid Equivalent
TFC	Total Flavonoid Content
QE	Quercetin Equivalent
TCC/TC	Total Tannin Content
DPPH	2,2-diphenyl-1-picrylhydrazyl

FRAP	Ferric Reducing Antioxidant Power
IC ₅₀	Half Maximal Inhibitory Concentration
AE	Atropine Equivalent
CRD	Completely Randomized Design
SD	Standard Deviation
PE	Plant Extract
MEPA	Methanolic Extract of <i>Phyllanthus acidus</i>
MEPM	Methanolic Extract of <i>Phyllanthus maderaspatensis</i>
PA	<i>Phyllanthus acidus</i>
PM	<i>Phyllanthus maderaspatensis</i>

Acknowledgments

The author expresses gratitude to the Department of Life Sciences, GIET University, Gunupur, for providing the necessary facilities and guidance during this research. I am also thankful to the Department of Botany, Binayak Acharya Govt. College, Berhampur, for allowing me to carry out research work and for continuous support throughout the study.

Author Contributions

Arpita Panda: Data curation, Writing – original draft

Y. Aparna: Supervision, Investigation

Subhashree Aparajita: Conceptualization, Resources, Writing – review & editing

Monoj Das: Visualization, Resources

Funding

This work is not supported by any external funding.

Data Availability Statement

The data is available from the corresponding author upon reasonable request. The data supporting the outcome of this research work have been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography



Arpita Panda is a Research scholar pursuing her Ph.D. in Botany at the School of Life Sciences, GIET University, Gunupur. She holds an M.Phil degree in Botany. Her doctoral research focuses on the evaluation of phytochemical and pharmacological activities of *Phyllanthus* species found in south odisha

aiming to validate their ethnomedicinal uses. Her research interests include phytochemical screening, secondary metabolite profiling, antimicrobial, antioxidant, anticancer, and antidiabetic activity studies and other pharmacological studies of medicinal plants. She has participated in several national and international conferences and has one research article in an international journal. She is actively engaged in field surveys of the Eastern ghat region and laboratory-based natural product research.



Aparna Yerra is currently working as an Assistant Professor at GIET University. She completed her M.Sc. and Ph.D. in Biotechnology from GITAM University. Her research contributions primarily focus on polyamines and sericulture, and she has published several research articles in these areas.

She has presented her research work at numerous national and international conferences and received the Young Scientist Conclave Award in recognition of her contributions. She was subsequently selected for the DST-SERB National Post-Doctoral Fellowship, during which she worked on silk-based biomaterials. Aparna also served as a Research Scientist at MNR Medical College and worked as a faculty member at the Central University of Andhra Pradesh. Her current research interests are centred on sericin protein and silk-based biomaterials.



Subhashree Aparajita is a dedicated academician and researcher currently serving as an Assistant Professor of Botany at Binayak Acharya (Govt.) College, Berhampur, Odisha. She completed her PhD in Botany from Utkal University, Bhubaneswar in 2013. Aparajita has over a decade of

experience in teaching and mentoring students. Aparajita has been actively involved in research with a strong background in molecular biology. She is carrying out a research project funded by OSHEC under the OURIIP scheme. Her work bridges the gap between traditional botany and molecular biotechnology. She has a significant number of research papers published in reputed national and international journals. She has been awarded as the best NSS officer from Berhampur University for her social service and leadership.



Manoj Das is an Associate professor and Head of the department of Biotechnology at GIET university. He obtained his PhD in Botany from Berhampur University, Berhampur in 2004. He received the Young

Scientist Award at the Orissa Bigyan Congress in 2002. He holds life memberships in CSI and ISTE. Since 2020, he has been serving as a Chief Coordinator of the E-YUVA Centre supported by BIRAC, DBT, New Delhi, and as a coordinator of an M.Sc. Biotechnology research project funded by DBT. He has extensive teaching and research experience with major research interests in green synthesis and characterization of nanoparticles. He has published numerous research articles in national and international journals.

Research Field

Arpita Panda: Plant science, medicinal plants, phytochemistry, pharmacognosy, microbiology, Ethnopharmacology, Natural product drug discovery

Aparna Yerra: Biomaterials, Sericulture, Molecular Biology, Microbiology.

Subhashree Aparajita: Phytochemical Diversity, Molecular Phylogeny, Secondary metabolites, plant biotechnology.

Manoj Das: Plant Science, plant biotechnology, phytochemistry, pharmacognosy, Nanobiotechnology