

Secondary metabolite profiling and genetic expression of *Andrographis paniculata* (Kalmegh)

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Abstract. *Andrographis paniculata* (Kalmegh) has long been used across Asia, America, and Africa to treat diseases such as diabetes, high blood pressure, ulcers, leprosy, and malaria. Like all plants, it synthesizes diverse secondary metabolites through pathways such as the phenylpropanoid pathway. Key enzymes include Phenylalanine Ammonia-Lyase (PAL), which converts phenylalanine to trans-cinnamic acid, Chalcone synthase (CHS), which initiates flavonoid biosynthesis, and Dihydroflavonol-4-reductase (DFR), which reduces dihydroflavonols to leucoanthocyanidins. These metabolites provide defense against biotic and abiotic stresses due to their antioxidant and antimicrobial properties, contributing to the plant's therapeutic value. This study profiles these metabolites and examines expression of phenylpropanoid genes using PCR. Metallothionein gene expression, linked to metal detoxification, was also analyzed, offering insights into the plant's metabolism.

Key words: Flavonoid, Phenylpropanoid pathway, Antioxidant, Antimicrobial.

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1 Introduction

Andrographis paniculata (Kalmegh) is a widely used medicinal herb known for treating ailments such as diabetes, hypertension, ulcers, malaria, and leprosy across Asia, America and Africa [1]. Its therapeutic value arises from diverse secondary metabolites [2].

Key enzymes, including *PAL*, *CHS* and *DFR*, regulate the biosynthesis of antioxidant and antimicrobial compounds that enhance plant defence and stress tolerance [3]. Studies report that *A. paniculata* leaves contain abundant flavonoids, alkaloids and phenolics with strong antioxidant and hepatoprotective effects [4]. Andrographolide, the major diterpene lactone, exhibits anti-inflammatory, anticancer, anti-obesity, and antidiabetic activities by modulating cellular signalling and boosting endogenous antioxidant enzymes [5]. Emerging research further underscores the role of transcriptional regulation and metabolite transport in controlling phenylpropanoid-derived compounds [6]. Understanding these mechanisms is crucial for improving the plant's medicinal potential through biotechnological approaches [7].

2. Materials and methods

2.1 Plant material

A. paniculata leaves were used for profiling secondary metabolites. Plants were obtained from Bidhan Chandra Krishi Viswavidyalaya, Nadia- 741252; Newtown, Kolkata; Contai, Purba Medinipur- 721401. 10 plants in each group (3 replicates) are used.

2.2 Preparation of plant extract

Plant extract was prepared with modifications. One gram of leaf tissue was crushed with 50% ethanol, centrifuged, and the supernatant was collected and sonicated for 20 minutes. Extract was stored at -20°C.

3. Determination of polyphenol content

By the Folin-Ciocalteu method as mentioned in Singleton *et al.* (1999) Polyphenol content was measured. A reaction mixture was prepared containing 300 µl of Folin-Ciocalteu reagent, 100 µl of plant extract, and 600 µl of 10% (W/V) sodium carbonate solution. After it was incubated in the dark for about 45 minutes, and at 760 nm the absorbance was measured.

3.1 Estimation of total flavonoid content

Determination of Total flavonoid content was performed using the method of Lin and Tang. (2007). A mixture of 500 µl of plant extract, aluminum trichloride hexahydrate, potassium acetate, and deionized water was incubated at room temperature. The optical density (OD) value was recorded and compared with a rutin calibration curve.

3.2 Total antioxidant assay

For the determination of the total antioxidant activity, the Phosphomolybdenum assay was performed (Prieto *et al.*, 1999). A mixture of 3 mL of reagent solution and plant extract was put in incubation for one hour at 95°C. After cooling to room temperature, absorbance was measured at 690 nm against a blank. Antioxidant activity was quantified as µg of ascorbic acid equivalents per gram of fresh weight tissue using a standard ascorbic acid calibration curve.

3.3 Lipid Peroxidation Assay

Lipid peroxidation levels were estimated according to the method of Heath and Packer. (1968) Malondialdehyde (MDA) content was determined by reaction of the leaf extract with thiobarbituric acid (TBA) which was followed by incubation of the sample for 30 minutes at 95°C. Then the absorbance was measured at 532 nm and it was corrected at 600 nm of the resulting complex.

3.4 Proline Estimation

Total proline content estimation was done by Bates *et al.* (1973) method of proline estimation. Leaf samples were homogenized in trichloroacetic acid, centrifuged, and mixed with acid ninhydrin, glacial acetic acid, and orthophosphoric acid. For 45 minutes the mixture was boiled at 90°C, then immediately to stop the reaction it was cooled on ice. Followed by the phase separation with toluene, the absorbance was measured at the wavelength of 520 nm.

3.5 Primer designing

Primers for *PAL*, *DFR*, *CHS*, *MT* and *ACTIN* were designed using NCBI sequences, aligned using ClustalW and generated using Primer3.

Table 1. Primer Sequences Used for PCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>PAL</i>	ACTGAAGAACG GCGAACATGA	CGATTCGCGATT GCTGGAT
<i>DFR</i>	CCAAAAGCGGAT ACAAACTTGAC	TCAGGATCCAAG GAATCAAATTC
<i>CHS</i>	TCCCGGCCTCAA ATCTAAAGA	GGCTTCAGGGCC AGCTTATC
<i>MT</i>	TGCTCATGTGGC TCAAGCTG	CAGGTGCAAGG GTCGCAC

<i>AC</i>	CACGAGACCACC	CAACCTTAATCT
<i>TI</i>	TACAACTCG	TCATGCTGCTC
<i>N</i>		

Table 2. Details of Thermal Cycle for RT-PCR

Primer (Forward & Reverse)	Denaturation	Go to cycle for 35 times		Final Extension	Storage
		Denaturation	Annealing		
<i>PAL</i>	94°C for 1.5 mins	68°C for 1 min	72°C for 1.5 mins	72°C for 10 mins	4°C
<i>ACT</i>	94°C for 1.5 mins	60°C for 1 min	72°C for 1.5 mins	72°C for 10 mins	4°C

<i>CH</i> <i>S</i>	94°C for 1.5 mins	67°C for 1 mins	72 °C for 1.5 min s	72 °C for 10 min s	4 °C
<i>DF</i> <i>R</i>	94°C for 1.5 mins	63°C for 1 mins	72 °C for 1.5 min s	72 °C for 10 min s	4 °C

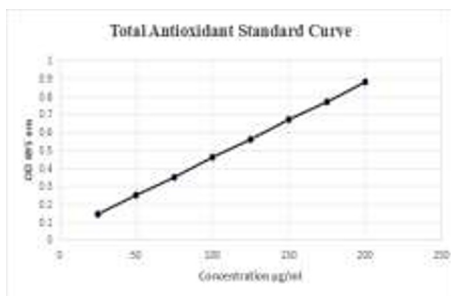
3.5 Gene isolation profiling using DNA and PCR

Total DNA was extracted following [8], and PCR was conducted for *PAL*, *DFR*, *CHS*, *MT* and *ACTIN* genes.

4. Results

4.1 Total chlorophyll content

Chlorophyll estimation was performed by measuring absorbance at 645nm and 662nm. The total chlorophyll content was 9.261 µg/mL (Chlorophyll-A) and 6.8729 µg/mL (Chlorophyll- B).



4.2 Total polyphenol content

Polyphenol content was calculated from a standard curve (50–300 µg/mL) using absorbance at 760 nm. The leaf sample showed a total polyphenol concentration of 4384.2 µg/g-FW (Fig. 1).

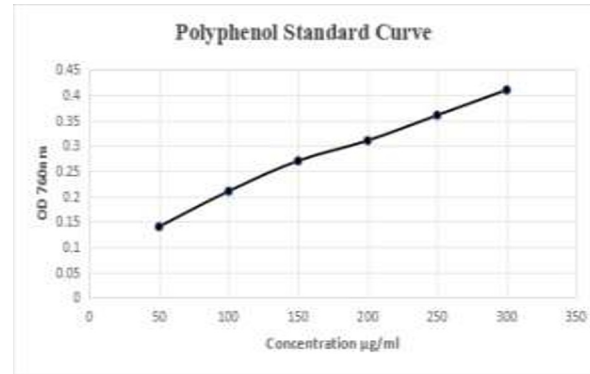


Fig. 1. Polyphenol Standard Curve

4.3 Total flavonoid content

Flavonoids were quantified using a rutin standard curve (20–100 µg/mL) at 415 nm. The flavonoid content of the sample was 1652.6 µg-RE/g-FW (Fig. 2).

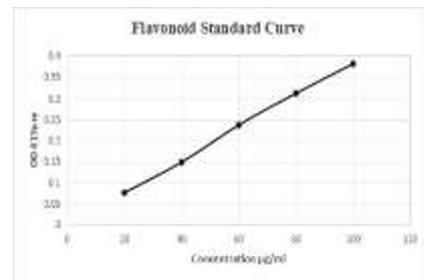


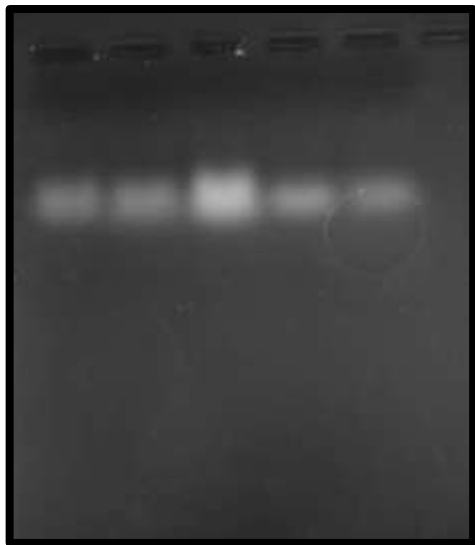
Fig. 2. Flavonoid Standard Curve

4.4 Total antioxidant content

Antioxidant activity was determined by using a phosphomolybdenum-based calibration curve at 695 nm. The total antioxidant content measured was 2007.6µg-AAE/g-FW content recorded was 179.56 µg/g-FW (Fig. 3).

Fig. 3. Antioxidant Standard Curve

4.5 Expression of important genes in secondary metabolism

**Fig. 4.** Pictorial data of Gel electrophoresis *PAL*, *ACTIN*, *MT*, *CHS* and *DFR* (left to right) gene in *A. paniculata*.

5 Discussion

This study investigates the secondary metabolite profile of *Andrographis paniculata*, a medicinal herb widely used across continents for treating conditions such as diabetes, hypertension, and malaria [9]. The research emphasizes the phenylpropanoid pathway, a central metabolic route responsible for synthesizing polyphenols and flavonoids that play key roles in plant defense and significantly contribute to the plant's therapeutic potential [10]. A series of biochemical assays was conducted to generate a comprehensive metabolite profile and identify major bioactive compounds present in *A. paniculata*. Quantification of these metabolites enhances understanding of the plant's

chemical composition and provides insights into its pharmacological relevance. Chlorophyll analysis revealed the presence of Chlorophyll A (9.261 $\mu\text{g/mL}$) and Chlorophyll B (6.8729 $\mu\text{g/mL}$), values comparable to reported ranges in *Ocimum sanctum* [11]. Total polyphenol content was measured at 4384.2 $\mu\text{g/g FW}$ [12]. Total flavonoid content reached 1652.6 $\mu\text{g RE/g FW}$ [13]. Antioxidant capacity, measured at 2007.6 $\mu\text{g AAE/g FW}$, further confirms the plant's high bioactivity.

Gene expression analysis of key phenylpropanoid genes—*PAL*, *CHS* and *DFR* was performed using PCR (Fig. 4), alongside Metallothionein to evaluate metal detoxification capability [14]. Together, these molecular and biochemical findings highlight the complexity and medicinal significance of *A. paniculata*, underscoring its relevance in traditional medicine and its potential applications in pharmaceutical and nutraceutical development [15].

6 Conclusion

The studies on *Andrographis paniculata* highlight its extensive medicinal value, largely attributed to flavonoids and polyphenols synthesized through the phenylpropanoid pathway. Key enzymes such as *PAL*, *CHS* and *DFR* drive the formation of bioactive compounds that provide strong antioxidant, antimicrobial, and antiviral properties [16]. Phytochemical analyses confirm notable antioxidant and hepatoprotective activities, primarily linked to andrographolide, which enhances *SOD* and *CAT* activity and reduces oxidative stress [17]. Research on metal-induced stress further supports the plant's ability to activate secondary metabolism and antioxidant defense genes, contributing to stress tolerance [18]. Additionally, microbial soil amendments have been shown to modulate phenylpropanoid gene expression and influence metabolite

production [19]. PCR-based analyses revealed stress-induced upregulation of major metabolic genes, offering insight into adaptive molecular responses. Analytical assays, including Folin–Ciocalteu and flavonoid quantification, reinforce the plant's antioxidant-rich profile. Collectively, these findings present *A. paniculata* as a promising candidate for therapeutic applications and biotechnological enhancement.

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References

1. V. Soumya, A. Sowjanya, P. Kiranmayi, Evaluating the status of phytochemicals within *Catharanthus roseus* due to higher metal stress. *Int. J. Phytoremediat.* **23**, 1391–1401 (2021).
2. M.L. Ramaroson, C. Koutouan, J.J. Helesbeux, V. Le Clerc, L. Hamama, E. Geoffriau, M. Briard, Role of phenylpropanoids and flavonoids in plant resistance to pests and diseases. *Molecules* **27**, 8371 (2022).
3. S. Hossain, Z. Urbi, H. Karuniawati, R.B. Mohiuddin, A.M.M. Allzrag, R. Capasso, *Andrographis paniculata* (Burm. f.) Wall. ex Nees: An updated review of phytochemistry, antimicrobial pharmacology, and clinical safety and efficacy. *Life* **11**, 348 (2021).
4. S. Kumar, B. Singh, V. Bajpai, *Andrographis paniculata* (Burm. f.) Nees: Traditional uses, phytochemistry, pharmacological properties and quality control/quality assurance. *J. Ethnopharmacol.* **275**, 114054 (2021).
5. Z. Liu, X. Hu, J. Nie, X. Li, Q. Wang, W. Liu, W. Zhang, Optimization of RNA in situ hybridization for mRNA localization detection in mature tissue of cucumber seedlings. *Plants* **9**, 1461 (2020).
6. M.A. Fazili, I. Bashir, M. Ahmad, S.A. Bukhari, A.M. Shah, G.H. Rather, In vitro strategies for the enhancement of secondary metabolite production in plants: A review. *Bull. Natl. Res. Cent.* **46**, 35 (2022).
7. P. Talukder, S. Talapatra, N. Ghoshal, S. Sen Raychaudhuri, Antioxidant activity and high-performance liquid chromatographic analysis of phenolic compounds during in vitro callus culture of *Plantago ovata* Forsk. *J. Sci. Food Agric.* **96**, 232–244 (2016). <https://doi.org/10.1002/jsfa.7086>
8. G. Khaksar, M. Sirijan, N. Suintichaikamolkul, S. Sirikantaramas, Metabolomics for agricultural waste valorization: Shifting toward a sustainable bioeconomy. *Front. Plant Sci.* **13**, 938480 (2022).
9. Z. Yue, V. Singh, J. Argenta, W. Segbefia, A. Miller, T. Ming Tseng, Use of plant secondary metabolites to reduce crop biotic and abiotic stresses: A review. *IntechOpen* (2022). <https://doi.org/10.5772/intechopen.104553>
10. S. Singh, V.P. Pandey, Seasonal variation in chlorophyll content of *Ocimum sanctum*. *Int. J. Bot. Stud.* **5**, 45–49 (2020).
11. A. Khan, F. Kanwal, S. Ullah, M. Fahad, L. Tariq, M.T. Altaf, A. Riaz, G. Zhang, Plant secondary metabolites—Central regulators against abiotic and biotic stresses. *Metabolites* **15**, 276 (2025).
12. K. Rabeih, M. Hnini, M. Oubohssaine, A comprehensive review of transcription factor-mediated regulation of secondary metabolites in plants under environmental

stress. *Stress Biol.* **5**, 15 (2025).

13. Y. Hao, Z. Zhang, E. Luo, J. Yang, S. Wang, Plant metabolomics: Applications and challenges in the era of multi-omics big data. *Trop. Plants* (2025).
14. W. Atwijukire, E.E. Ayogu, H. Onohuean, Immunomodulatory potentials of *Andrographis paniculata* and *Allium sativum* in managing plasmodium infections. *Discover Appl. Sci.* **7**, 1–14 (2025).
15. S.B.P. Adiguna, J.A. Panggabean, R.T. Swasono, S.I. Rahmawati, F. Izzati, A. Bayu, C. Giuseppina, Evaluations of andrographolide-rich fractions of *Andrographis paniculata* with enhanced antioxidant, anticancer, antihypertensive, and anti-inflammatory activities. *Plants* **12**, 1220 (2023).
16. A. Intharuksa, W. Arunotayanun, W. Yooiin, P. Sirisa-Ard, A comprehensive review of *Andrographis paniculata* (Burm. f.) Nees and its constituents as potential lead compounds for COVID-19 drug discovery. *Molecules* **27**, 4479 (2022).
17. A.O. Ayenitaju, F.M. Omokhuale, *Andrographis paniculata*: Review of its curative activities. *J. Pharm. Dev. Ind. Pharm.* **5**, 2023 (2023).
18. K.R.L. Saranya, K.K. Mandal, T. Kar, C.S. Reddy, K.V. Satish, Effects of disturbance regimes on phytodiversity of Similipal Biosphere Reserve, India. *J. Indian Soc. Remote Sens.* **51**, 1213–1226 (2023).
19. D. Palengara, S. Krishnan, S. Eenthavally, B. Vadakkangara, From invaders to innovators: Biological potential of AgNPs biosynthesized using invasive alien plant species. *Discover Plants* **2**, 1–27 (2025).