GMR
The Original

MOLECULAR CHARACTERIZATION OF SECONDARY METABOLITE PATHWAYS IN INDIGENOUS BOTANICAL SPECIES

Dr. Sofia S[1], Anil Kumar[2*], A. Xalxo[3], Vijay Kumar[4], Mohammed A. Al-Anber[5], Sagar Tanaji Kale[6]

[1]Assistant Professor in Botany, Fatima Mata National College (Autonomous), Kollam, Kerala, India, Email id: sraigil@gmail.com, Orcid I'd: 0000-0003-1682-4007
[2]Associate Professor in Botany, Shaheed Durga Mall Government Post Graduate College Doiwala (Dehradun), Email Id: singhaniya.akr@gmail.com, Orcid Id: https://orcid.org/0000-0001-7697-8118
[3]Asst Professor, Department of Botany, SAGEMMC College Ambikapur C.G., Email Id: a.m.xalxo@gmail.com, Orcid Id: https://orcid.org/0009-0003-5498-531
[4]Genetics and Plant Breeding (Assistant Professor), School of Agricultural Sciences, IIMT University Meerut Uttar Pradesh-250001.
Email id: vijayram9092@gmail.com Orcid id: 0000-0003-1531-7126
[5]Professor, Department of Chemistry, Faculty of Sciences, Applied Science Private University, P.O. Box 166, Amman 11931, Jordan,
Email id: m_alanber@asu.edu.jo, Orcid id: https://orcid.org/0000-0001-5613-2107
[6]Research Scholar, Savitribai Phule Pune University, Pune Email Address: sagarkale08@yahoo.com , Orcid id: https://orcid.org/0009-0005-5149-6637
*Corresponding Author - Anil Kumar, E-mail ID: singhaniya.akr@gmail.com

ABSTRACT

Malaxis acuminata was evaluated as a representative indigenous medicinal botanical species to characterize transcriptome-derived secondary metabolite pathway potential. This study used a publicly available de novo transcriptome annotation dataset containing transcript/scaffold identifiers, KEGG orthology identifiers, pathway annotations, protein accessions, alignment scores, and E-values. The dataset was cleaned, standardized, and screened for secondary metabolite-associated pathways using terms related to phenylpropanoid, flavonoid, stilbenoid, gingerol, steroid, terpenoid, diterpenoid, alkaloid, tryptophan, phenylalanine, and zeatin metabolism. Descriptive pathway analysis identified 147 transcript-level annotation records, including 130 unique KEGG orthology identifiers and 70 pathway names. Among these, 23 records were associated with secondary metabolite biosynthesis or precursor metabolism, representing 10 pathway categories. Phenylalanine metabolism was the most represented pathway, followed by stilbenoid, diarylheptanoid, and gingerol biosynthesis. Pathway-class analysis showed that phenolic and phenylpropanoid-related pathways accounted for 60.9% of secondary metabolite-associated records, followed by alkaloid-related, terpenoid/steroid-related, flavonoid-related, and phytohormone-related pathways. High-confidence annotations were observed for stilbenoid/gingerol biosynthesis, zeatin biosynthesis, phenylalanine metabolism, phenylpropanoid biosynthesis, and flavone/flavonol biosynthesis. These findings indicate diverse molecular pathway potential in *M. acuminata,* with dominant phenylalanine-derived and phenolic biosynthetic signatures. The analysis supports transcriptome annotation as a useful approach for molecular characterization of secondary metabolite pathways in non-model indigenous medicinal plants. However, findings require future targeted validation using expression profiling, metabolomics, and enzyme assays.

KEYWORDS: Malaxis acuminata, secondary metabolites, transcriptome annotation, KEGG pathways, medicinal orchid

1. INTRODUCTION

Indigenous plants have proved to be one of the most significant sources of secondary metabolites with structural diversity. The phenolics, flavonoids, alkaloids, terpenoids, steroids, phytohormones, and their derivatives play an essential role in the ecological, pharmacological, and biotechnological aspects. Secondary metabolites of medicinal plants are of great importance as they are used for both therapeutic applications and commercial production. Their production is influenced by genetics, development, tissues, environment, and the interactions between plants and microbes. Scientific research shows that secondary metabolites cannot only be regarded as isolated molecules, but they also belong to the complex network of metabolism, transportation, storage, and ecological signaling (Pang et al., 2021).

The molecular elucidation of secondary metabolic pathways is crucial in the study of medicinal plants because many indigenous and non-model botanical organisms do not have full genome data. While conventional phytochemical techniques enable one to determine the classes of metabolites present in the plant, they do not provide an understanding of how such compounds are formed through genetics and enzymatic processes. Transcriptome sequencing provides a pragmatic way of identifying potential transcripts, assigning KEGG orthologs, determining enzymatic function, and constructing biosynthetic pathways in cases where there is no available genomic information for the organism being studied.

Advancements made recently in the field of genomics of medicinal plants have increased the capability to explore the evolution of secondary metabolism biosynthesis pathways. With genome and transcriptome data available for medicinal

plants, it has been possible to find pathway-specific genes and understand metabolic pathways associated with medicinal features, and also relate molecular signatures to medicinal properties. This is important as secondary metabolite biosynthetic pathways can be specific for certain lineages and can also be responsive to external stimuli and developmental regulation (Alami et al., 2022).

Studies on the genomes of medicinal plants have helped enhance our understanding of the biosynthetic complexity through linking structural genes to their regulatory network and evolutionary history. This strategy has proven helpful in explaining how medicinal plants synthesize their chemical diversity via gene duplication, pathway acquisition, enzyme divergence, and transcriptional regulation. The advancements have provided an ideal platform for studying indigenous plants known for their medicinal uses but poorly described at the molecular level (Cheng et al., 2021).

Secondary metabolism in plants is affected by transport, as many metabolites need to be transported through the cell membranes, deposited in certain organs, or released into the intercellular space. The role of transporters in secondary metabolism lies in metabolite localization, detoxification, storage, and transport inside plant bodies. Thus, metabolic pathway characterization should include studies on the functions performed by biosynthetic proteins as well as those performed by other physiological processes (Nogia & Pati, 2021).

Regulatory systems also complicate the regulation of secondary metabolism. The role of transcription factors and microRNAs in the regulation of gene expression in pathway-related genes in response to various stresses results in effects on the synthesis of secondary metabolites and pathway activation. This regulatory system is particularly important in medicinal plants, which often exhibit stress-induced secondary metabolites playing roles in the plant's antioxidative, antibacterial, anti-inflammatory, or defensive activities (Kajla et al., 2023).

Second-generation sequencing techniques have expedited the study of medicinal plants through cost-effective transcription discovery, functional analysis, and pathway prediction of non-model organisms. This approach is most applicable for native species of plants, as molecular biology is possible without a pre-existing genomic structure of the species in question (Singh et al., 2025). It has also been revealed through integrated metabolomics and transcriptomic studies that pathway-level understanding provides insights into plant quality and metabolism (Yan et al., 2024).

Among the groups of secondary metabolites, flavonoids, alkaloids, and terpenoids are particularly noteworthy for their roles in medicinal properties, defense, coloration, stress responses, and ecological adaptations. The regulation of the synthesis of these compounds involves the cooperation of certain structural genes and regulatory proteins, which makes the pathway transcript annotation valuable for detecting potential components involved in the synthesis of the biologically active compounds (Singh et al., 2025). In addition, tissue-specific transcriptome studies revealed differences in gene expression in relation to secondary metabolism in medicinal plants, indicating the usefulness of pathway-based transcript annotation (Biswal et al., 2021).

M. acuminata (Jeevak) is a therapeutically valuable orchid, which represents a useful subject of study to understand the capabilities of secondary metabolite synthesis pathways in indigenous medical plants. It was established through the analysis of de novo transcriptome data that there were genes responsible for secondary metabolite biosynthesis pathways within the species of interest, as well as those for metabolites used for therapeutic purposes (Bhattacharyya et al., 2022). The current study seeks to elucidate secondary metabolite biosynthetic pathways in M. acuminata using de novo transcriptome annotations, with a focus on biochemical characterization and pathway identification.

Objectives of the study:

- To identify transcriptome-derived annotations associated with secondary metabolite pathways in Malaxis acuminata.
- To classify identified pathways into major biochemical groups, including phenolic, flavonoid, terpenoid, steroid, and alkaloid pathways.
- To characterize the dominant secondary metabolite pathway patterns using KEGG-based annotation metrics.

2. METHODOLOGY

2.1 Study Design

This study was conducted as a secondary bioinformatics-based descriptive study for the characterization of the secondary metabolite pathway in an indigenous medicinal botanical species. Pathway annotation data were used from the transcriptome of the therapeutically important orchid Malaxis acuminata (Jeevak) for analysis. The study emphasized molecular pathway characterization, not experimental validation, and all interpretations were limited to the pathway level and translations based on transcript annotations and KEGG orthology.

2.2 Dataset Selection and Source

A publicly available supplementary dataset associated with de novo transcriptome analysis of Malaxis acuminata was chosen for analysis (Bhattacharyya et al., 2022). The dataset that was selected was one that had annotations at the transcript level regarding secondary metabolite biosynthesis, such as KEGG orthology identifier, pathway names, pathway map identifiers, protein accessions, annotation descriptions, alignment score, and E-values. The dataset was suitable as it was small, directly analyzable, and specifically associated with specific medicinal indigenous botanical species.

2.3 Data Preprocessing and Cleaning

The consistency of the structure of the uploaded CSV file was checked before analysis. The dataset was filtered to remove identifiers for transcript, KEGG orthology, pathway, protein accession, annotations, alignment scores, and E values. The following was done during preprocessing to check for empty entries, malformed rows, duplicated annotations, and inconsistently formatted pathway names. Names of pathways were harmonized when necessary to enable grouping of

similar annotations under common biochemical pathways. Records with information on paths that could be interpreted were saved for downstream classification and summary.

2.4 Identification of Secondary Metabolite-Associated Pathways

Annotated pathways were screened for records of any secondary metabolite biosynthesis and any pathway for secondary metabolite precursors. The biologically relevant terms of pathway names, such as phenylpropanoid, flavonoid, flavone, flavonol, stilbenoid, gingerol, steroid, terpenoid, diterpenoid, alkaloid, tryptophan, phenylalanine, and zeatin, were used to evaluate the pathway names. Routes directly involved in specialised metabolite biosynthesis were selected, but primary metabolic pathways were only selected when they were known to be precursors for secondary metabolite production.

2.5 Pathway Classification and Molecular Characterization

Pathways identified were grouped into major biochemical classes by their various functions in secondary metabolism. Phenolic and phenylpropanoid-related pathways were combined into the metabolism of phenylalanine, biosynthesis of phenylpropanoids and stilbenoids, diarylheptanoids, and gingerols. Attempts were made to classify flavone and flavonol biosynthesis under the heading of flavonoid metabolism. Terpenoid and steroid-related pathways were considered and grouped together as steroid biosynthesis and diterpenoid biosynthesis, respectively. Alkaloid-related pathways included indole alkaloid biosynthesis, tropane, piperidine, and pyridine alkaloid biosynthesis. The biosynthesis of zeatin was considered a specialized metabolic pathway related to phytohormones. With this classification, the dataset was molecularly characterized at both the transcript-to-pathway and the pathway-class levels.

2.6 Data Analysis and Interpretation

Data from the cleaned dataset were analyzed using Python and descriptive statistics. The totals of annotated records, unique transcripts, unique KEGG orthology identifiers, unique pathway names, secondary metabolite-associated records, and secondary metabolite pathway categories were summarized. The frequency of each secondary metabolite-associated pathway was calculated using Python, and dominant pathway groups were identified. The identified pathways were functionally interpreted through their relation to known biochemical functions in medicinal plants, like stress response, production of phenolic compounds, biosynthesis of flavonoids, biosynthesis of terpenoids, biosynthesis of steroids, metabolism of alkaloids, plant defence, and antioxidant potential. Results were not necessarily interpreted as evidence of metabolite abundance, but rather as evidence for the potential of the secondary metabolite pathway in M. acuminata based on molecular annotation.

3. RESULTS

3.1 Dataset Characteristics

After cleaning, the Malaxis acuminata dataset yielded 147 transcript-level annotation records, which were made up of 147 unique transcript/scaffold IDs, 130 KEGG orthology IDs, 70 pathway names, and 136 protein accession entries. The dataset was appropriate for pathway-level molecular characterization of the data, but lacked expression data, biological replicates, and metabolite abundance data. The general structure of the annotations in the analyzed M. acuminata dataset is presented in Table 1.

Table 1. Dataset summary

Parameter	Value
Total annotated records	147
Unique transcript/scaffold IDs	147
Unique KEGG orthology IDs	130
Unique pathway names	70
Unique protein accessions	136
Secondary metabolite-associated records	23
Non-secondary/general records	124

3.2 Secondary Metabolite-Associated Annotations

The secondary metabolite-associated records were found by the screening with a percentage of 15.6% (23 records). Such records included those of pathways involved in phenolic, flavonoid, terpenoid, steroid, alkaloid, phytohormone-related, and amino-acid-derived metabolism. Table 2 gives the frequencies of the secondary metabolite-associated records and non-secondary metabolite records.

Table 2. Secondary metabolite record distribution

Annotation category	Records	Percentage
Secondary metabolite-associated records	23	15.6%
Non-secondary/general pathway records	124	84.4%
Total	147	100.0%

3.3 Secondary Metabolite Pathway Frequency

The most represented pathways were those involved in phenylalanine metabolism, followed by stilbenoid, diarylheptanoid, and gingerol biosynthesis. It is noteworthy that both pathways combined provided 13 of 23 records for metabolites. The frequency of the secondary metabolite-associated pathways identified is shown in Table 3. The frequency distribution of secondary metabolite-associated pathways found in M. acuminata is shown in Figure 1.

Table 3. Identified secondary metabolite-associated pathways

Pathway	Records	Best E-value	Class
Phenylalanine metabolism	7	1.0E-134	Phenolic/phenylpropanoid precursor
Stilbenoid, diarylheptanoid, and gingerol biosynthesis	6	1.0E-160	Phenolic specialized metabolism
Flavone and flavonol biosynthesis	2	2.0E-91	Flavonoid
Steroid biosynthesis	2	2.0E-10	Terpenoid/steroid
Diterpenoid biosynthesis	1	9.0E-33	Terpenoid
Indole alkaloid biosynthesis	1	1.0E-42	Alkaloid
Phenylpropanoid biosynthesis	1	1.0E-103	Phenylpropanoid
Tropane, piperidine, and pyridine alkaloid biosynthesis	1	1.0E-26	Alkaloid
Tryptophan metabolism	1	3.0E-40	Alkaloid precursor
Zeatin biosynthesis	1	1.0E-138	Phytohormone-related

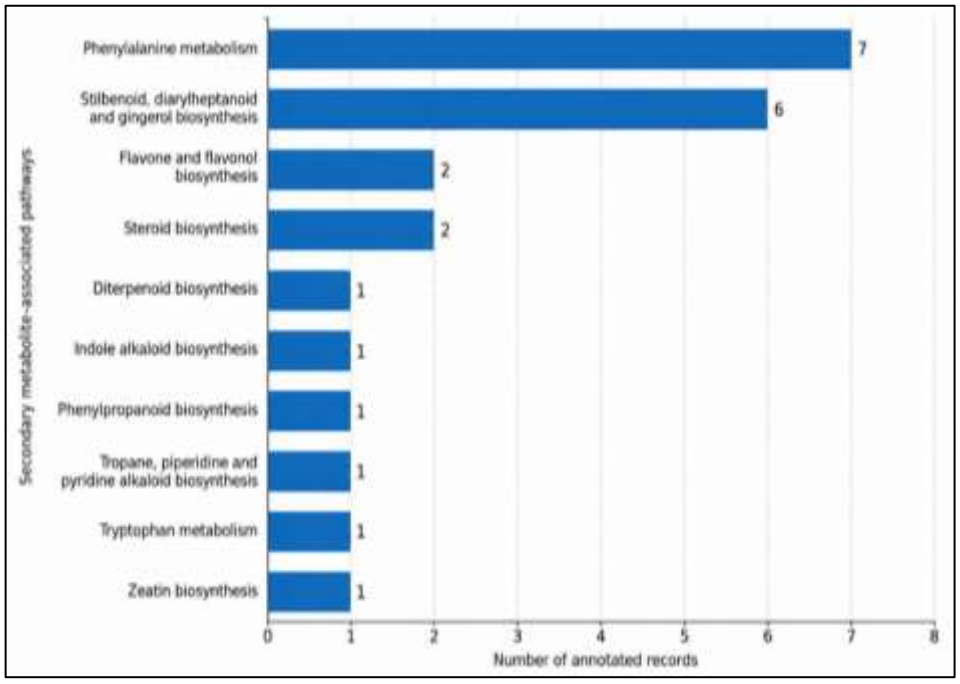

Figure 1. Frequency distribution of secondary metabolite-associated pathways in Malaxis acuminata.

3.4 Pathway Class Distribution

The pathways were dominated by phenolic and phenylpropanoid-related pathways, which accounted for 60.9% of all the secondary metabolite-associated pathways. The pathways related to the alkaloids accounted for 13.0%, and those related to terpenoids/steroids accounted for 8.7%. The distribution of biochemical classes of secondary metabolite-associated records is presented in Table 4. The biochemical class distribution of records that are associated with a secondary metabolite is shown in Figure 2.

Table 4. Biochemical class distribution

Pathway class	Records	Percentage
Phenolic/phenylpropanoid-related	14	60.9%
Alkaloid-related	3	13.0%
Terpenoid/steroid-related	3	13.0%
Flavonoid-related	2	8.7%

Phytohormone-related specialized metabolism	1	4.3%
Total	23	100.0%

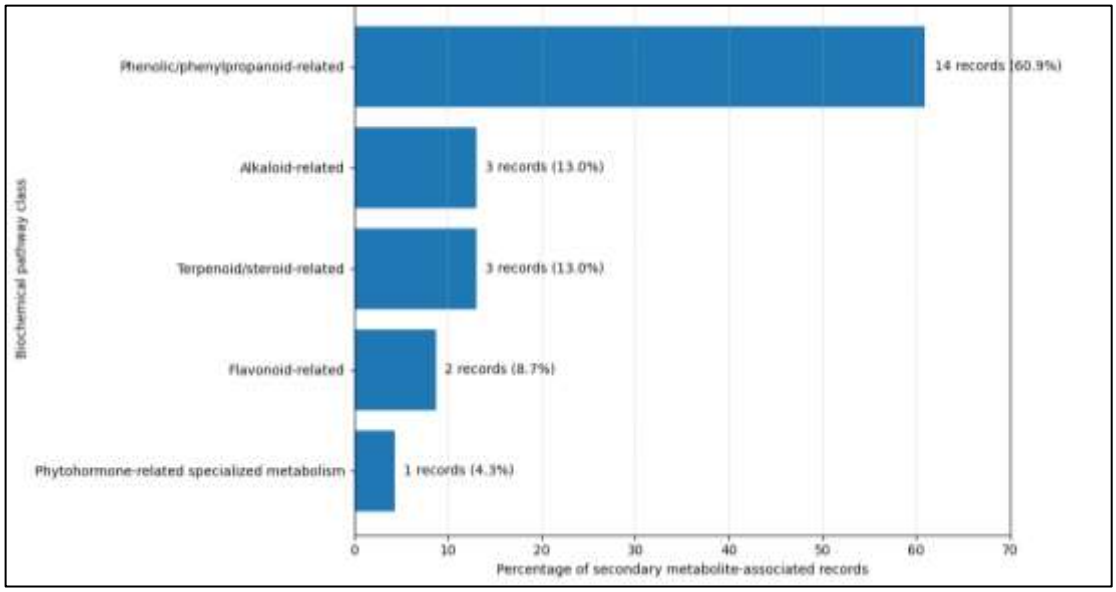

Figure 2. Biochemical class distribution of secondary metabolite-associated records in Malaxis acuminata

3.5 Representative Transcript-Level Annotations

The highest level of annotation evidence was seen for stilbenoid, diarylheptanoid, and gingerol biosynthesis, with a best E-value of 1.0E-160. There was also strong evidence for zeatin biosynthesis, phenylalanine metabolism, phenylpropanoid biosynthesis, and flavone/flavonol biosynthesis observed. The following representative high-confidence pathway annotations at the transcript level are shown in Table 5. Figure 3 shows the correlation structure of the eight pathway-level annotation metrics.

Table 5. Representative high-confidence pathway annotations

Transcript/scaffold ID	KO ID	Pathway	Score	E-value
C110706	K00517	Stilbenoid, diarylheptanoid, and gingerol biosynthesis	479.0	1.0E-160
C111736	K00279	Zeatin biosynthesis	425.0	1.0E-138
C96306	K00430	Phenylalanine metabolism	391.0	1.0E-134
scaffold10989	K00129	Phenylalanine metabolism	359.0	1.0E-109
scaffold10594	K09757	Phenylpropanoid biosynthesis	255.0	1.0E-103
C107344	K05280	Flavone and flavonol biosynthesis	296.0	2.0E-91
scaffold12255	K01757	Indole alkaloid biosynthesis	159.0	1.0E-42
C99578	K04124	Diterpenoid biosynthesis	130.0	9.0E-33

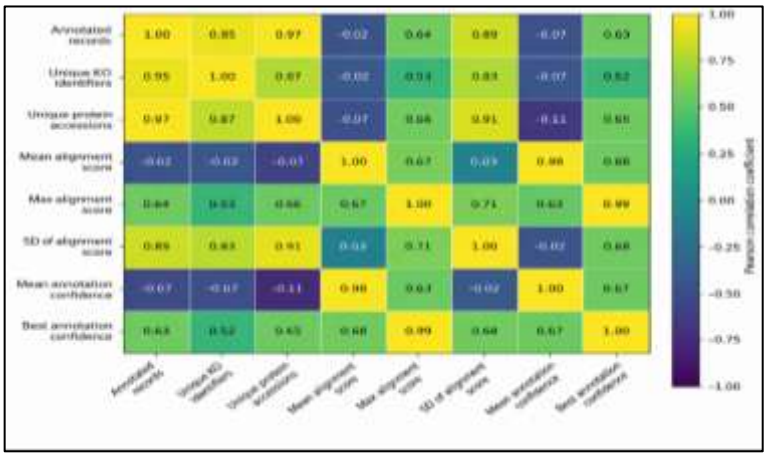

Figure 3. Eight-variable correlation heatmap of pathway-level annotation metrics in Malaxis acuminata.

3.6 Integrated Molecular Profile

The transcriptome dataset provided molecular evidence, based on its gene expression, for a number of secondary metabolite pathway classes in M. acuminata. The most prominent was related to phenylalanine-derived metabolism and phenolic/phenylpropanoid metabolism, suggesting a prominent role for aromatic amino-acid-derived secondary biosynthesis. Specialized metabolism, including flavonoid metabolism and alkaloid, terpenoid/steroid, and phytohormone metabolism, was supported by further evidence. The results indicated the utilization of M. acuminata as a representative medicinal plant species for the indigenous species for molecular characterization of potential secondary metabolite pathways. The results were still annotation-based with no confirmation of abundance or differential pathway activity.

4. DISCUSSION

In this study, molecular data were used to characterize the secondary metabolite-associated pathway annotations of Malaxis acuminata with the aid of the transcriptome. A total of 23 secondary metabolite-associated records were found from a total of 147 transcript-level annotations, covering 10 pathway/process categories. The top pathway signal was associated with phenylalanine metabolism, metabolism of phenylpropanoids, and biosynthesis of stilbenoids, diarylheptanoids, and gingerols. These results suggest that the pathway group of major secondary metabolites was the biosynthesis of aromatic amino acids and phenols. This finding corroborates transcriptome analysis in medicinal plants, elucidating candidate molecular components for the biosynthesis of pharmacologically relevant specialized metabolites, which can be annotated by pathways (Biswal et al., 2021).

Most of the pathways were phenolic and phenylpropanoid pathways, indicating that M. acuminata might have considerable molecular potential to produce phenolic compounds. Phenylpropanoid metabolism is closely related to the central precursor pathway of phenylalanine metabolism, responsible for the synthesis of flavonoids, lignin-related secondary metabolites, stilbenoids, and other specialized metabolites. Therefore, the relatively high number of annotations in the dataset for phenylalanine metabolism was indicative of phenolic metabolism being a significant part of the molecular secondary metabolite profile of this indigenous medicinal orchid. It was further supported by the identification of stilbenoid, diarylheptanoid, and gingerol biosynthesis, which are linked to the synthesis of special phenolic compounds that could be involved in plant defense and medicinal benefits.

A few pathways of flavone and flavonol biosynthesis were also identified in the data set, but with less frequency than those of phenylalanine and stilbenoid biosynthesis. Although the annotation of flavonoids is related to the flavonoids, the presence of these annotations is still of biological importance due to the known protective activities of flavonoids: antioxidant, pigmentation, UV protection, stress response, and therapeutic potential. Yuan et al. (2022) revealed that an integrated transcriptome and metabolome study enabled the better resolution of flavonoid biosynthesis in medicinal orchids by correlating transcript annotation with metabolite accumulation. For the present study, the molecular evidence for biosynthetic potential from the flavonoid pathway annotations hindered the interpretation of actual flavonoid accumulation due to the lack of metabolite abundance data.

Tryptophan metabolism, indole alkaloid biosynthesis, tropane biosynthesis, piperidine biosynthesis, and pyridine biosynthesis were identified as pathways for the identification of alkaloid-related annotations. These pathways were only recorded for a few pathways, but they added to the profile of secondary metabolites. The transcriptome and metabolome studies are useful for identifying key biosynthetic components of alkaloid production in medicinal plants, as are integrated profiling studies, which have led to the frequent association of these two groups of compounds with bioactivity in medicinal plants (Liu et al., 2023). The number of annotations related to alkaloids was not sufficiently high in the present dataset to suggest a high representation of pathways in the analyzed supplementary file and/or a low density of annotations for specialized metabolism containing nitrogen.

Some pathways, such as terpenoid and steroid-related pathways, were also noted in the process, like steroid biosynthesis and biosynthesis of diterpenoids. The relevance is due to terpenoid metabolites being involved in plant defence, membrane structure, plant growth regulation, and medicinal bioactivity. A combination of transcriptome and metabolome analyses has been used in medicinal plants to identify key genes and coordinated modules that are involved in the production of secondary metabolites from different plants by using network-based approaches (Moghadam et al., 2023). The terpenoid/steroid annotations were used to support the presence of isoprenoid-derived metabolic potential in this study, but additional validation would need to be done to determine if the pathways were being transcriptionally expressed or chemically expressed.

The pathway distribution also suggested that the secondary metabolite biosynthesis of M. acuminata was not restricted to any particular biochemical pathway. However, the data revealed specialized metabolism of phenolics, flavonoids, alkaloids, terpenoids, steroids, and phytohormones. A pathway annotation-driven approach for the identification of secondary metabolite biosynthesis-related genes was also applied to the non-model plant Digitalis purpurea, which demonstrated the relevance of the annotation-driven molecular characterization approach for non-model plants (Amiri et al., 2023). The present study thus makes a contribution to the increasing trend in using small transcriptome-derived datasets in determining candidate biosynthetic pathways in medicinal botanical species.

Results also showed the usefulness of combining the annotation confidence measures with a pathway-level interpretation. The stilbenoid biosynthesis, diarylheptanoid biosynthesis, zeatin biosynthesis, phenylalanine metabolism, phenylpropanoid biosynthesis, and flavone/flavonol biosynthesis pathways had strong E-value evidence. A metabolome and transcriptome integrated analysis of Panax japonicus has revealed that structural similarity among flavonoids and related compounds can be explained by the combination of pathway annotation and metabolite evidence, thereby highlighting that flavonoid biosynthesis pathways are structurally similar and might share their biosynthesis mechanisms

(Chen et al., 2024). In this current analysis, metabolomics data were not used, but the high number of annotation signals gave a logical framework in which pathways could be selected to be prioritized in future validation analyses.

One of the shortcomings was the fact that the data analysed were of an "annotation" type. It did not consist of values for expression abundance, matrices of expression in different tissues, biological replicates, or metabolite concentration profiles. Metabolomics and transcriptomics integration studies have been done in medicinal plant species, and the accumulation pattern of metabolites is a more reliable pathway for gene–metabolite connection, highlighting the advantages of this approach (Zhao et al., 2024). Thus, the results of this study do not provide proof of pathway activity or metabolite accumulation but rather indicate the molecular pathways' potential.

In conclusion, this study revealed that M. acuminata harboured molecular evidence from the transcriptome for a variety of secondary metabolic pathway classes, with the molecular signals being mostly phenolic and phenylpropanoid-related. The results indicate the potential value of transcriptome annotation to molecularly characterize secondary metabolite pathways in native plants. Further investigations are needed to integrate transcript abundance, metabolomics, enzyme annotation, and comparative analysis of related medicinal plant species or genera to enhance biological inferences and pathway validation, as has been recently done for multi-species medicinal plants (Li et al., 2025).

5. CONCLUSION

The molecular characterization of Malaxis acuminata was performed for secondary metabolite-associated pathways, based on the annotation data from the transcriptome. 23 records of secondary metabolites were identified with analysis, among 147 transcript-level annotations, in 10 categories of pathways. The most represented pathways were those related to the metabolism of phenylalanine and stilbenoid, diarylheptanoid, and gingerol biosynthesis, which suggested that pathways related to phenolics and phenylpropanoid metabolism represented the most important representation of the molecular signals in the dataset. The presence of flavonoid, alkaloid, terpenoid, steroid, and phytohormone-related specialized metabolic pathways was confirmed with additional annotations. The results indicated that M. acuminata has multiple molecular pathway potentials in secondary metabolite biosynthesis, which justify the use of this plant species as an indigenous medicinal plant. The analysis also proved the effectiveness of small transcriptome-derived datasets for pathway-level characterization in non-model plants. The results continued to be annotated data and thus could not be used to validate gene expression, the level of metabolites, or experimentally validated activity of pathways. In further studies, it is proposed that the levels of transcripts, metabolomics, enzyme activity, as well as comparative studies, be conducted on related indigenous medicinal species to validate the activity of pathways and enhance biological interpretation.

6. REFERENCES

1. Alami, M. M., Ouyang, Z., Zhang, Y., Shu, S., Yang, G., Mei, Z., & Wang, X. (2022). The current developments in medicinal plant genomics have enabled the diversification of secondary metabolites' biosynthesis. International journal of molecular sciences, 23(24), 15932.
2. Amiri, F., Moghadam, A., Tahmasebi, A., & Niazi, A. (2023). Identification of key genes involved in secondary metabolite biosynthesis in Digitalis purpurea. PloS One, 18(3), e0277293.
3. Bhattacharyya, P., Sharma, T., Yadav, A., Lalthafamkimi, L., Ritu, Swarnkar, M. K., ... & Kumar, S. (2022). De novo transcriptome based insights into secondary metabolite biosynthesis in Malaxis acuminata (Jeevak)–A therapeutically important orchid. Frontiers in Plant Science, 13, 954467.
4. Bhattacharyya, Paromik; Sharma, Tanvi; Yadav, Abhinandan; Lalthafamkimi, Lucy; Ritu; Swarnkar, Mohit Kumar; et al. (2022). Data_Sheet_3_De novo transcriptome based insights into secondary metabolite biosynthesis in Malaxis acuminata (Jeevak)–A therapeutically important orchid.csv. Frontiers. Dataset. https://frontiersin.figshare.com/articles/dataset/Data_Sheet_3_De_novo_transcriptome_based_insights_into_secondary_metabolite_biosynthesis_in_Malaxis_acuminata_Jeevak_A_therapeutically_important_orchid_csv/21352893?file=37896726
5. Biswal, B., Jena, B., Giri, A. K., & Acharya, L. (2021). De novo transcriptome and tissue specific expression analysis of genes associated with biosynthesis of secondary metabolites in Operculina turpethum (L.). Scientific Reports, 11(1), 22539.
6. Chen, C., Wang, P., Yan, Y., Jiao, Z., Xie, S., Li, Y., & Di, P. (2024). Integrated metabolome and transcriptome analysis provide insight into the biosynthesis of flavonoids in Panax japonicus. Frontiers in Plant Science, 15, 1432563.
7. Cheng, Q. Q., Ouyang, Y., Tang, Z. Y., Lao, C. C., Zhang, Y. Y., Cheng, C. S., & Zhou, H. (2021). Review on the development and applications of medicinal plant genomes. Frontiers in plant science, 12, 791219.
8. Guo, J., Huang, Z., Sun, J., Cui, X., & Liu, Y. (2021). Research progress and future development trends in medicinal plant transcriptomics. Frontiers in plant science, 12, 691838.
9. Kajla, M., Roy, A., Singh, I. K., & Singh, A. (2023). Regulation of the regulators: transcription factors controlling biosynthesis of plant secondary metabolites during biotic stresses and their regulation by miRNAs. Frontiers in Plant Science, 14, 1126567.
10. Li, H., Chen, N., Zhang, H., & Xu, D. (2025). Multidimensional regulation of transcription factors: decoding the comprehensive signals of plant secondary metabolism. Frontiers in Plant Science, 16, 1522278.
11. Li, W. Y., Li, C. Z., Huang, J. L., Zhan, J., Wang, A. Q., Xiao, D., & He, L. F. (2025). Transcriptome and metabolome reveal the accumulation of secondary metabolites in different species of Dioscorea. BMC Plant Biology, 25(1), 1174.
12. Liu, J., Han, L., Li, G., Zhang, A., Liu, X., & Zhao, M. (2023). Transcriptome and metabolome profiling of the medicinal plant Veratrum mengtzeanum reveal key components of the alkaloid biosynthesis. Frontiers in Genetics, 14, 1023433.

13. Moghadam, A., Foroozan, E., Tahmasebi, A., Taghizadeh, M. S., Bolhassani, M., & Jafari, M. (2023). System network analysis of Rosmarinus officinalis transcriptome and metabolome—Key genes in biosynthesis of secondary metabolites. PLoS One, 18(3), e0282316.
14. Nogia, P., & Pati, P. K. (2021). Plant secondary metabolite transporters: diversity, functionality, and their modulation. Frontiers in plant science, 12, 758202.
15. Pang, Z., Chen, J., Wang, T., Gao, C., Li, Z., Guo, L., ... & Cheng, Y. (2021). Linking plant secondary metabolites and plant microbiomes: a review. Frontiers in plant science, 12, 621276.
16. Singh, D., Mathur, S., Prasad, M., & Ranjan, R. (2025). Next-generation sequencing in medicinal plants: recent progress, opportunities, and challenges. Journal of Plant Growth Regulation, 44(4), 1448-1464.
17. Singh, D., Singh, N., Dwivedi, S., & Trivedi, P. K. (2025). Transcriptional regulation of secondary plant product biosynthesis: insights into flavonoid, alkaloid, and terpenoid pathways. Plant Cell, Tissue and Organ Culture (PCTOC), 160(1), 6.
18. Yan, Q., Zhang, G., Zhang, X., & Huang, L. (2024). A review of transcriptomics and metabolomics in plant quality and environmental response: From bibliometric analysis to science mapping and future trends. Metabolites, 14(5), 272.
19. Yuan, Y., Zuo, J., Zhang, H., Li, R., Yu, M., & Liu, S. (2022). Integration of transcriptome and metabolome provides new insights to flavonoids biosynthesis in Dendrobium huoshanense. Frontiers in Plant Science, 13, 850090.
20. Zhao, X., Ge, W., & Miao, Z. (2024). Integrative metabolomic and transcriptomic analyses reveals the accumulation patterns of key metabolites associated with flavonoids and terpenoids of Gynostemma pentaphyllum (Thunb.) Makino. Scientific Reports, 14(1), 8644.