

Metagenomic and Metabolomic Analysis Reveals Organ- and Population-Specific Interactions Between Bacterial Communities and Metabolites in Medicinal Plants *Tinospora cordifolia* and *Gynura procumbens*

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ABSTRACT

Background: *Tinospora cordifolia* and *Gynura procumbens* are important medicinal plants native to South and Southeast Asia. This study integrates metagenomic and metabolomic approaches to compare microbial communities and bioactive compound profiles in wild and cultivated populations of *T. cordifolia* and *G. procumbens*.

Methods: Microbiome analysis using 16S rRNA amplicon sequencing and metabolomic profiling via LC-MS/MS were performed on root and leaf of both plants.

Results: Bacterial communities in *T. cordifolia* and *G. procumbens* varied significantly between leaf and root. Proteobacteria dominated all samples, while roots harbored higher bacterial diversity, including Actinobacteria, Firmicutes, and Chloroflexi, particularly in wild populations. Metabolomic analysis revealed distinct profiles between organs, with leaves showing greater population-dependent variability, especially in *T. cordifolia*, where 482 metabolites differed significantly between wild and cultivated plants. Notably, *turmerone* was upregulated in wild leaves, while *cinnamic acid* was downregulated. Root metabolomes were more stable but still exhibited population-specific patterns in *G. procumbens*. In *T. cordifolia*, *bis(4-ethylbenzylidene)sorbitol* positively correlated with Actinobacteria and Chloroflexi, while *vicenin* and *2-methoxyestradiol* showed negative correlations with several phyla, suggesting antimicrobial potential. In *G. procumbens*, *ethamivan* positively correlated with Firmicutes and Chloroflexi.

Conclusion: These findings highlight the role of ecological and organ identity in driving plant-microbiome-metabolite dynamics, with implications for medicinal plant quality, bioactivity, and cultivation.

INTRODUCTION

Tinospora cordifolia (heart-leaved moonseed) and *Gynura procumbens* (longevity spinach) are native Indonesian medicinal plants with high potential application in the pharmaceutical and food industries due to their diverse bioactive compounds. Various studies have identified important secondary metabolites in the roots and leaves of both plants. Methanolic leaf extracts of *T. cordifolia* have been shown to contain phenols, 4-vinylguaiacol, guaiacol, syringol, and vanillin, all of which exhibit significant antioxidant activity [1,2]. Ethanol extracts from *T. cordifolia* leaves contain phenols, aldehydes, esters, alkanes, and terpenes [3] demonstrating significant immune-boosting, anticarcinogenic, anti-inflammatory, and antimicrobial properties [4]. A total of 54 metabolites have been identified in *G. procumbens* leaf extracts, including flavonols such as kaempferol 3-O-rutinoside, 4-hydroxybenzaldehyde, and 3,5-dicaffeoylquinic acid, contributing to its antioxidant and phenolic properties [5,6]. Additional studies have reported its pharmacological [7], toxicological [8], antioxidant [9,10] and anti-inflammatory activities [11]. Several studies have reported that endophytic microbiota play a role in modulating the production of bioactive compounds in plants. Nevertheless, despite the well-documented pharmacological properties of *T. cordifolia* and *G. procumbens*, the specific influence of their microbiome on metabolite production remains unexplored.

Environmental factors and metabolite production can both influence plant's interactions with microbes. Variations in soil characteristics, temperature, and salinity can significantly affect the diversity and abundance of microorganisms associated with plant organs [12-15]. Environmental stresses such as drought and salinity can also alter microbial communities and impact the biosynthesis of bioactive metabolites [13,16,17]. Plant growth stages also play a role in shaping these dynamic interactions. By understanding the interplay between environmental factors, plant development, and microbiomes, the cultivation of medicinal plants can be optimized [9,18]. Such information can support the development of more effective cultivation strategies to enhance plant productivity and increase the content of high-value bioactive compounds. This approach can promote the sustainable use of *T. cordifolia* and *G. procumbens* as sources of phytochemicals for pharmaceutical industries.

Plant endophytic bacterial communities are shaped by both biotic and abiotic factors, which determine species composition, community structure, diversity, and functional role [19]. Endophytic bacteria have numerous applications in agriculture, medicine, and other industries [20]. For instance, the isolation of 38 endophytic bacteria from the leaves and stems of *T. cordifolia*, including genera such as *Bacillus*, *Aneurinibacillus*, and *Pseudomonas*, demonstrated their potential to produce bioactive metabolites similar to those of the host plant [21]. These bacterial communities can also promote plant growth and enhance disease resistance [22]. Endophytic bacteria isolated from the leaf of *G. procumbens* have been reported to produce cytokinin-like compounds that influence the growth and development of the plant. Certain bacterial species, such as *Klebsiella* spp. and *Bacillus* spp., exhibit plant growth-promoting and biocontrol activities, including antagonistic effects against plant pathogens. Similarly, leaf endophytic bacterial communities also reported to accelerate plant growth in medicinal plant *Pulicaria incisa* [23].

Although the pharmacological properties of *T. cordifolia* and *G. procumbens* have been widely studied and many bioactive compounds have been successfully identified, the role of endophytic microbial communities in their secondary metabolite production remains underexplored. Previous studies suggest that plant microbiomes, especially endophytes, can significantly influence the biosynthesis of bioactive compounds through mechanisms such as gene expression modulation and elicitor production. Metagenomics and metabolomics have emerged as powerful tools to investigate the complex interactions between plants and their microbial communities. Combining these techniques enables comprehensive profiling of microbial diversity and mapping of specific metabolites produced in plant organs. A deeper understanding of these interactions can support biotechnological strategies such as functional microbial inoculation or environmental manipulation to optimize bioactive compound production. This integrated approach holds promise not only for improving the yield and quality of herbal raw materials but also for reinforcing the role of *T. cordifolia* and *G. procumbens* as sustainable sources of phytopharmaceuticals in modern medicine.

METHODS

Sample Collection and DNA Extraction

Samples of *T. cordifolia* and *G. procumbens* were collected from both wild and cultivated populations. Wild *T. cordifolia* samples were obtained from three distinct populations in the mountainous forests of Mojokerto, East Java, Indonesia, located at 275–299 m above sea level (masl): TC1 (7°36.129204' S, 112°33.295146' E; 288 masl), TC2 (7°36.043080' S, 112°33.221051' E; 275 masl), and TC3 (7°36.326057' S, 112°33.404845' E; 299 masl). Wild *G. procumbens* samples were collected from two populations in the same region at higher elevations: GP1 (7°40.968031' S, 112°34.674508' E; 1075 masl) and GP2 (7°40.868580' S, 112°34.007903' E; 1010 masl). These sites represent humid montane forest habitats with shaded conditions and well-drained soils. For each population, three healthy and mature individual plants showing no visible symptoms of disease or herbivore damage were selected. Cultivated samples of both species were obtained from Taman Husada Farm (7°18'13.0" S, 112°41'12.6" E), where plants were grown under managed cultivation conditions. All cultivated individuals were also at a comparable mature vegetative stage at the time of sampling.

Root and leaf tissues were collected from each plant for microbial community analysis, metabolomic profiling, and bacterial isolation. To eliminate surface-associated microorganisms, all plant tissues were sterilized using a PBS solution [24]. The sterilized samples were stored at -20°C until further analysis. For DNA extraction, the plant tissues were ground in liquid nitrogen using a sterile mortar and pestle. Genomic DNA was then extracted from the powdered tissue using the ZymoBIOMICS DNA Miniprep Kit, according to the manufacturer's instructions.

Library Preparation and Sequencing

Amplicon libraries were prepared using high-quality genomic DNA extracts in accordance with the Illumina PCR Quantification Protocol. To target the bacterial community, the V3–V4 hypervariable regions of the 16S rRNA gene were amplified using 30 ng of template DNA for each reaction. The resulting amplicons, expected to be around 400 base pairs in length, were purified with Agencourt AMPure XP magnetic beads to remove unwanted by-products and ensure clean fragments. Following purification, the DNA fragments were eluted and barcoded through an indexing step to construct the final sequencing libraries. The integrity and quality of the libraries were evaluated using the Agilent 2100 Bioanalyzer, which provided detailed information on fragment size distribution and overall DNA concentration. Only libraries that met the established quality thresholds were selected for sequencing, which was carried out using paired-end reads (2 × 250 bp) on the Illumina HiSeq 2500 sequencing platform.

Analysis of Bacterial Community Structure

The microbial community structure was analyzed using the QIIME 2 platform (Bolyen et al., 2019). Raw sequencing reads underwent quality filtering using a sliding window approach (25 bp) with a minimum Phred quality score of 20. Reads were discarded if they: (1) were trimmed to less than 75% of their original length, (2) contained ambiguous nucleotides, (3) exhibited low sequence complexity (defined as ten or more consecutive identical bases), or (4) showed residual adapter contamination. Primer sequences were removed during the demultiplexing process. Denoising and dereplication of sequences were carried out using the DADA2 algorithm [25]. High-quality sequences were then clustered into operational taxonomic units (OTUs) at a 99% similarity threshold using the VSEARCH plugin, which also included chimera detection and removal [26]. Shared OTUs across different samples were illustrated using an online Venn diagram generator. Taxonomic classification was assigned using the classify-sklearn method against the SILVA reference database [27].

Metabolomic Analysis

The metabolomic profiling process includes sample preparation, LC-MS/MS analysis, and subsequent bioinformatic analysis. Initially, 50 µg of lyophilized sample material was subjected to extraction using a methanol-water solution containing internal standards. This was followed by a sequence of homogenization, ultrasonication, and centrifugation steps. The resulting supernatant was filtered and prepared for LC-MS/MS analysis, with quality control (QC) samples included to ensure consistent analytical performance. Chromatographic separation was carried out on a Waters UPLC I-Class Plus system coupled with a Q Exactive mass spectrometer, utilizing a Hypersil GOLD aQ column. A gradient elution system was employed, using water with formic acid (Phase A) and acetonitrile (Phase B) as the mobile phases. Mass spectrometric detection was conducted in both positive and negative ionization modes, capturing high-

resolution full scan data along with targeted MS/MS fragmentation of selected precursor ions within a specified mass range.

Metabolite identification and data processing were performed using Compound Discoverer 3.3, integrating compound annotation from multiple databases such as BMDB, mzCloud, and ChemSpider. The generated data matrix, consisting of peak intensities and metabolite annotations, was processed using MetaX software. This included data normalization through Probabilistic Quotient Normalization (PQN), batch effect correction, and rigorous quality filtering to eliminate highly variable features. Principal Component Analysis (PCA) was used to assess data integrity and identify clustering patterns among samples. System stability and reproducibility were evaluated via QC samples, with LOESS signal correction applied to mitigate any batch-related variation.

RESULTS

Distribution of Bacterial Communities in *Tinospora cordifolia* and *Gynura procumbens* Organs

The bacterial community structure was analyzed in *T. cordifolia* and *G. procumbens* samples obtained from three different populations: two wild populations located in the Mojokerto forest and one cultivated population from the Taman Husada Farm in Surabaya, Indonesia.

The analysis revealed distinct bacterial community compositions between leaf and root of *T. cordifolia*, with Proteobacteria emerging as the predominant phylum in all samples (Figure 1A). In the leaf samples, Cyanobacteria dominated the community beside Proteobacteria, together they account for more than 99% of total bacterial abundance, followed by a smaller presence of Actinobacteria. In leaf samples from wild populations, Cyanobacteria represent the dominant phylum, accounting for over 50% of the total bacterial abundance. In contrast, leaf samples from cultivated plants are dominated by Proteobacteria, comprising more than 90% of the total bacterial community. Root harbored a more diverse bacterial community, with higher relative abundances of Proteobacteria, Actinobacteria, and Cyanobacteria, along with the presence of Acidobacteria, Bacteroidetes, Firmicutes, and Chloroflexi. These phyla were notably underrepresented or absent in leaf, indicating that the below ground environment supports a more complex and ecologically rich microbiome. Chloroflexi are more abundant in root samples from cultivated plants, whereas Firmicutes are more prevalent in root samples from wild populations. Minor phyla such as Planctomycetes, Verrucomicrobia, and Gemmatimonadetes were detected at low abundances, particularly in root.

The analysis also revealed clear differences in bacterial community composition between leaf and root of *G. procumbens*, with Proteobacteria, Actinobacteria, and Cyanobacteria emerging as the predominant phylum across all samples (Figure 1B). In the leaf of cultivated plants, Proteobacteria dominated the microbial community, followed by notable contributions from Actinobacteria, Cyanobacteria, Firmicutes, and Acidobacteria. In the leaf of wild plants, although Proteobacteria remained dominant, their relative abundance slightly decreased, allowing for an increase in Actinobacteria and a more balanced representation of Cyanobacteria. In contrast, root particularly those from wild plants (G.P.R.Wild), exhibited a more diverse bacterial community structure. In addition to high levels of Proteobacteria, these samples showed elevated abundances of Actinobacteria and Cyanobacteria, along with the presence of other phyla such as Firmicutes, Acidobacteria, Bacteroidetes, and Chloroflexi. These groups were either less prominent or absent in the leaf samples, indicating that the rhizosphere environment supports a more complex and functionally diverse microbiome. The root of cultivated plants also reflected broader microbial diversity compared to the leaf samples but remained less diverse than the wild root sample, with Proteobacteria and Actinobacteria as the major contributors and lower levels of Cyanobacteria.

Variation in Metabolite Profiles Among Different Organs and Populations of *Tinospora cordifolia*

Principal Component Analysis (PCA) was utilized to assess differences in metabolite composition among various organ types and population origins. In *T. cordifolia*, the resulting PCA plot demonstrates a distinct separation between leaf and root samples along the first principal component (PC1), reflecting organ-specific metabolic variation in this plant. Additionally, a moderate divergence between cultivated and wild leaf samples is evident along the second principal component (PC2), whereas cultivated and wild root samples cluster closely together (Figure 2A). These results suggest a significant environmental influence on the leaf

metabolite profile of *T. cordifolia*, with minimal impact on the root metabolome. Building upon the PCA analysis, a detailed differential metabolite expression analysis was performed to further explore variations between leaf samples from wild and cultivated populations. Since the PCA results indicate metabolite differences between the leaves of cultivated and wild plants, we conducted a differential metabolite analysis between the two sample groups. The results are presented as a volcano plot, illustrating the significant metabolite variations between the two populations. A total of 482 metabolites exhibited statistically significant changes in relative concentration between wild and cultivated populations (Figure 2B). Of these, 213 metabolites were upregulated in the wild population (marked in red), while 269 metabolites were downregulated (marked in blue) compared to the cultivated plant. Notably, among the upregulated metabolites are plant homologs of *ketotestosterone*, *turmerone*, and *fluoxetine*. On the other hand, *2-amino-1,3,4-octadecanetriol*, *flufenacet*, and *cinnamic acid* were among the significantly downregulated metabolites in wild leaf.

Similar to what observed in *T. cordifolia*, The PCA analysis of *G. procumbens* showed a distinct separation between leaf and root samples along the first principal component (PC1), indicating that organ types strongly influences the plant's metabolic composition (Figure 3A). However, in contrast to what observed in *T. cordifolia*, leaf samples from wild and cultivated plants tended to cluster closely together, suggesting a relatively consistent metabolite profile across individuals from different populations. Nevertheless, root samples were more widely dispersed, especially along PC1, reflecting greater variability in metabolite expression within this organ (Figure 3B). Building upon the PCA results, differential metabolite expression analysis was conducted to further explore the metabolic differences in the root of *G. procumbens*. In root samples, 200 metabolites exhibited significant differences in relative concentration. Among these, a number were significantly upregulated in wild samples – such as *dihydrotanshinone* and *2,4-Bis(2,4-dimethylphenyl)-6-(2-hydroxy-4-n-octyloxyphenyl)-1,3,5-triazine* – while others like *cis-mefentanyl* and *octadecanamine* were downregulated (Figure 3B). Overall, these findings highlight the influence of cultivation environment and organ-specific responses on the metabolite profiles of *T. cordifolia* and *G. procumbens*, emphasizing the plant's chemical diversity and adaptive metabolic regulation.

Correlation analyses between metabolite composition and microbial communities

To explore the relationship between microbiome communities and key bioactive compounds in *T. cordifolia* and *G. procumbens*, correlation analysis was performed between dominant bacterial phyla and major metabolites identified in the positive ion mode using LC-MS/MS. In the *T. cordifolia* sample, notable positive correlations were observed, particularly between plant homologs of drofenine, 2-amino-1,3,4-octadecanetriol, and bis(4-ethylbenzylidene)sorbitol with Actinobacteria and Chloroflexi. These findings suggest that these bacterial taxa may contribute to, or benefit from, the accumulation of these compounds, potentially influencing their growth and colonization within *T. cordifolia* organs. On the other hand, negative correlations were observed between plant homologs of 2-methoxyestradiol, coniferin, ibuprofen, trigonelline, and vicerin with Actinobacteria, Chloroflexi, Gemmatimonadetes, and Acidobacteria. This suggests that these metabolites may exert antimicrobial effects, thereby suppressing the growth or colonization of these bacterial groups, or reflect a conserved microbial response to specific plant chemicals that shape community composition

In *G. procumbens*, distinct patterns emerged, with Firmicutes, Bacteroidetes, and Chloroflexi showing positive correlations with plant homologs of 2-amino-1,3,4-octadecanetriol, DEET, and ethamivan, respectively. These results suggest a possible facilitative role of these microbes in the presence or bioavailability of this metabolite. In contrast, plant homologs of α -propylaminopentiphenone were strongly negatively associated with Verrucomicrobia, Planctomycetes, Gemmatimonadetes, and Acidobacteria, indicating potentially inhibitory or metabolically incompatible relationships. Overall, these findings highlight plant-specific microbiome-metabolite interactions, where certain bacterial groups are positively associated with particular compounds in one plant but negatively associated or neutral in another, reflecting a complex interplay between host plant metabolism and microbial community composition.

Figures

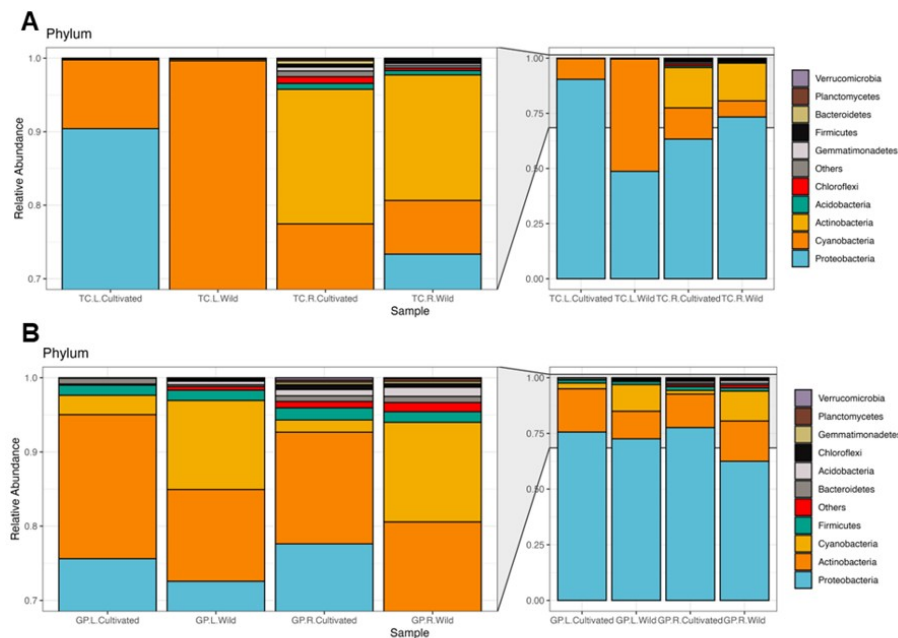


Figure 1: The bacterial taxonomic composition and relative abundance at the phylum level for bacteria associated with the leaves and root of *Tinospora cordifolia* (A) and *Gynura procumbens* (B) were analyzed across two sampling populations. The sample abbreviations are as follows: TC.L.Cultivated and GPL.Cultivated refer to *T. cordifolia* and *G. procumbens* collected from the Taman Husada Farm; TC.L.Wild and GPL.Wild refer to *T. cordifolia* and *G. procumbens* collected from the Mojokerto forest; L—leaf samples; and R—root samples.

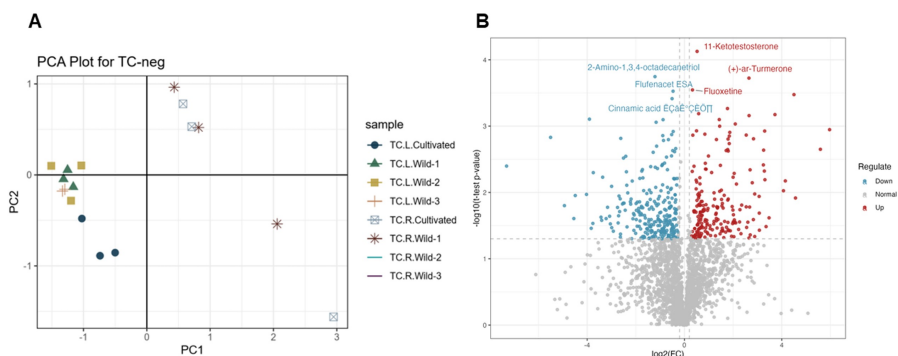


Figure 2: Analysis of differential metabolites between organ types and populations of *Tinospora cordifolia*. (A) Principal Component Analysis (PCA) of *Tinospora cordifolia* metabolites from root and leaf samples. (B) Volcano plots illustrating differential metabolites between leaves of wild and cultivated plants. The sample abbreviations are as follows: TC.L.Cultivated and GPL.Cultivated refer to *T. cordifolia* and *G. procumbens* collected from the Taman Husada Farm; TC.L.Wild and GPL.Wild refer to *T. cordifolia* and *G. procumbens* collected from the Mojokerto forest; L—leaf samples; and R—root samples.

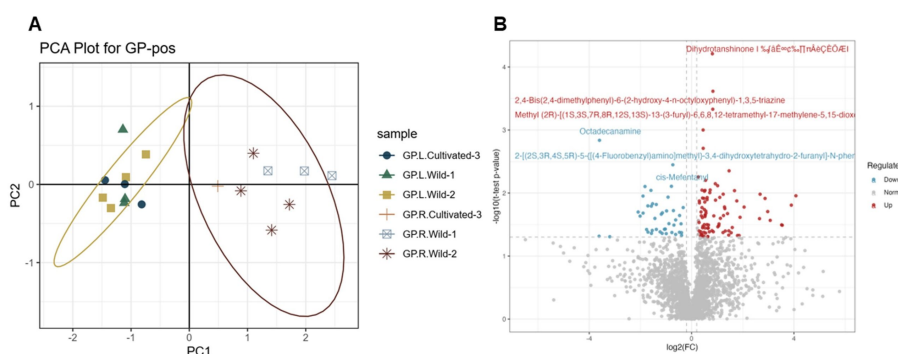
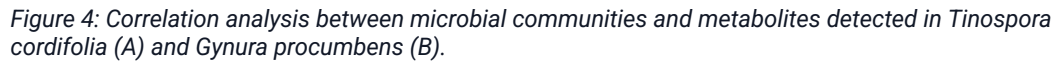


Figure 3: Analysis of differential metabolites between organ types and populations of *Gynura procumbens*. (a) Principal Component Analysis (PCA) of *Gynura procumbens* metabolites from root and leaf samples. Volcano plots illustrating differential metabolites between leaves of wild and cultivated plants. The sample

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The integration of bacterial community profiling and untargeted metabolomic analyses in *T. cordifolia* and *G. procumbens* reveals substantial variation shaped by plant organ type and population origin. Distinct microbial assemblages and metabolite compositions observed between roots and leaves, as well as between wild and cultivated populations, highlight the influence of ecological and physiological factors on plant associated microbiomes and metabolomic expression. In both plant species, the bacterial community composition varied significantly between leaf and root, consistent with previous studies showing that plant organs create distinct microhabitats that influence microbial colonization [28-30]. Proteobacteria emerged as the dominant phylum in all samples, a common feature of plant-associated microbiomes due to their versatile metabolic capabilities and symbiotic potential [31]. In *T. cordifolia*, Cyanobacteria were highly abundant in wild leaf samples, while cultivated leaves were dominated by Proteobacteria. Root samples exhibited higher bacterial diversity, harboring additional phyla such as Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi, and Bacteroidetes. This supports the idea that root environments provide more complex and nutrient-rich niches, facilitating greater microbial diversity [32]. The greater prevalence of Firmicutes in wild roots and Chloroflexi in cultivated roots further underscores how environmental conditions selectively shape microbiome composition [33]. Similar trends were observed in *G. procumbens*, where roots hosted a more diverse bacterial community compared to leaves. While Proteobacteria remained dominant in all organs, the relative abundance of Actinobacteria and Cyanobacteria varied, particularly between wild and cultivated samples. These patterns suggest that both host organ type and environmental origin are strong determinants of microbial assembly, a conclusion supported by similar findings in other medicinal and crop plants [34].

Metabolomic analysis revealed pronounced organ-specific and environmental effects on metabolite profiles. In *T. cordifolia*, PCA and volcano plot analyses demonstrated clear differentiation between leaves and roots, and between wild and cultivated leaf samples. Notably, 213 metabolites were upregulated in wild leaves, including *turmerone*, this compound is known for its antimicrobial properties [35]. The detected metabolites could be broadly grouped into major phytochemical classes, including terpenoids, phenolic compounds, phenylpropanoids (such as cinnamic acid derivatives and flavonoid glycosides), nitrogen-containing/alkaloid-like compounds, and other aromatic secondary metabolites. In contrast, root metabolomes were less affected by population origin, implying a degree of biochemical stability in below-ground organs, possibly due to their critical role in primary metabolism and plant-microbe signalling [36]. The downregulation of cinnamic acid in cultivated leaves suggests potential attenuation of defence-related pathways under cultivated conditions, as phenylpropanoid derivatives such as cinnamic acid are widely recognized for their role in plant defense [37,38]. Similarly, *G. procumbens* showed a strong organ-specific metabolic signature, but less pronounced differences between wild and cultivated leaves. Instead, root samples exhibited greater variability and population-dependent divergence. Several bioactive compounds, including *dihydrotanshinone* and *triazine* derivatives, were significantly upregulated in wild roots, reflecting enhanced secondary metabolism possibly triggered by environmental stressors or microbial interactions [39]. These metabolites can be mainly associated with quinone/terpenoid-derived and nitrogen-containing secondary metabolite groups, which are frequently linked to stress

adaptation and bioactivity in medicinal plants. Previous phytochemical studies in *G. procumbens* also reported organ- dependent accumulation of phenolics and flavonoids related to environmental responses [40]. In *T. cordifolia*, organ-dependent metabolite accumulation has been previously reported, with different organs showing variation in the abundance of diterpenoids, alkaloids, and phenolic compounds [41], supporting our observation of clear tissue-specific metabolic separation. These reports strengthen our conclusion that the detected metabolomic divergence between leaf and root represents intrinsic biochemical compartmentalization rather than solely environmental effects.

The correlation analyses between bacterial phyla and plant metabolites further support the hypothesis of reciprocal interactions between host chemistry and microbiome structure. In *T. cordifolia*, metabolites such as *drofenine* and *bis(4-ethylbenzylidene)sorbitol* showed positive correlations with Actinobacteria and Chloroflexi, suggesting either microbial contribution to their biosynthesis or preferential colonization in response to these compounds. Conversely, the negative correlation of *vicenin* and *2-methoxyestradiol* with certain bacterial phyla may reflect antimicrobial properties or selective pressures imposed by plant chemistry [42]. *G. procumbens* exhibited distinct microbial-metabolite relationships, such as the positive association of *DEET* and *ethamivan* with Firmicutes and Chloroflexi, and the negative correlation of *α-propylaminopentiphenone* with Verrucomicrobia and Acidobacteria. These plant- specific patterns emphasize the complexity of host-microbiome interactions and highlight the role of specific metabolites in shaping microbial communities [43]. Collectively, this study demonstrates that plant organ type and environmental origin significantly influence both the bacterial communities and metabolite profiles of *T. cordifolia* and *G. procumbens*. Roots consistently harbored greater microbial and chemical diversity, while wild plants supported more complex microbiomes and enhanced accumulation of defense- related secondary metabolites, particularly terpenoids and phenolic compounds. The observed correlations between microbial taxa and specific metabolites suggest a dynamic interaction that may affect plant health and medicinal properties. These findings provide a foundation for future exploration of functional microbiomes and metabolic engineering in medicinal plants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

AUTHOR CONTRIBUTIONS

Conceptualization, Arif Luqman, Anjar Tri Wibowo, Yosephine Sri Wulan Manuhara, and Almando Gerald; methodology, Arif Luqman, Anjar Tri Wibowo, Hoang Dang Khoa Do, Minh Thiet Vu, Le Minh Bui, and Almando Gerald; validation, Arif Luqman, Anjar Tri Wibowo, Hoang Dang Khoa Do, and Almando Gerald; formal analysis, Arif Luqman, Anjar Tri Wibowo, Edo Danilyan, Fahmi Ikhlasul Amalludin, Hoang Dang Khoa Do, Minh Thiet Vu, Le Minh Bui, and Almando Gerald; investigation, Edo Danilyan, Fahmi Ikhlasul Amalludin, Yohanes Kartjito Putro, Elsalisa Ainur Rofiq; resources, Arif Luqman, Anjar Tri Wibowo, Yosephine Sri Wulan Manuhara, and Almando Gerald; data curation, Edo Danilyan, Fahmi Ikhlasul Amalludin, Yohanes Kartjito Putro, Elsalisa Ainur Rofiq; writing original draft preparation, Anjar Tri Wibowo, Edo Danilyan, Fahmi Ikhlasul Amalludin, and Arif Luqman; writing review and editing, Anjar Tri Wibowo, Edo Danilyan, and Arif Luqman; visualization, Edo Danilyan, and Fahmi Ikhlasul Amalludin; supervision, Anjar Tri Wibowo, Arif Luqman, Yosephine Sri Wulan Manuhara, and Almando Gerald; project administration, Anjar Tri Wibowo, Yosephine Sri Wulan Manuhara, and Wahyu Aristyaning Putri; funding acquisition, Anjar Tri Wibowo, Arif Luqman, Almando Gerald, Yosephine Sri Wulan Manuhara, Wahyu Aristyaning Putri. All authors have read and agreed to the published version of the manuscript

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Statement of generative AI and AI- assisted technologies in the writing process and transparency declaration

The authors declare that, during the preparation of this manuscript, generative artificial intelligence tools (ChatGPT by OpenAI) were used for language editing, grammar correction, and improvement of readability and clarity of the text. No artificial intelligence tools were used for the generation, analysis, interpretation, or manipulation of research data, results, figures, or

scientific conclusions. Furthermore, no AI-generated images were included in this manuscript.

Following the use of these tools, the authors carefully reviewed, revised, and validated all content to ensure its scientific accuracy and integrity. The authors take full responsibility for the content of the published article, including the accuracy of the data, analyses, interpretations, and conclusions presented therein.

The corresponding author, acting as the guarantor of this work, affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted.

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