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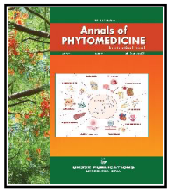

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Phytochemical profiling of two distinct stem parts of *Cissus quadrangularis* L. and exploration of their metabolic pathways

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Abstract

Cissus quadrangularis L., a climbing succulent that is perennial in nature and belongs to the Vitaceae family, is well recognized for its medicinal applications, especially in promoting bone recovery, treating osteoporosis, and addressing fractures. This plant contains an abundance of bioactive substances which enhance its properties as an antioxidant, anti-inflammatory agent, and osteogenic promoter. The study aims to explore the role of nodal structures in the plant's medicinal efficacy by comparing two different stem types from the same accession. The first node exhibits a three-dimensional structure, while the second node shows a two-dimensional structure. Both stem types were harvested, shade-dried, and subjected to GC-MS analysis to profile their phytochemical composition. A total of 105 metabolites were identified in the 3F stem, while 139 were found in the 2F stem. Statistical analysis revealed clear variations in metabolite levels between the two types of stems, with significant compounds such as tocopherol, myristic acid, and octodecane exhibiting different accumulation patterns. Pathway analysis emphasized the roles of fatty acid biosynthesis, as well as sesquiterpenoid and triterpenoid pathways, in the observed metabolic alterations. These results indicate that structural differences in *C. quadrangularis* affect its metabolite distribution, which could influence its potential for treating conditions like osteoporosis and diseases related to oxidative stress.

1. Introduction

Plants produce a wide range of secondary metabolites in response to environmental factors including water deficit, high/low temperatures, UV-B and the organisms and pathogens that affect them. For centuries, the bioactive compounds have formed the foundation of plant-based medical systems, which continue to meet primary healthcare needs for over 80% of the global population, particularly in developing nations, and serve as the basis for many proven modern pharmaceuticals (Maroti *et al.,* 2022). They not only aid plant survival but are also bioactive compounds with enormous medical applications, especially in plants with pharmacological uses (Pradhan *et al.,* 2024). Of such plants, *C. quadrangularis* shows a lot of potential for its pharmacological uses and its usage in ethnomedicine. This plant has different names such as veld grape, Hadjod in Ayurveda, bone setter and this is a perennial succulent climber that falls under the Vitaceae family. Originally found in India and Southeast Asia, it has been naturalized elsewhere in the tropical and sub-tropical regions and highlights its importance in the two categories of medical practices; namely, traditional and modern medicine (Piyush *et al.*, 2021). *C. quadrangularis* is best known for its therapeutic uses in ailments such as bone fractures, osteoporosis, and any osseous injury. Its inclusion in of the conventional Ayurveda and Siddha medical systems reveal its ability to stimulate new bone formation and as well aid the process of fracture healing (Jaspreet *et al.,* 2024). It is best identified by its angular stem, its leaves with serrated margins and small berries when it is in the berry stage. More recent study has posited its multi-purpose use and attributed its impact to the compound constituents. These bioactive compounds encompass phytoestrogens that replicate estrogen's function of maintaining bone density, flavonoids and triterpenoids for their proved antioxidant as well as anti-inflammatory properties, carotenoids for their privileged action of neutralizing free radicals besides ascorbic academics that facilitates collagen synthesis and the subsequent repair of tissue (Jeganath *et al*., 2020). In this case, the study sought to establish the role played by specific nodal structures of *C. quadrangularis* through which this plant may exercise its medicinal attributes.

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The choice of source of explants is required for optimization of yield of phytochemicals as biosynthesis and accumulation of phytochemicals in response to ectopic environment is tissue/organ specific (Ankita *et al.,* 2021). Two types of stems from one of the accessions was harvested to examine its GC-MS profile of nodal differentiation. The first node exhibited three-dimensional structure whereas the second node presented in two-dimensional format. These nodal segments were collected shade dried in order to conduct phytochemical study in an attempt to establish the bioactive compound composition of two different stem parts from a single accession of *C. quadrangularis*. This targeted approach is designed to shed light on whether differences in plant structure play any role in distribution or molecule density. More so, due to elevated rates of osteoporosis and bone fractures caused by the increasing ageing population, lack of exercise and abnormal diet there is an increasing need for natural plant products to address such ailments. These ailments often involve joint cartilage and bone degeneration, causing pain, stiffness, and reduced mobility, with oxidative stress and inflammation as key contributors (Firoz *et al.*, 2023).

Bone remodeling is an active process involving the ongoing growth of new bone by osteoblasts and the breakdown of bone by osteoclasts. When these steps are uneven then ailments like osteoporosis occur and therefore there is a need to think of osteogenic agents (Dimitrios *et al.,* 2006). Subsequently, *C. quadrangularis* has revealed potential to stimulate osteoblast and minerals deposition, hence serving as a complementary treatment. Apart from its modulatory activity on bone formation, *C. quadrangularis* possesses the wide range of pharmacological activities. They enhanced the ability to combat oxidation stress and inflammation, considered significant in chronic diseases (Alexander *et al.,* 2024). It also possesses antidiabetic attributes which consist of the ability to regulate blood glucose levels and enhance state of insulin resistance, as well as gastro-protective properties which strengthen the gut lining and lessen instances of gastric ulcers. Also, it has shown to have a lipid regulation activity that helps control obesity and cardiovascular disorders (Hasni *et al.,* 2023). Phytochemicals are active compounds with therapeutic properties, making them highly valuable as medicines or drugs (Pankaj *et al*., 2022).

The research on *C. quadrangularis* therefore not only encompasses the phytochemical properties of the plant but also its therapeutic potential in the solution of health problems affecting the world. Thus, understanding the distinct stem part properties of this plant as well as their content of bioactive compounds provides new evidence for including traditional plants in contemporary healthcare services.

2. Materials and Methods

2.1 Plant collection and sample preparation

Fresh stems of *C. quadrangularis* were harvested from a germplasm collection comprising 31 accessions maintained at the Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Among these, the accession TNCq 60 exhibited distinct phenotypic traits (Figure 1). Two specific stem types from one of these accessions were collected for GC-MS analysis: the first node shows the three-dimensional (3F) and the second node shows two dimensional (2F), which were separated for analysis (Figure 2). The stems were shade-dried for 10 days and then powdered for extraction.

2.2 Plant Authentication

The plant material, including flowers, stems, and leaves, was submitted to the Botanical Survey of India (BSI), Coimbatore, India, for identification and authentication. The species was confirmed and authenticated, and a Voucher Specimen was deposited with the Authentication Number - BSI/SRC/5/23/2021/Tech/331.

Figure 1: *C. quadrangularis* - TNCq 60.

Figure 2: 3F and 2F.

2.3 Solvent extraction

The powdered samples were subjected to Soxhlet extraction using hexane and methanol as solvents. The extracts were concentrated by using Rotary evaporator at 60 rpm, 60-degree temperature and stored at 4°C until further analysis.

2.4 GC-MS analysis

The analysis was conducted using a Perkin Elmer Clarus SQ 8C gas chromatography mass spectrometer (GC-MS). The instrument parameters were configured as follows: the source temperature was maintained at 220°C, the injector port temperature was set to 220°C,

and the interface temperature was 250°C. The oven temperature program started at 75°C for 2 min, ramped to 150°C at a rate of 10°C per min, and further increased to 250°C at the same rate. A split ratio of 1:12 was used with the injector operating in split-less mode. The analysis employed a DB-5 MS capillary column (0.25 mm OD × 0.25 µm ID × 30 m length), a standard non-polar column procured from Agilent Co., USA. Helium was used as the carrier gas at a flow rate of 1 ml per min. The mass spectrometer scanning range was set between 50-550 Da, with the source maintained at a vacuum pressure of 4.5 × 10 m Torr and a temperature of 220°C. Ionization was performed at -70 eV. The MS system featured an internal pre-filter to minimize neutral particle interference and included a data system with built-in libraries for spectral searching and matching. The NIST MS Search 2.2v library, containing over 500,000 references, was utilized for compound identification (Mastan *et al.,* 2022). Compound identification was performed by comparing the obtained mass spectra with the NIST 14 library database.

2.5 Data analysis

Statistical analysis and pathway analysis was carried out using MetaboAnalyst version 6 to identify difference between the samples. Chemical classification was achieved using ClassyFire, which automated the structural categorization of compounds into superclasses and subclasses based on InChIKey identifier.

3. Results

3.1 Metabolite profiling of 3F and 2F stem of *C. quadrangularis*

A total of 105 and 139 metabolites were identified in both methanol and hexane solvent of 3F and 2F stem, respectively (Table.1). In which, 2F containing a total of 79 unique metabolites, while the 3F, which contains 45 unique metabolites. A total of 60 metabolites were common between the 3F and 2F. This indicates that the stem's dimensional structure influences metabolite variation (Figures 3 and 4), (Table 2).

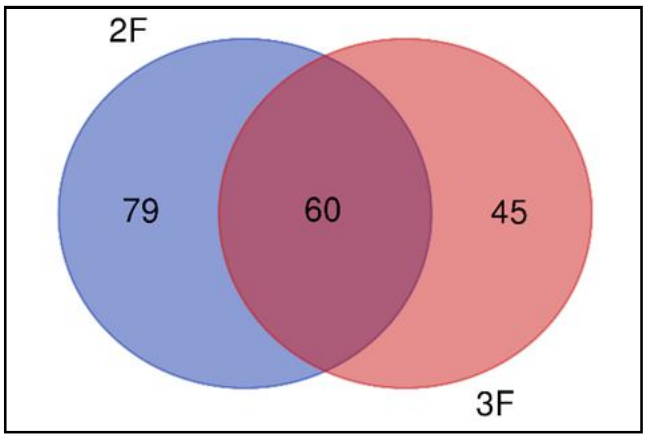

Figure 3: Venn diagram of unique and shared metabolites in 2F and 3F stems.

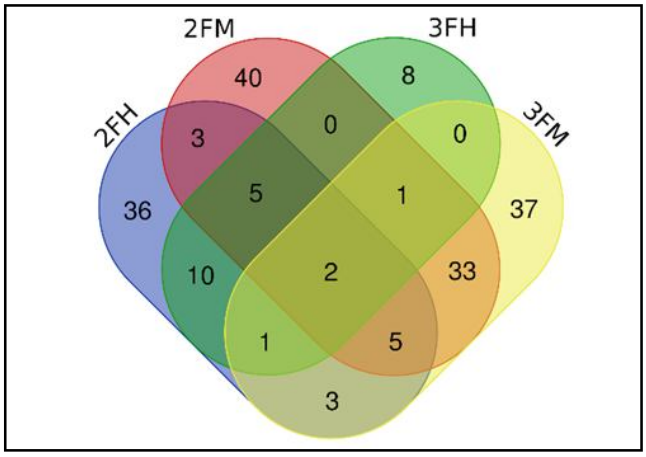

Figure 4: Venn diagram: solvent based-unique and shared metabolites in 2F and 3F stems.

Table 1: Comprehensive list of metabolites and their PubChem IDs categorized by solvent conditions (2F and 3F)

Samples	No. of metabolites	PubChem IDs
2F and 3F	60	5461492 18950 5364924 522224 7311 11902 68346 8181 649 439710 15600 11005 2959275 541562 5365022 173183 226486 566657 634514 985 1135 72491 3026 5921 12389 12329 558221 292285 1017 141085 196216 5280934 712914 14900 6452096 537255 144863 5280794 3893 92158 519764 549041 537294 12403 5283469 10467 225688 5280435 82330 5281 6782 92729 119838 11006 237332 1549095 5370028 92139 8042 10465
2F	79	12410 5280590 79052 12406 10408 11636 580756 5366014 538186 64674 5363092 159643 6432173 92094 91745072 122857 15612 12592 5459879 85214 101465 5364677 12401 90232 91701516 72326 24699 12390 8215 8346 5282772 567149 13849 545627 536786 5363101 74416 12408 80281 6989 314392 581229 41920 5364713 23831 8180 544066 610888 579931 17218 17835 552030 5363222 7838 23494 41208 7714 6428538 296566 12412 8091 439503 581840 40924 13585 1197 141084 545593 73791 7742 545963 552140 536377 107297 534625 638072 31236 14257 580376
3F	45	11197 5363161 559066 597057 552071 88389 588574 520159 340 277822 586537 7858 70133 91696297 19309 225689 5565575 573939 11052245 79075 5363633 12523 585743 14795191 568041 5281515 78093 597795 518616 28473 5280662 8872 111037 10104370 540542 5366008 602351 8182 135703343 566188 91701118 99931 16003 538757 8343

Table 2: List of metabolites and their PubChem IDs based on individual solvent conditions (Hexane and Methanol) for 2F and 3F

Samples	No.of metabolites	PubChem IDs
2FH 2FM 3FH 3FM	2	8181 5280435
2FH 2FM 3FH	5	7311 558221 292285 196216 8042
2FH 2FM 3FM	5	5365022 14900 6452096 5280794 92729
2FH 3FH 3FM	1	519764
2FM 3FH 3FM	1	11005
2FH 2FM	3	24699 6428538 638072
2FH 3FH	10	68346 15600 12389 1017 141085 12403 82330 6782 11006 92139
2FH 3FM	3	173183 3026 537255
2FM 3FM	33	5461492 18950 5364924 522224 11902 649 439710 2959275 541562 226486 566657 634514 985 1135 72491 5921 12329 5280934 712914 144863 3893 92158 549041 537294 5283469 10467 225688 5281 119838 237332 1549095 5370028 10465
2FH	36	12410 5280590 79052 12406 11636 538186 64674 6432173 12592 5364677 12401 567149 545627 5363101 12408 80281 581229 41920 5364713 544066 17218 17835 5363222 23494 41208 296566 12412 13585 141084 545593 73791 545963 536377 534625 31236 14257
2FM	40	10408 580756 5366014 5363092 159643 92094 91745072 122857 15612 5459879 85214 101465 90232 91701516 72326 12390 8215 8346 5282772 13849 536786 74416 6989 314392 23831 8180 610888 579931 552030 7838 7714 8091 439503 581840 40924 1197 7742 552140 107297 580376
3FH	8	12523 5281515 28473 111037 10104370 540542 8182 91701118
3FM	37	11197 5363161 559066 597057 552071 88389 588574 520159 340 277822 586537 7858 70133 91696297 19309 225689 5565575 573939 11052245 79075 5363633 585743 14795191 568041 78093 597795 518616 5280662 8872 5366008 602351 135703343 566188 99931 16003 538757 8343

3.2 Statistical analysis of identified metabolites in 3F and 2F stem of *C. quadrangularis*

To understand the difference between the 3F and 2F, PCA and PLS-DA analysis were carried out. PCA analysis revealed the variance between the 3F and 2F. PC1 explains 52.8% of the variance and PC2 33.5 % of variance, which indicating that 3F and 2F has metabolite level variation (Figure 5).

To distinguish the metabolite contribution in the variation between the 3F and 2F, PLS-DA analysis was carried out. PLS-DA visualizes the separation of groups (2FH, 2FM, 3FH, 3FM) in the first two latent components. The x-axis (Component 1) explains 48.9% of the variance, and the y-axis (Component 2) explains 28.3%, indicating the contribution of these components to group differentiation (Figure 6). If, the metabolites VIP score is greater than or equal to one were considered as the important metabolites which contribute for the sample difference.

Octodecane, myristic acid and tocopher showed highest VIP score in the PLS-DA analysis. Octodecane was highly accumulated in the 2F, myristic acid was higher in the 3F and tocopher was high in 2F (Figure 7).

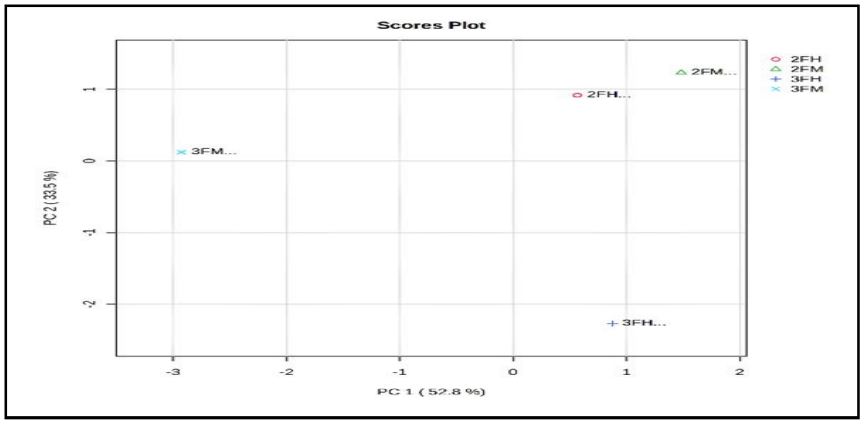

Figure 5: PCA plot showing variancebetween 3F and 2F stems.

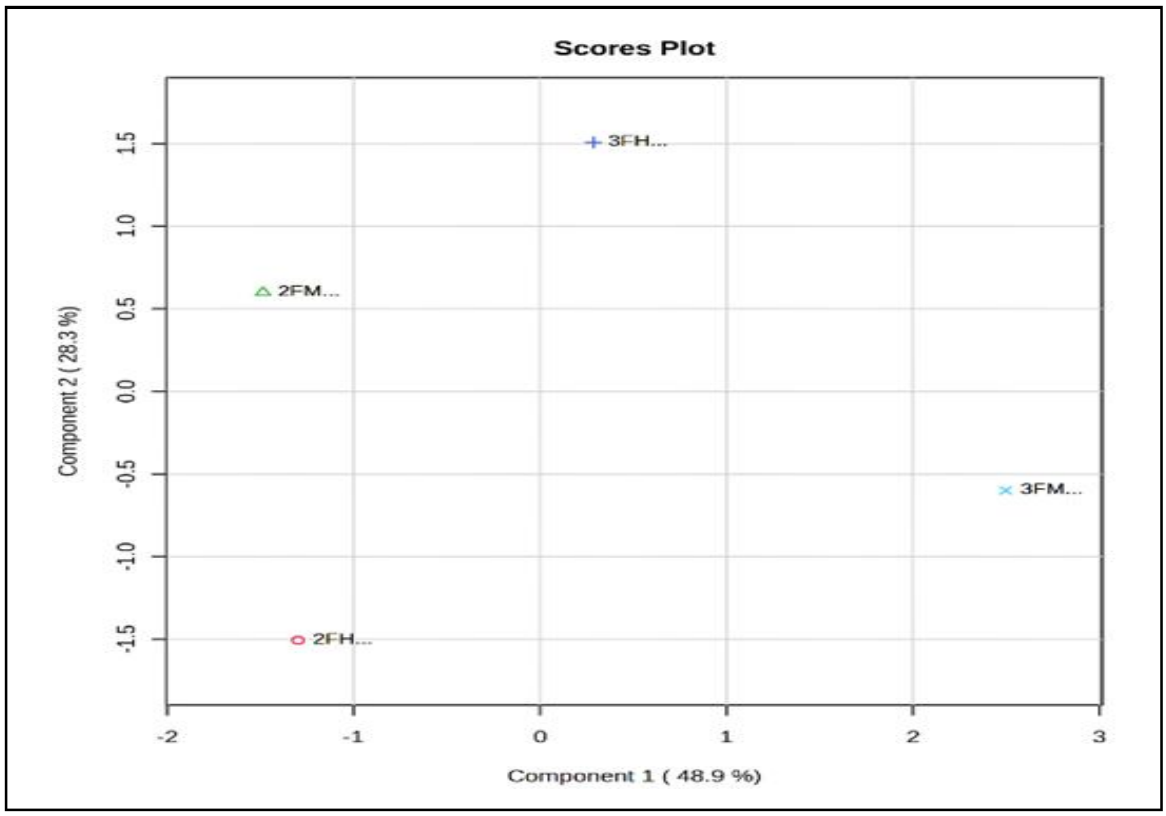

Figure 6: PLS-DA score plot showing variance between 3F and 2F stems.

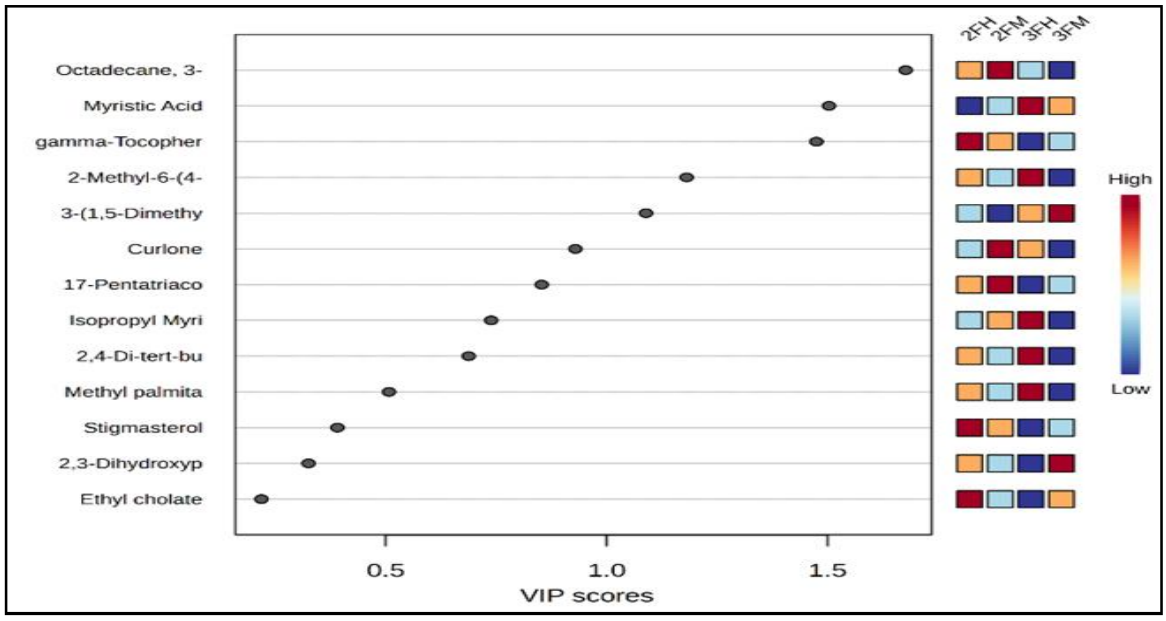

Figure 7: VIP scores showing variance between 3F and 2F stems.

3.3 Differentially accumulated metabolites

The GC-MS analysis revealed a several metabolites that exhibit differential regulation. The list of upregulated metabolites included gamma-tocopherol, 2,3-dihydroxypropyl hexadecanoate, 17-pentatriacontene, ethyl cholate, and stigmasterol. However, in contrast there is down-regulation of myristic acid, isopropyl myristate, octadecane-3-ethyl-5-(2-ethylbutyl)-, curlone, 2,4-di-tert-butylphenol, 2-methyl-6-(4-methylphenyl)hept-2-en-4-one, and 3-(1,5-dimethyl-4 The results of the present study offer understanding of the metabolic shifts taking place under the investigated conditions (Table 3).

Table 3: Insights into upregulated and downregulated compounds across different stem regions

S. No.	Name	Fold change	Log 2 (FC)	Regulation (up/down)
1.	Myristic acid	0.012553	-6.3159	Down regulated
2.	Gamma-tocopherol	60.03	5.9076	Up regulated
3.	2,3-dihydroxypropyl hexadecanoate	38.365	5.2617	Up regulated
4.	17-pentatriacontene	6.9649	2.8001	Up regulated
5.	Ethyl cholate	5.631	2.4934	Up regulated
6.	Stigmasterol	5.0131	2.3257	Up regulated
7.	Isopropyl myristate	0.29813	-1.746	Down regulated
8.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	3.1381	1.6499	Up regulated
9.	Curlone	0.3512	-1.5097	Down regulated
10.	2,4-Di-tert-butylphenol	0.3525	-1.5043	Down regulated
11.	2-methyl-6-(4-methylphenyl)hept-2-en-4-one	0.47504	-1.0739	Down regulated
12.	3-(1,5-dimethyl-4-hexenyl)-6-methylene-1-cyclohexene	0.47735	-1.0669	Down regulated

3.4 Functional annotation

3.4.1 Chemical classification

Metabolite distribution has significantly differed between the two conditions, 2F and 3F. Alkanes were significantly higher in 2F (17) as compared with 3F (6), and fatty acids were also higher in 2F (9 vs 2). In contrast, the identification of triterpenoids and carbohydrates was higher in 3F with 5 and 4 metabolites, respectively, compared to 2F with only 1 metabolite. The metabolite level of carbohydrates and carbohydrate conjugates were also found to be higher in 3F (4) than 2F (1). Changes in benzoic acids and derivatives, methoxy-phenols and linoleic acids were minimal and their respective counts were recorded at 2 or below in both conditions. From this data, it is evident how different experimental conditions also affect certain metabolite classes, with triterpenoids and carbohydrates increased in extracts/organisms exposed to 3F, alkanes and fatty acids of 2F (Figure 8).

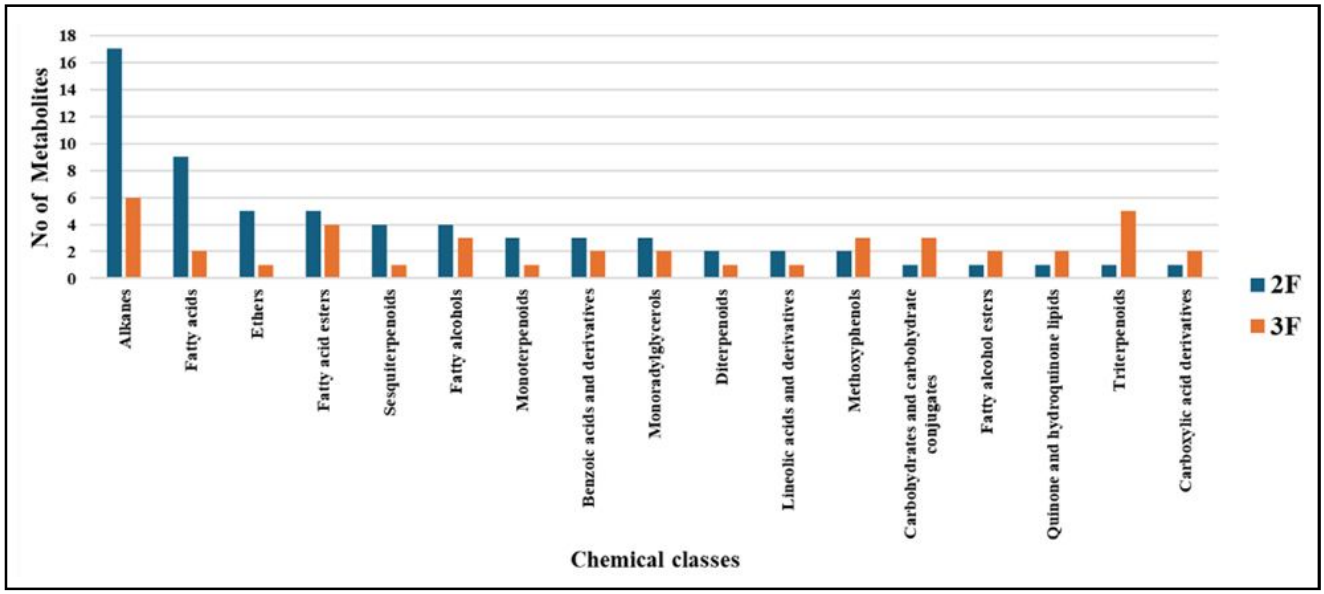

Figure 8: Chemical classification of 2F and 3F metabolites.

3.4.2 Pathway

3.4.2.1 Pathways involved in 2F *C. quadrangularis*

Metabolic profiling analysis showed that there was a profound change across biomolecules in different biosynthetic and degradative routes. Out of these pathways, sesquiterpenoid and triterpenoid biosynthesis appeared to be significantly involved (p = 0.025686, impact = 0.21622) in the metabolic changes patients undergoing hypoxic conditions. All the involved pathways are listed in the Table 2 with direction of change depicting the final pattern of metabolism process. The lipid metabolism pathways were significantly highlighted, more particularly fatty acid biosynthesis, p = 0.0022711 and biosynthesis of unsaturated fatty acids, p = 5.64E-05. Other pathways encompassing the roles of SCD1 include steroid biosynthesis (p = 0.010102, impact = 0.04963) α-linolenic acid metabolism (p = 0.24511, impact = 0.125) which also shifted the

phases of sterols and essential fatty acid metabolism. Two biosynthetic pathways of secondary metabolites, phenylpropanoid and ubiquinone, were found to have moderate *p*-values ($p = 0.37359$ and $p = 0.087268$, respectively) and low effect sizes suggesting additional roles in generating the observed metabolic profile (Table 4).

Table 4: Biochemical pathways involved in 2F *C. quadrangularis* and their associated *p*-values

S.No.	Pathway name	*p*-value
1	Sesquiterpenoid and triterpenoid biosynthesis	0.025686
2	Alpha-linolenic acid metabolism	0.24511
3	Beta-alanine metabolism	0.17646
4	Steroid biosynthesis	0.010102
5	Phenylpropanoid biosynthesis	0.37359
6	Ubiquinone and other terpenoid-quinone biosynthesis	0.087268
7	Pyrimidine metabolism	0.068692
8	Pantothenate and CoA biosynthesis	0.23683
9	Fatty acid biosynthesis	0.0022711
10	Biosynthesis of unsaturated fatty acids	5.64E-05
11	Cutin, suberine, and wax biosynthesis	0.01478
12	Fatty acid elongation	0.22002
13	Brassinosteroid biosynthesis	0.24511
14	Fatty acid degradation	0.33079

3.4.2.2 Pathways involved in 3F *C. quadrangularis*

The pathway analysis highlighted the changes of the metabolic processes with the focus on fatty acids biosynthesis and biosynthesis of unsaturated fatty acids ($p = 0.0017517$, impact = 0.01123), ($p = 4.27E-05$, impact = 0.0), respectively. Secondary metabolite biosynthesis pathway analysis also highlighted sesquiterpenoid and triterpenoid biosynthesis ($p = 0.0014451$, impact = 0). Low alterations detected in metabolism of alpha-linolenic acid ($p = 0.23166$, impact = 0.125), beta-alanine metabolism ($p = 0.16635$, impact = 0.07143), and phenylpropanoid biosynthesis ($p = 0.35491$, impact = 0.0451), whereas some pathways, such as pyrimidine metabolism ($p = 0$). Other networks like ubiquinone biosynthesis, steroid biosynthesis, and fatty acid elongation also were affected only slightly, implying that their contribution towards metabolic changes is comparatively insignificant. The identified alterations prioritize lipid and secondary metabolite biosynthesis pathways as the most reactive to the discussed conditions (Table 5).

Table 5: Biochemical pathways involved in 3F *C. quadrangularis* and their associated *p*-values

S. No.	Pathway name	*p*-value
1	Alpha-linolenic acid metabolism	0.23166
2	Beta-alanine metabolism	0.16635
3	Phenylpropanoid biosynthesis	0.35491
4	Biosynthesis of various plant secondary metabolites	0.25489
5	Pyrimidine metabolism	0.061142
6	Pantothenate and CoA biosynthesis	0.22377
7	Ubiquinone and other terpenoid-quinone biosynthesis	0.38109
8	Fatty acid biosynthesis	0.0017517
9	Biosynthesis of unsaturated fatty acids	4.27E-05
10	Sesquiterpenoid and triterpenoid biosynthesis	0.0014451
11	Steroid biosynthesis	0.069333
12	Cutin, suberine and wax biosynthesis	0.16635
13	Fatty acid elongation	0.20775
14	Brassinosteroid biosynthesis	0.23166
15	Fatty acid degradation	0.31369

Based on the results of the 2F condition, the pathways with *p*-value less than 0.05 and non-zero impact are fatty acid biosynthesis (p = 0.0022711, impact = 0.01123), steroid biosynthesis (p = 0.010102, impact = 0.04963), and sesquiterpenoid and triterpenoid biosynthesis (p = 0.025686, impact factor = 0.012110). The pathways with *p*-values lower than 0.05 for the 3F condition are biosynthesis of unsaturated fatty acids with *p*-value of 4.27E-05, sesquiterpenoid and triterpenoid biosynthesis with p = 0.0014451 and fatty acid biosynthesis with p = 0.0017517, with no impact value of 0. These data suggest that both conditions 2F and 3F exhibit significant metabolic reprogramming of fatty acid synthesis, sesquiterpenoid, and triterpenoid synthesis, although 2F has the unique pattern of steroid biosynthesis.

3.5 Node structure and metabolite distribution

Structural differences in the nodes of *C. quadrangularis* influence metabolite distribution by affecting biosynthesis, storage, and transport pathways. Three-dimensional (3F) nodes, with their larger surface area, intricate vascular systems, and specialized tissues, facilitate the accumulation of complex metabolites like triterpenoids and sterols, supported by higher activity in triterpenoid biosynthesis pathways. In contrast, two-dimensional (2F) nodes, with simpler structures and less complex vascular systems, prioritize primary metabolic functions, resulting in elevated levels of simpler compounds such as fatty acids and alkanes, consistent with their dominance in fatty acid biosynthesis pathways. These structural variations not only dictate metabolic profiles but also reflect adaptive mechanisms for environmental interaction and resource allocation, offering insights for targeted utilization in pharmaceutical and nutraceutical applications.

3.6 Bioactive compounds and their therapeutic benefits

Bioactive compounds in plants, including flavonoids, phenols, and terpenoids, are closely associated with a wide range of therapeutic properties. Phenolic compounds, particularly abundant in species like *C. quadrangularis*, demonstrate potent antioxidant activity by neutralizing free radicals and mitigating oxidative stress. This activity is crucial in protecting cells from damage, slowing the aging process, and reducing the risk of chronic conditions such as cancer and cardiovascular diseases. Furthermore, flavonoids and tannins have been shown to enhance osteogenic properties by promoting osteoblast differentiation and activity. These actions contribute to bone formation, mineralization, and fracture healing, underscoring their potential as therapeutic agents in managing osteoporosis and other bone-related disorders.

3.7 Key metabolites in osteoblast function

Gamma-tocopherol, stigmasterol, and octadecane are key metabolites with significant therapeutic potential in the management of osteoporosis and the promotion of osteoblast function. Gamma-tocopherol, a potent antioxidant, helps mitigate oxidative stress, a key factor in bone degradation, by protecting osteoblasts and promoting bone mineralization. Stigmasterol, a plant sterol, enhances osteoblast differentiation and activity, supporting bone formation while also reducing inflammatory cytokines that accelerate bone loss. Octadecane, a long-chain alkane, contributes to lipid metabolism, modulating osteoblast function and promoting the synthesis of key bone matrix components. Collectively, these metabolites support bone health through antioxidant, anti-inflammatory, and osteogenic effects, making them promising candidates for the development of plant-based therapies for osteoporosis and other bone-related disorders.

4. Discussion

In metabolite profiling, 2F nodes show a higher number of unique metabolites, emphasizing their role in metabolic diversity and specialized functions. These metabolites are linked to therapeutic properties like antioxidant, anti-inflammatory, and osteogenic activities, highlighting the pharmacological potential of 2F nodes. The statistical analyses conducted on the 3F and 2F stems of *C. quadrangularis* revealed significant metabolic variations that are likely influenced by the physical dimensions of the stems. It was hypothesized that these stems' metabolic functions, specifically the CAM, play a role in their adaptation to dry environments. Also, the physical size of the stems has probably contributed to water storage and metabolic activities that are critical in their survival under extreme conditions (Irwin *et al.*, 1983) (Qingyun *et al.*, 2024).

PCA indicated a clear separation between the metabolic profiles of the two stem types, with distinct variance patterns suggesting that the stem dimension plays a key role in shaping metabolic activity. This separation indicates that morphological traits that are localized to certain parts of the plant like stem size, may affect the overall metabolic pathways in all tissues of the plant. This means that even a single gene modification affecting stem size could alter metabolite content in many tissues due to the complex interconnections between plant metabolism and metabolite transport in specific tissues (Nicole *et al.,* 2015). PLS-DA analysis further identified important metabolites that contributed to this differentiation, including octodecane, myristic acid, and tocopherol, which showed the highest VIP scores. The higher accumulation of octodecane and tocopherol in the 2F stem suggests enhanced metabolism of aliphatic compounds and antioxidants, possibly as an adaptive response to environmental stress. Similar findings by Stiffie *et al.* (2017) indicate that tocopherols, as vitamin E forms, helps to protect plants from oxidative damage caused by stressors like drought or heat. These compounds may offer biological protection against cellular degeneration, contributing to the resilience of the 2F stem in challenging conditions. In contrast, myristic acid was more abundant in the 3F stem, which may reflect its role in fatty acid synthesis or storage. Purohit *et al.* (2023) also highlighted myristic acid as a key metabolite in *Cissus rotundifolia*, specifically within the fatty acid biosynthetic pathways, supporting its potential role in metabolic regulation and adaptation. Nitesh *et al.* (2021) found that tocopherol acts as an antioxidant, protecting plants from oxidative stress, while myristic acid plays a role in membrane fluidity and signaling, contributing to stress response mechanisms. PCA and PLS-DA revealed distinct metabolic profiles between 2F and 3F stems of *C. quadrangularis*, with key metabolites like tocopherol and myristic acid. These findings highlight the influence of stem morphology on metabolic functions, suggesting potential for optimizing plant breeding to enhance stress resilience and therapeutic benefits. The results of chemical classification highlighted that experimental conditions significantly influence metabolite distribution, with 3F favouring triterpenoids and carbohydrates, while 2F shows higher levels of alkanes and fatty acids. According to Nupur (2021), the chemical structure of each phytochemical enables it to promote well-being. The concentration of these phytochemicals in plants, herbs,

or spices varies depending on factors such as season, plant cultivar, soil conditions, and nutrient availability.

The identified metabolic pathways in 2F and 3F conditions have wide-ranging industrial applications. Fatty acid biosynthesis plays a key role in the pharmaceutical, cosmetic, and biofuel industries, while steroid biosynthesis (2F) is crucial for hormone therapies. Fatty acids are vital for nutrition and heart health, with unsaturated fatty acids offering particular benefits for skin and bone health (Masrat *et al.,* 2023). Sesquiterpenoids and triterpenoids (2F) have potential in pharmaceuticals, cosmetics, and agriculture. Unsaturated fatty acids (3F) are important for nutrition, heart health, and skincare. These pathways enable sustainable production of bioactive compounds for multiple industries. These findings align with the work of Shengxin *et al.* (2024), which highlights the diverse applications of sesquiterpenoids and triterpenoids in agriculture, cosmetics, and pharmaceuticals. The study also emphasizes the role of sesquiterpene synthases in facilitating their heterologous biosynthesis. Sesquiterpenoids and triterpenoids in *C. quadrangularis* contribute to its medicinal value by offering anti-inflammatory, antioxidant, and antimicrobial benefits, while also supporting bone health through enhanced osteoblast activity and mineralization. These compounds, particularly triterpenoids, are known for their therapeutic properties and are effective in treating osteoporosis and chronic diseases, as highlighted by Sibi *et al.* (2020). This study highlights the functional specialization between the two stem types of *C. quadrangularis*, revealing how stem dimensions influence the distribution and regulation of key metabolites. Using PCA and PLS-DA, the research identifies unique metabolites, such as tocopherols and octodecane, linked to metabolic pathways like sesquiterpenoid and triterpenoid biosynthesis. These findings offer new insights into the plant's therapeutic potential, particularly in bone health and provide a foundation for future pharmacological studies.

5. Conclusion

This study provides valuable insights into the metabolic differentiation of *C. quadrangularis* stems based on their physical structure. The distinct accumulation of metabolites such as tocopherols, myristic acid, and octodecane in different stem types underscores the importance of plant morphology in shaping its biochemical composition. These findings highlight that the variation in metabolite content across stem types is crucial for understanding the plant's potential therapeutic applications, especially for bone health and oxidative stress. Furthermore, the diverse phytochemical profile, including flavonoids, polyphenols, and vitamins, suggests that *C. quadrangularis* holds promise not only in bone health but also in broader areas such as anti-inflammatory and antioxidant therapies. This suggests that the plant's wide-ranging bioactive properties can be leveraged for multiple therapeutic purposes. The nodal structures, with their unique biochemical properties, provide valuable insights into the plant's potential as a functional food or dietary supplement. The study emphasizes the significance of nodal differences in influencing the plant's overall metabolic profile, which could guide future developments in plant-based therapeutics. In conclusion, these findings underscore the need for further research on the pharmacological benefits of *C. quadrangularis,* particularly in the context of structural and environmental influences on its bioactive properties. This study paves the way for the development of targeted therapies based on the plant's metabolic and structural characteristics, contributing to the growing field of plant-based medicinal research.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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