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RESEARCH ARTICLE

Study of variation in floral metabolite profiles of *Tabernaemontana divaricata* (L.) R. Br. genotypes based on gas chromatography- mass spectrometry

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Abstract

Tabernaemontana divaricata (L.) R. Br. is a popular ornamental shrub. Each and every part of the plant is medicinally very significant. The floral metabolome of it remains largely uncharacterized. In this study, gas chromatography-mass spectrometry (GC-MS) analysis was performed on methanolic floral extracts from four genotypes leading to the identification of 68-99 metabolites out of which 30 metabolites based on notable peak area percentages from all 4 genotypes were taken to further study. The detected compounds included methyl salicylate, myo-inositol, cis-vaccenic acid, squalene, geraniol, phytol, n-hexadecanoic acid and benzene derivatives. The metabolites belong to diverse chemical classes such as esters, terpenoids, lactones, fatty acid derivatives and aromatic alcohols. These metabolites are well known for their antioxidant, antimicrobial and anti-inflammatory properties. Chemometric tools including principal component analysis (PCA), hierarchical clustering and Venn diagrams revealed clear genotype-specific variation and distinct grouping based on metabolite profiles. Whereas previous reports have focused on alkaloid-rich leaf and latex extracts, this study provides first characterization of the floral metabolite diversity and identifies genotype specific metabolic profiles of *T. divaricata*, thereby enhancing its chemotaxonomic understanding. Potential applications in breeding, fragrance or therapeutics are suggested as future avenues for research.

Keywords: Apocynaceae; biological activities; hierarchical clustering; metabolites; principal component analysis

Introduction

Metabolic profiling enables detailed characterization of plant chemotypic variation by analysing a wide array of metabolites. This includes primary metabolites essential to cellular function and also secondary metabolites known for their pharmacological properties (1). Because plant metabolites are chemically diverse, a range of analytical platforms are used for their isolation and identification, including GC-MS, liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR) and capillary electrophoresis-MS (2, 3). The use of multiple platforms is necessary because no single technique can capture the entire spectrum of metabolites; each method offers unique advantages for detecting specific classes of compounds, ensuring comprehensive metabolome coverage. GC-MS is particularly effective for profiling volatile and semi-volatile compounds due to its high chromatographic resolution and comprehensive spectral libraries (4). This technique supports the robust detection and quantification of volatile organic compounds (VOCs) which can function as biomarkers within complex biological samples (5). Within the Apocynaceae, *Tabernaemontana* species exemplify phytochemically diverse taxa of significant pharmacological interest (6).

Tabernaemontana divaricata (L.) R. Br. also known as Pinwheel or Crepe Jasmine, belongs to the family Apocynaceae. This species is an indigenous, evergreen ornamental shrub of India, with approximately 100 species distributed across tropical regions worldwide (7). Distinctive morphological traits of the genus include tubular white flowers, follicular fruits containing seeds enveloped in yellow to reddish arils and a characteristic milky latex exuded upon injury. Often referred to as milkweed, members of this genus are extensively recognized for their broad spectrum of bioactivities including antimicrobial, antioxidant, anti-inflammatory, anticholinesterase, anti-neurodegenerative, anticancer, antidiabetic, antivenom, larvicidal, antihypertensive, wound-healing and analgesic properties (8). The plant is traditionally employed in the treatment of various ailments such as abdominal tumours, epilepsy, ocular infections, fever, headache, inflammation, leprosy, asthma, diarrhoea, paralysis, rheumatic pain, ulceration and

emesis. Globally, it holds a prominent place in ethnomedicine and is esteemed not only for its ornamental appeal but also for its substantial therapeutic value (9).

Tabernaemontana has extensively investigated for its pharmacological attributes, most 330 studies have predominantly concentrated on the leaves, roots or latex. Despite the extensive pharmacological investigations into *Tabernaemontana* species, a significant knowledge gap persists in the metabolomic characterization of their floral tissues. Very few of investigations have resulted in fragmented and preliminary profiles of flower metabolites (10, 11). Systematic and comparative studies on the chemical diversity and inter-genotypic variation of floral metabolites remain scarce, impeding a deeper understanding of their unique bioactive compounds and chemotaxonomic roles. Addressing this gap is essential for validating traditional uses, advancing drug discovery and guiding ornamental breeding efforts, as the flowers likely harbor uncharacterized compounds with promising pharmacological and horticultural potential. The present study aims to provide a comprehensive GC-MS based profiling of floral metabolites across four *T. divaricata* genotypes. The elucidation of inter-genotypic metabolic variation advances the chemotaxonomic resolution of the species.

Materials and Methods

Plant species studied

The present investigation was conducted during 2024-2025 at the Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. It is situated at latitude of 10°00'N, longitude of 77°00'E and an elevation of 412 m above mean sea level (MSL). Four morphologically distinct genotypes of *Tabernaemontana divaricata* namely Td-Acc.04, Td-Acc.07, Td-Acc.08 and Td-Acc.09 maintained in the department's germplasm repository were selected for floral metabolite profiling as detailed in Table 1. Freshly bloomed flowers were randomly harvested at 6:00 am as maximum volites emission takes place in morning hours. They were collected from five individual plants per genotype. The collected floral samples were promptly transported to the laboratory for further biochemical analysis.

Preparation of methanolic extract of flowers

Following the removal of extraneous floral parts and thorough rinsing with clean water, 50 g of whole flowers were flash-frozen with liquid nitrogen and ground into a fine powder. The powdered sample was then extracted with 50 mL of methanol in conical flasks. The mixture was agitated in an orbital shaker for 96 hrs at 100 rpm and 28 °C. After incubation, the extract was filtered through Whatman No. 3 filter paper. The resulting filtrate was concentrated using a rotary flash evaporator (Model HV-1224) at 80 rpm and 55 °C. Subsequently, 1 mL of the concentrated extract was reconstituted in HPLC-grade methanol and filtered through a hydrophilic polyvinylidene fluoride (PVDF) membrane (0.22 µm pore size, HiMedia) to ensure sample purity prior to GC-MS analysis.

GC-MS Analysis

Volatile constituents of the methanolic flower extract were characterized using a GC-MS system comprising an Agilent 7890A gas chromatograph coupled with a 5975C Mass Selective Detector (MSD) (Agilent Technologies, Tirupur, Tamil Nadu) operating under electron ionization (EI) at 70 eV. The ion source temperature was maintained at 250 °C. Separation was achieved using an Agilent DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). High-purity helium (99.9 %) was employed as the carrier gas at a constant flow rate of 1.0 mL/min. The injection volume was 1 µL, with the injector operated in split mode at a ratio of 1:60. The oven temperature program was initiated at 100 °C and held for 0.5 min, ramped to 140 °C at 20 °C/min (held for 1 min) and further increased to 280 °C at 11 °C/min over a period of 20 min. Data acquisition and peak integration were performed using Mass Hunter Workstation Software (version 11.0, Agilent Technologies).

Table 1. *Tabernaemontana divaricata* genotypes

Genotype accession no.	Location of collection			Nature of open flower	Description
	Area	Latitude	Longitude		
Td-Acc.04	Kollam, Kerala	8.8932° N	76.6141° E		Single type, fragrant, long buds, twisted petals to form a pinwheel shape
Td-Acc.07	Kalpakkam, Chengalpet	12.5238° N	80.1568° E		Single type, round petals and non-fragrant used as control to compare the volatile compounds in fragrant types
Td-Acc.08	Vadavalli, Coimbatore	11.0268° N	76.9058° E		Semi double type, overlapping petals, delicate whorled petals with crepe texture and fragrant
Td-Acc.09	Madhavaram, Chennai	13.1488° N	80.2306° E		Double type, ruffled, curled, wavy edged tightly arranged and twisted petals that form lush intricate rosette like with fragrant blooms

Compound identification

Peak identification was performed using the Wiley Mass Spectral Library (W9N11) and the National Institute of Standards and Technology (NIST), GC-MS databases to determine the identity of unknown volatile compounds. Information regarding the biological activities of the identified metabolites was obtained from Dr. Duke's Phytochemical and Ethnobotanical Databases (access April 2025). Molecular weights and chemical formulas of the compounds were cross-verified using the PubChem database.

Chemometric analysis

The GC-MS derived metabolic profiles of the four *T. divaricata* genotypes were subjected to chemometric analysis to evaluate chemotypic variation based on floral volatile composition. The dataset was compiled using the relative peak area percentages of 30 metabolites from GC chromatograms. PCA and Hierarchical Cluster Analysis (HCA) were employed to explore clustering patterns and assess the discriminatory power of these techniques in differentiating closely related genotypes. Both analyses were conducted utilising Metabo Analyst software version 6.0 (12). To facilitate a comprehensive comparison, a heat map was constructed to depict the abundance and distribution patterns of the identified metabolites across the genotypes. Additionally, a Venn diagram was generated using j Venn software (13) to illustrate the shared and unique metabolites among the different genotypes, thereby facilitating visualization of genotype specific and common metabolic features.

Results

Metabolites analysed by GC-MS

In the current investigation, GC-MS analysis of *T. divaricata* floral extracts revealed a diverse array of volatile metabolites. A total of 68, 70, 71 and 99 compounds were detected in Td-Acc.04, Td-Acc.07, Td-Acc.08 and Td-Acc.09 respectively. The identified metabolites were validated by molecular formulas, peak area percentage and retention time. GC-MS chromatograms of the genotypes Td-Acc.04, Td-Acc.07, Td-Acc.08 and Td-Acc.09 are shown in Fig. (1- 4) respectively. To assess the distribution of identified floral metabolites across genotypes, a Venn diagram was constructed (Fig. 5). The analysis identified 6 metabolites that were commonly shared among all 4 genotypes, indicating a conserved core volatile profile. Among these, squalene and phenol were present in substantial quantities, while ibogaine, ethyl ester, hexane and linalool were detected in trace amounts. Conversely, a number of compounds were found to be genotype specific reflecting considerable chemical divergence. Td-Acc.04 exhibited 42 unique metabolites, Td-Acc.07 had 44, Td-Acc.08 possessed 37 and Td-Acc.09 displayed the highest number with 67 unique compounds. This chemotypic variation underscores the metabolic complexity among genotypes which may influence floral fragrance profiles, ecological interactions or pharmacological potential. For subsequent chemometric and comparative analyses, only 30 metabolites were selected based on significant peak area percentages indicative of their relative abundance across all genotypes. Metabolites exhibiting consistent and prominent peak areas were chosen to ensure the focus remained on compounds most relevant to genotype differentiation and biological interpretation.

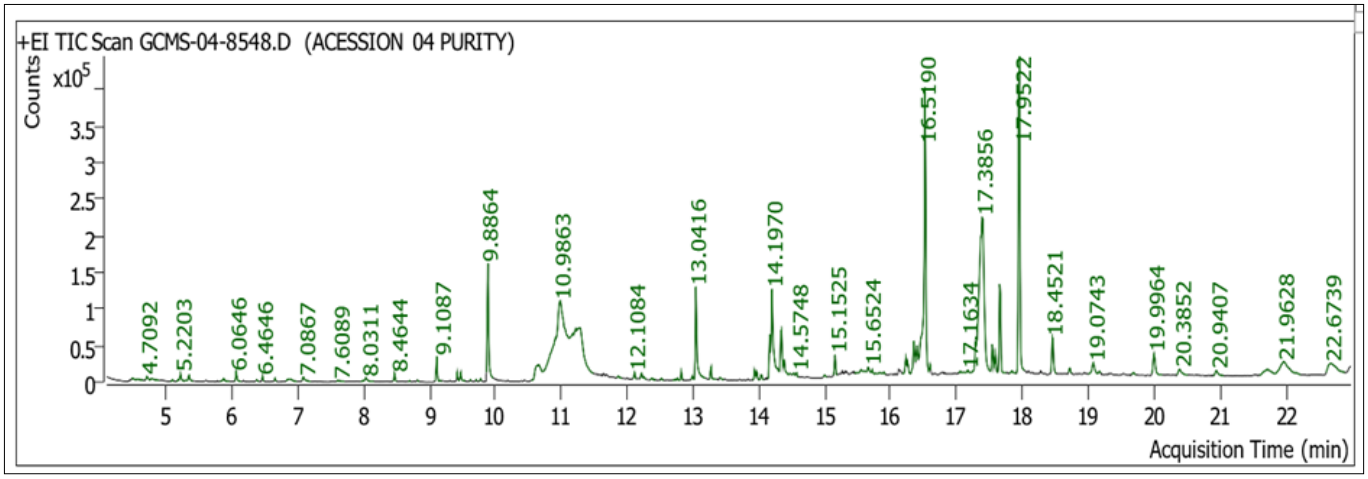

Fig. 1. Metabolite profiling of VOCs in flowers of *Tabernaemontana divaricata* Td-Acc.04.

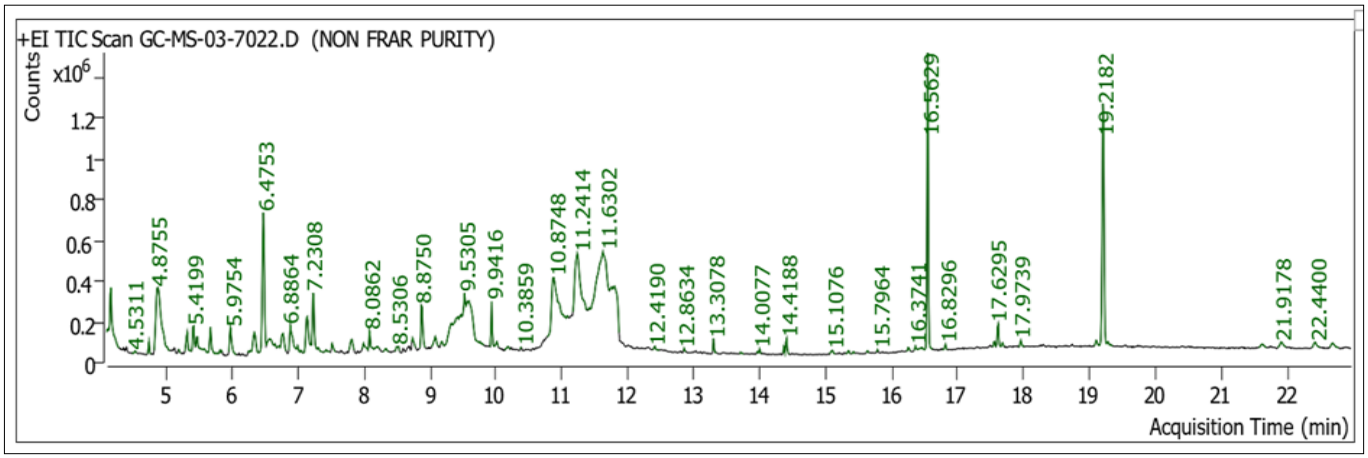

Fig. 2. Metabolite profiling of VOCs in flowers of *Tabernaemontana divaricata* Td-Acc.07.

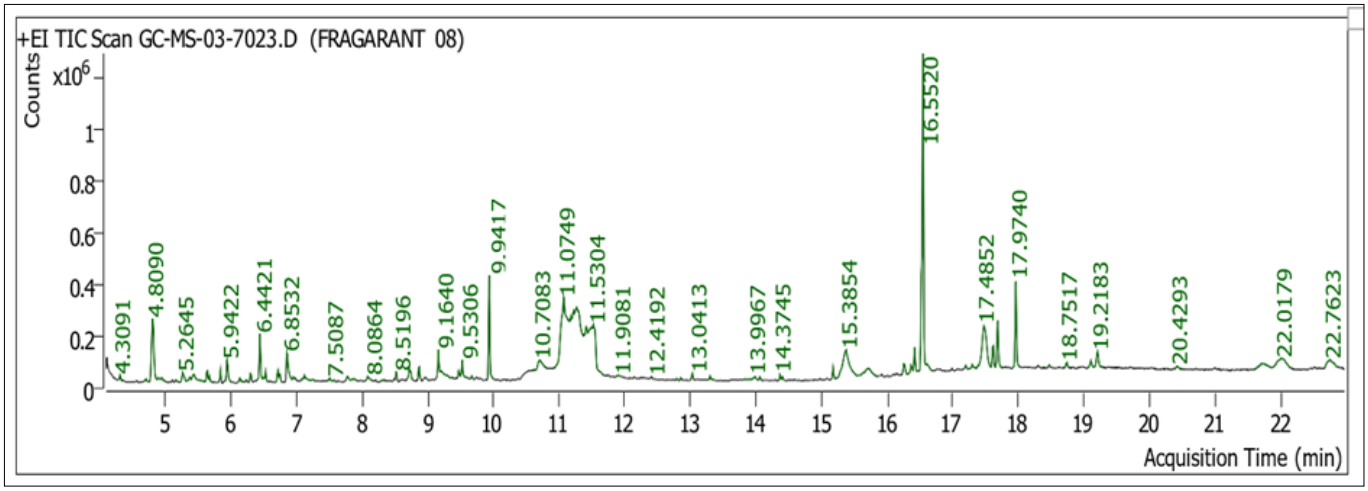

Fig. 3. Metabolite profiling of VOCs in flowers of *Tabernaemontana divaricata* Td-Acc.08.

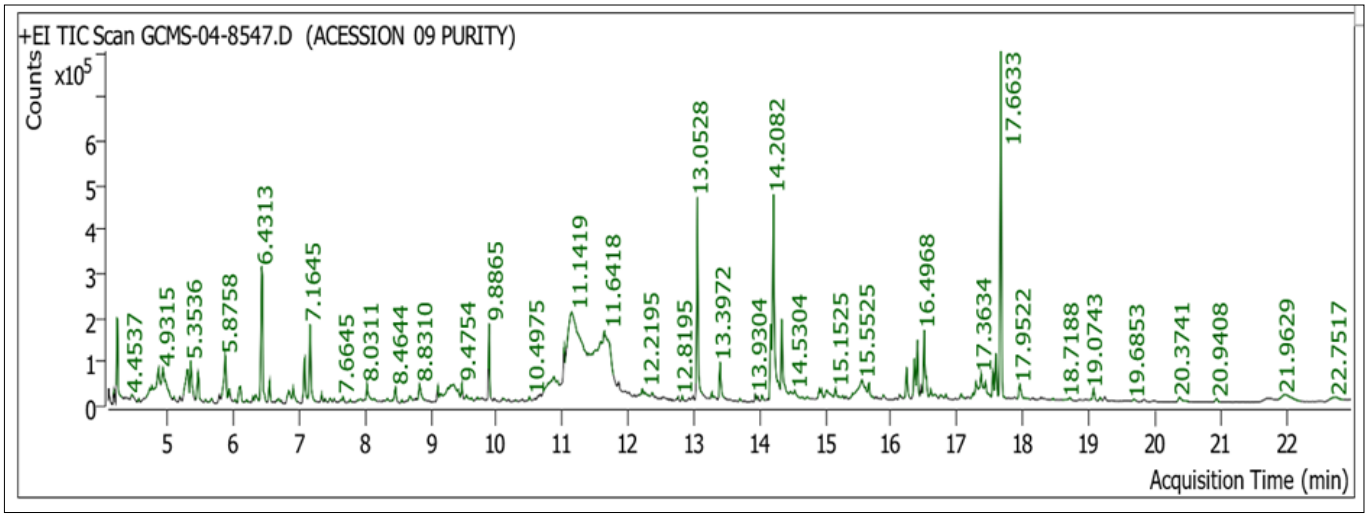

Fig. 4. Metabolite profiling of VOCs in flowers of *Tabernaemontana divaricata* Td-Acc.09.

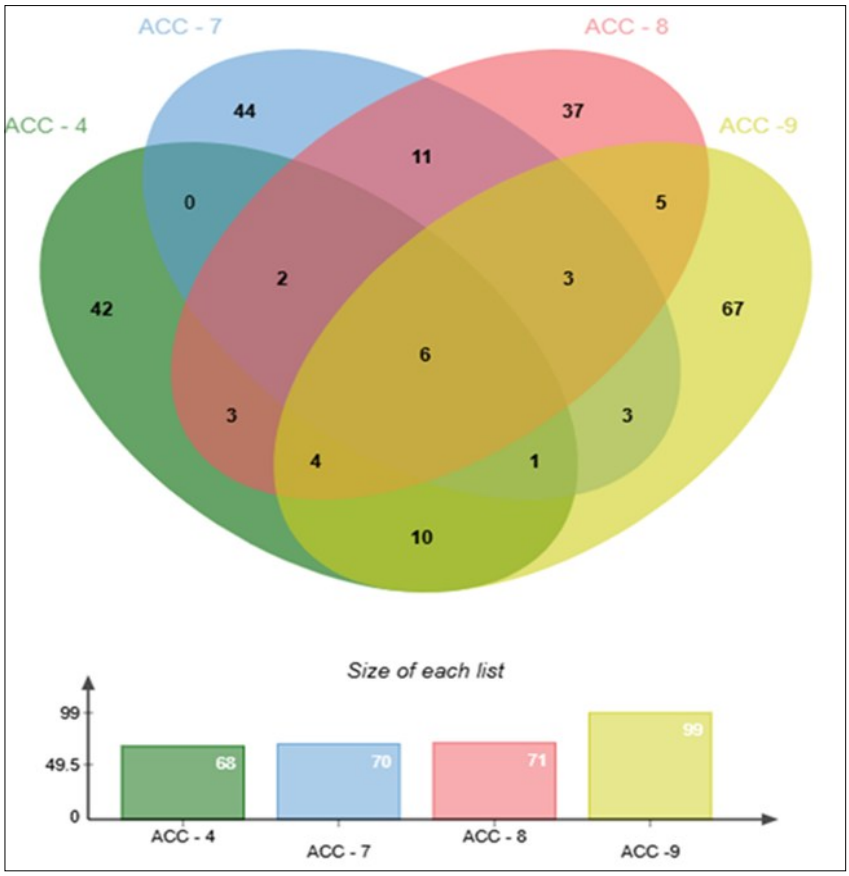

Fig. 5. Venn diagram showing shared and unique floral metabolites among 4 *Tabernaemontana divaricata* genotypes based on GC-MS analysis.

The metabolites of all the 4 genotypes of *Tabernaemontana* differed significantly. Table 2 summarizes the floral metabolites detected in *T. divaricata* genotypes through GC-MS profiling providing a detailed overview of the chemical diversity present in the floral extracts across different genotypes.

Table 3 presents the relative abundance of floral metabolites expressed as peak area percentages from the GC-MS chromatograms representing the contribution of each compound to the total ion chromatogram, reflecting its relative concentration in the floral metabolite profile. Variations in peak area across genotypes indicate differences in metabolite expression which may contribute to genotype-specific floral scent or bioactivity. Among the volatile compounds identified and compared myo-inositol 2-C-methyl- was the most abundant compound obtained in Td-Acc.04, Td-Acc.07and Td-Acc.08 with varied peak area of 16.36 %, 13.33 % and 21.63 % respectively. In Td-Acc.09, the compound cis-vaccenic acid recorded the highest peak area of 10.65 %.

Heat map

The heat map (Fig. 6) provides a visual comparison of the relative abundance of 30 floral metabolites identified across four *T. divaricata* genotypes. Variations in color intensity reflect differences in peak area percentages, indicating metabolic diversity among the genotypes. Hierarchical clustering grouped genotypes with similar chemical compositions and clustered metabolites showing comparable expression patterns. The color gradient reflects relative abundance levels of each metabolite across genotypes, with blue indicating higher abundance and yellow indicating lower abundance. Metabolite clustering along rows and genotype grouping along columns were generated using hierarchical clustering with Euclidean distance.

Principal component analysis

The PCA biplot (Fig. 7) demonstrates that PC1 and PC2 capture 80 % of the total metabolic variance (50 % and 30 % respectively) among the four *T. divaricata* genotypes. Genotype clustering patterns reveal Td-Acc.07 and Td-Acc.08 positioned in close proximity indicating high metabolic similarity, while Td-Acc.04 shows substantial divergence in the opposing quadrant. Td-Acc.09 exhibits intermediate characteristics with distinct metabolic features.

Loading vector analysis identifies key discriminatory compounds like benzaldehyde, benzyl alcohol, catechol, guanosine and 2-hydroxy-gamma-butyrolactone characterize the Td-Acc.07 and 08 cluster. Td-Acc.04 is distinguished by myo-Inositol and squalene accumulation indicating unique primary and secondary metabolic profiles. Td-Acc.09 shows variation by accumulation of caryophyllene, methyl salicylate, cis-vaccenic acid, aspidofractinine and ρ-menthane-1,2-diol associations.

Vector length analysis reveals compounds with greatest discriminatory power particularly myo-inositol, squalene, benzaldehyde and propylene glycol which demonstrate substantial contributions to genotype differentiation and serve as potential chemotaxonomic markers for genotype authentication.

Hierarchical Cluster Analysis (HCA)

The HCA of 4 genotypes of *T. divaricata* was performed using their GC-MS-derived metabolite profiles. The dendrogram (Fig. 8) was constructed applying Ward linkage on z-score standardized data, with Euclidean distance as the metric. Genotypes Td-Acc.07 and Td-Acc.08 clustered together at the shortest distance, indicating highly similar metabolite profiles suggesting potential similarities in metabolic pathways or expression patterns of secondary metabolites within them. Td-Acc.04 was markedly distinct, clustering separately from the others at a larger distance. This separation reflects a unique metabolite composition compared to the other genotypes possibly due to genetic, epigenetic or environmental factors influencing its metabolic expression. Td-Acc.09 joined the Td-Acc.07 and Td-Acc.08 grouping at a greater distance implying moderate similarity. While it shares metabolic characteristics with the Td-Acc.07 and 08 pair, distinguishable differences in certain metabolite concentrations set it apart.

Discussion

This study presents a comprehensive metabolite profiling of *T. divaricata* floral extracts across 4 distinct genotypes using GC-MS. A total of 68 to 99 volatile compounds were detected revealing considerable inter-genotypic variation in both the concentration and composition of floral metabolites. 30 metabolites with noteworthy peak area percentage were considered for this study. The identified compounds encompassed a broad spectrum of bioactive chemical classes including aromatic aldehydes, alcohols, lactones and terpenoids. These classes exhibit antimicrobial, antioxidant, anti-inflammatory and insect repellent activities, contributing to plant defense and offering promising therapeutic benefits. (28, 30, 32).

Previous GC-MS investigations on *T. divaricata* have largely concentrated on leaf and latex extracts with limited emphasis on the floral metabolome. Leaf-based studies have documented the presence of various phytoconstituents with notable bioactivities. For instance, 96 compounds in leaf extracts including key bioactive molecules such as n-hexadecanoic acid, phytol, linoleic acid, squalene, cedrol and vitamin E (37). Similarly, compounds such as 3,7,11,15-tetramethyl-2-hexadecen-1-ol, urs-12-en-24-oic acid methyl ester and squalene in the leaf profiles of *Tabernaemontana* (38). Latex-based analyses have revealed an abundance of alkaloids, phenolics, flavonoids and proteins. Notably, the presence of α-linolenic acid, pentadecanoic acid, 13-docosenamide, lupeol acetate, cycloartenol and β-amyrin compounds known for their antibacterial and anticancer potential (39).

In contrast, the present study specifically focused on floral metabolites across multiple *T. divaricata* genotypes offering novel perspectives on its chemotypic diversity and informing future pharmacological exploration. While compounds such as hexadecanoic acid, squalene, phytol and vitamin E were also detected in floral tissues as previously reported in leaf studies several metabolites unique to flowers were identified. The metabolite profile of latex exhibits a distinct chemical composition compared to that of the floral tissues. Notably methyl salicylate, caryophyllene, myo-Inositol, geraniol, stigmasterol, propylene glycol and various benzene derivatives were prominent in the floral extracts but either absent or minimally represented in leaf and latex-based profiles.

Myo-inositol was the most abundant compound

Table 2. Floral metabolites in *Tabernaemontana divaricata* genotypes

Chemical compound	Molecular formula	Chemical class	Retention index	Retention time	Genotypes source	Biological activity	References
1,2-Cyclopentanedione	$C_5H_6O_2$	Diketone	1741.6	4	Td-Acc.07 Td-Acc.08 Td-Acc.09	Antioxidant activity	(14)
2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	Lactone	2142	5	Td-Acc.07 Td-Acc.08 Td-Acc.09	Antioxidant, analgesic, anti-diabetic, antibacterial and antifungal activity	(15)
Propylene Glycol	$C_3H_8O_2$	Aliphatic Alcohol	1599	5	Td-Acc.09	antimicrobial	(16)
Benzyl alcohol	C_7H_8O	Aromatic alcohol	1057	5	Td-Acc.04	Antimicrobial	(5)
Benzene acetaldehyde	C_8H_8O	Aromatic aldehyde	1014.1	5	Td-Acc.04 Td-Acc.09	Antimicrobial, antioxidant and anti-inflammatory	(17)
Phenylethyl Alcohol	$C_8H_{10}O$	Aromatic alcohol	1125	6	Td-Acc.04 Td-Acc.09	antibacterial	(18)
4H-Pyran-4-one	$C_6H_8O_4$	Pyrone	971.9	6	Td-Acc.07 Td-Acc.08 Td-Acc.09	Antimicrobial, anti-inflammatory, antiproliferative antioxidant, automatic nerve activity	(19)
Phenol	$C_8H_{10}O$	Aromatic alcohol	1090.1	6	Td-Acc.04 Td-Acc.07 Td-Acc.08 Td-Acc.09	Antioxidant, anti-inflammatory, antimicrobial and anticancer	(20)
Catechol	$C_6H_6O_2$	Phenol	1196.24	7	Td-Acc.07 Td-Acc.08 Td-Acc.09	Anticarcinogenic, Cytotoxic and anti-oxidant	(21)
Methyl salicylate	$C_8H_8O_3$	benzoate esters	1191.3	7	Td-Acc.09	Analgesic, anti-inflammation, antipyretic,	(22)
Benzaldehyde	C_8H_8O	Aromatic aldehyde	964.21	7	Td-Acc.04	Antibacterial, antifungal	(23)
5-Hydroxymethylfurfural	$C_6H_6O_3$	Furanone	1124.1	7	Td-Acc.07 Td-Acc.09	Antioxidant, antiproliferative activity	(15)
Geraniol	$C_{10}H_{18}O$	monoterpenoid	1256.1	8	Td-Acc.09	Antimicrobial, anti-inflammatory, antioxidant, anti-cancer	(24)
Caryophyllene	$C_{15}H_{24}$	Bicyclic sesquiterpene	1415.9	9	Td-Acc.08 Td-Acc.09	Antioxidant, anti-inflammatory, anticancer, cytotoxic, antimicrobial, hypolipidemic, neuroprotective and cardioprotective properties	(25)
Guanosine	$C_{10}H_{13}N_5O_5$	Purine nucleoside	1588	9	Td-Acc.07	Antibiotic, anti-tumour and anti-mycoplasmal activity	(26)
Benzoic acid	$C_{11}H_{14}O_3$	Benzoate	193.9	10	Td-Acc.04 Td-Acc.08 Td-Acc.09	Antimicrobial, antioxidant and antibacterial	(27)
p-Menthane-1,2,3-triol	$C_{10}H_{20}O_3$	monoterpenoid triols	1015.32	11	Td-Acc.09	Antimicrobial, antioxidant, anti-inflammatory	(28)
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	Methylated monosaccharide	-	11	Td-Acc.07 Td-Acc.08 Td-Acc.09	Phosphorylation and gene regulation	(8)
Myo-Inositol	$C_7H_{14}O_6$	Polyol	195	11	Td-Acc.04 Td-Acc.07 Td-Acc.08	Antioxidant and antimicrobial	(19)
Pentadecanal-	$C_{15}H_{30}O$	fatty aldehyde	1713.2	12	Td-Acc.04	Antimicrobial	(29)
Hexadecanoic acid	$C_{16}H_{32}O_2$	Saturated fatty acid	1960	13	Td-Acc.04 Td-Acc.07 Td-Acc.09	Antioxidant, antibacterial and antifungal	(30)
Phytol	$C_{20}H_{40}O$	Diterpene alcohol	2138	14	Td-Acc.04 Td-Acc.09	Antioxidant, anti-inflammatory, antimicrobial, antinociceptive-anxiety, antiparasitic, anticancer, anti-	(31)
cis-Vaccenic acid	$C_{18}H_{34}O_2$	long-chain fatty acids	2034	14	Td-Acc.09	Antibacterial and hypolipidemic	(8)
Dansyl glycine	$C_{14}H_{16}N_2O_4S$	Amino acid	-	16	Td-Acc.09	Antibacterial	(28)
Aspidofractinine	$C_{20}H_{26}N_2O$	Indole alkaloid	-	17	Td-Acc.04 Td-Acc.09	Cardiovascular cytotoxic antibacterial, anti-inflammatory and analgesic	(29)
Squalene	$C_{30}H_{50}$	Triterpene	2832	18	Td-Acc.04 Td-Acc.07 Td-Acc.08 Td-Acc.09	Antioxidant, emollient antitumor, antibacterial and detoxification activities	(32)
Nerolidol 2	$C_{15}H_{26}O$	Sesquiterpene alcohol	1555.5	19	Td-Acc.04	Anti-microbial, anti-biofilm, antioxidant, anti-inflammatory and anti-cancer	(33)
Ibogamine-18-carboxylic acid	$C_{22}H_{28}N_2O_3$	Indole alkaloid	-	19	Td-Acc.07	Psychoactive & neuromodulator	(34)
Vitamin E	$C_{29}H_{50}O_2$	Toc chromanols	2914.8	20	Td-Acc.04	Antioxidant	(35)
Stigmasterol	$C_{29}H_{48}O$	Phytosterol	3145	22	Td-Acc.04 Td-Acc.08 Td-Acc.09	Anti-inflammatory, anti-cancer, anti-diabetic and antimicrobial	(36)

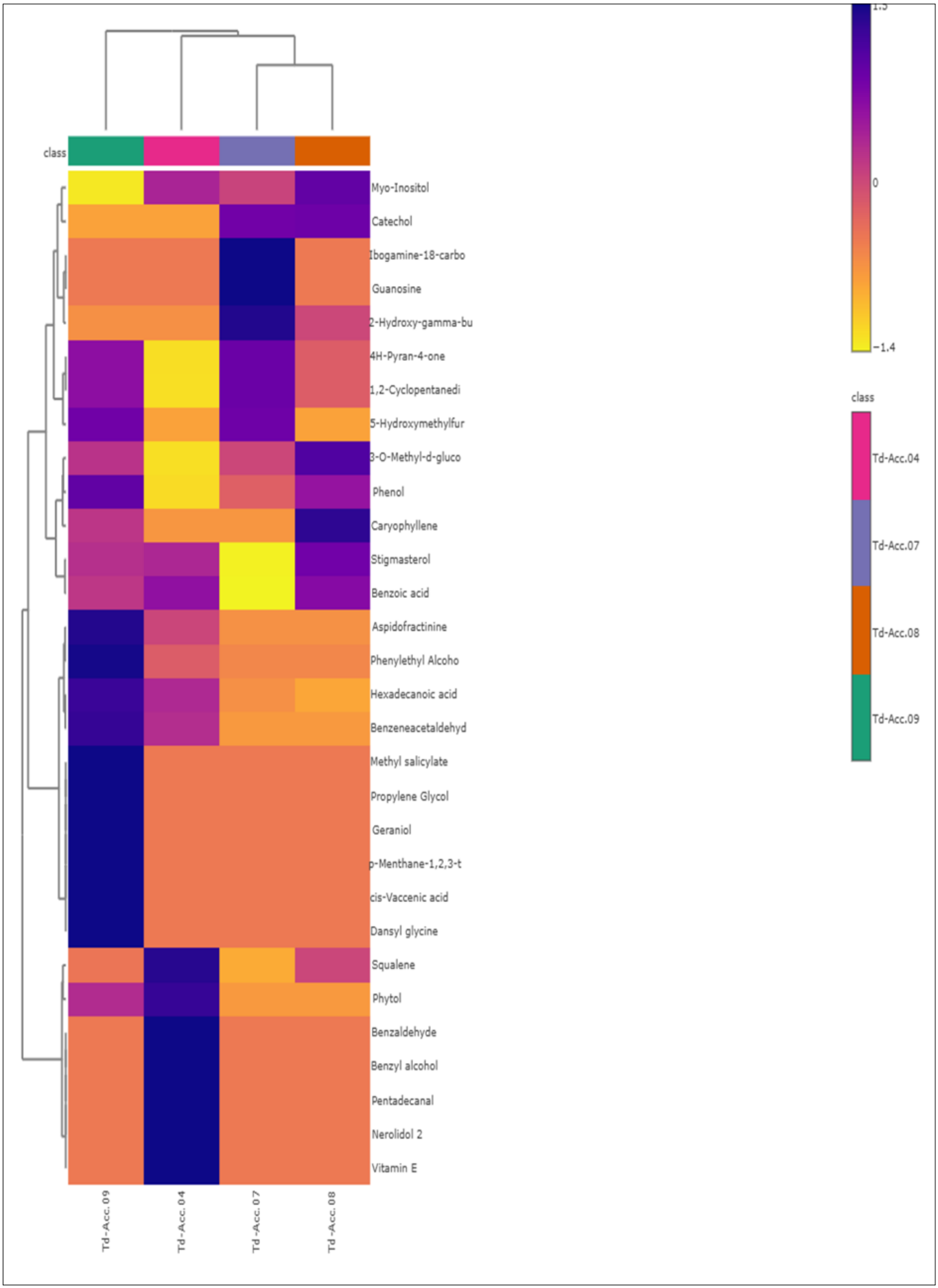

Fig. 6. Heat map representation of floral metabolites detected in four *Tabernaemontana divaricata* genotypes based on GC-MS peak area percentage.

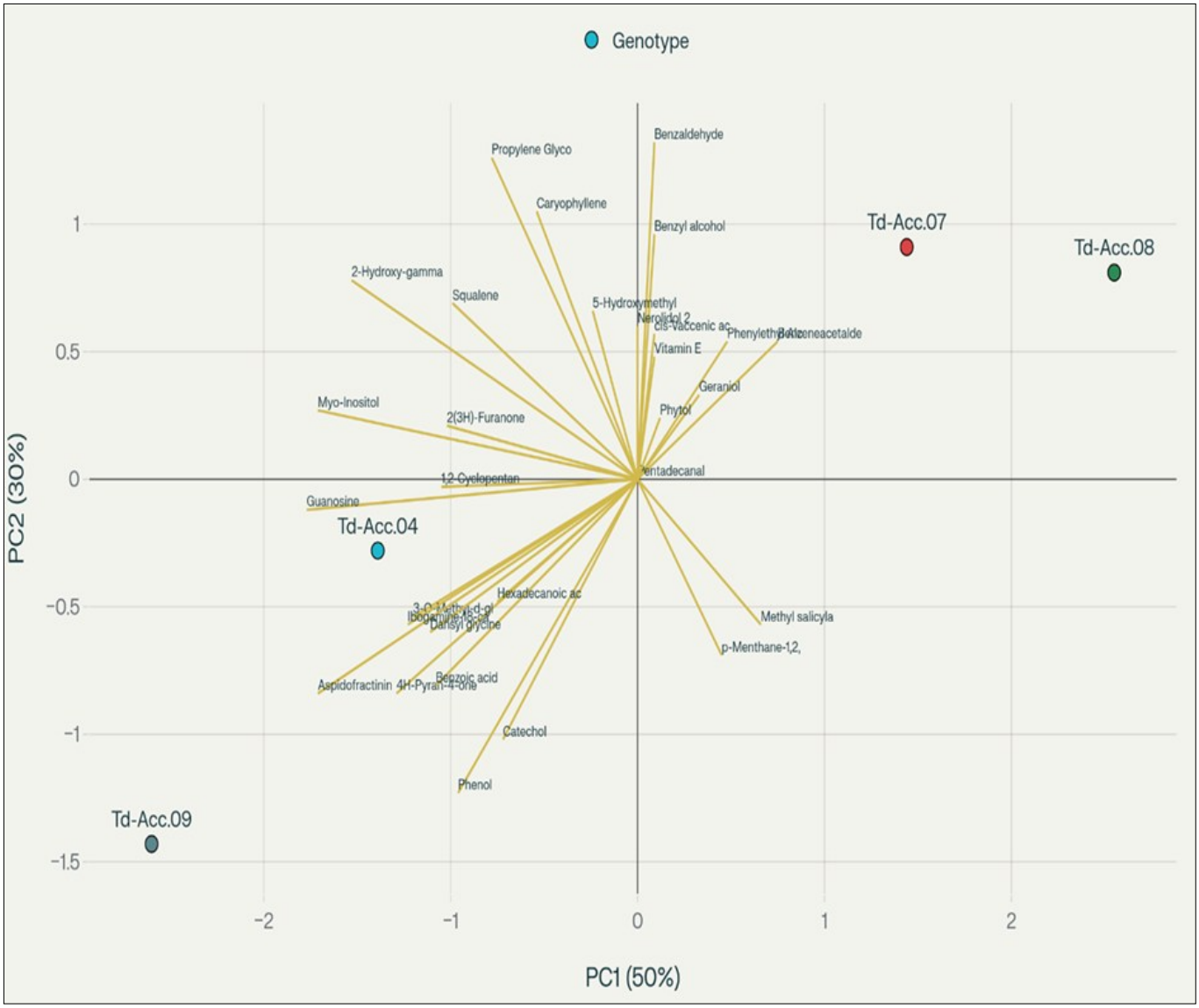

Fig. 7. PCA Biplot of *Tabernaemontana divaricata* genotypes based on GC-MS analysis.

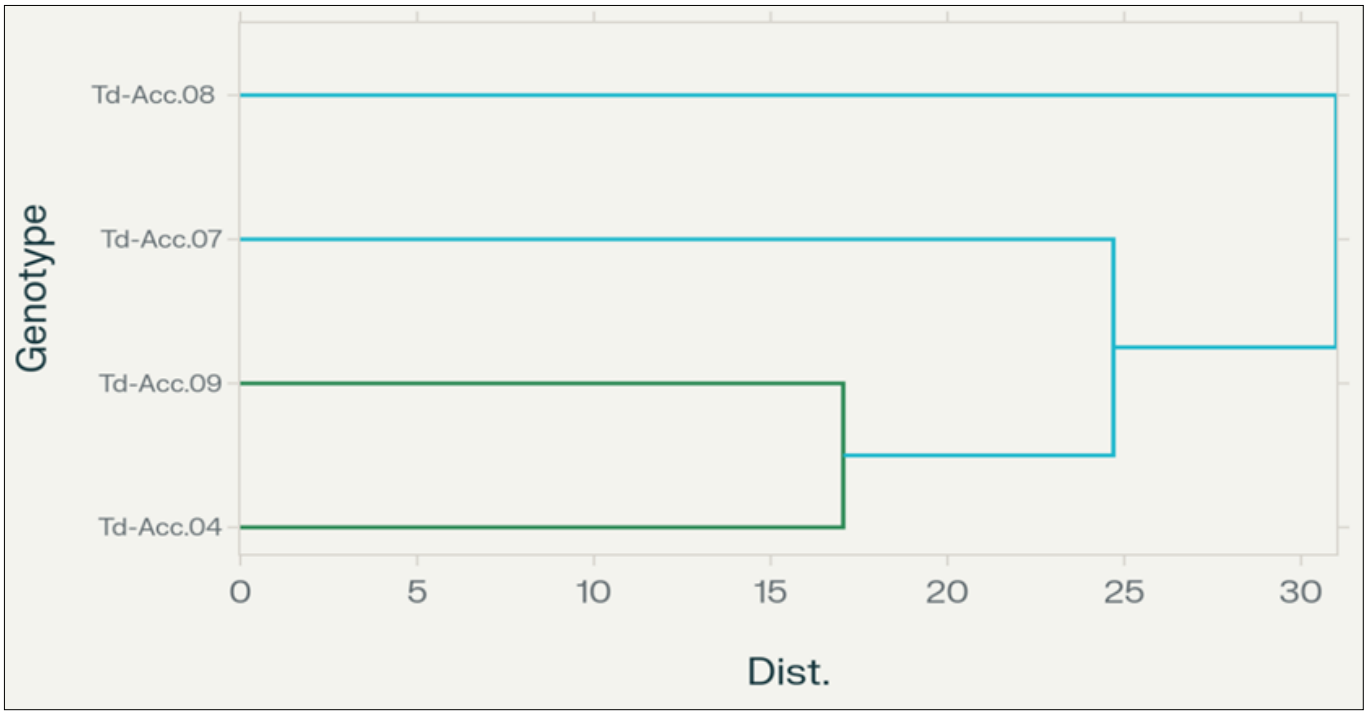

Fig. 8. HCA dendrogram of *Tabernaemontana divaricata* genotypes based on GC-MS metabolic profiles.

Table 3. Relative peak area percentages of floral metabolites identified in different *Tabernaemontana divaricata* genotypes based on GC-MS analysis

Chemical compound	Td-Acc.04	Td-Acc.07	Td-Acc.08	Td-Acc.09
1,2-Cyclopentanedione	0	4.35	1.32	3.65
2-Hydroxy-gamma-butyrolactone	0	7.32	4.21	0
Propylene glycol	0	0	0	3.5
Benzyl alcohol	0.96	0	0	0
Benzeneacetaldehyde	0.35	0	0	2.61
Phenylethyl alcohol	0.24	0	0	1.73
4H-Pyran-4-one	0	6.21	2.77	5.5
Phenol	0.13	0.02	0.5	0.51
Catechol	0	2.4	2.22	0
Methyl salicylate	0	0	0	1.49
Benzaldehyde	0.32	0	0	0
2(3H)-Furanone	0	2.93	0	0
5-Hydroxymethylfurfural	0	3.39	0	3.25
Geraniol	0	0	0	0.5
Caryophyllene	0	0	2.89	0.96
Guanosine	0	13.96	0	0
Benzoic acid	3.31	0	3.89	2.29
p-Menthane-1,2,3-triol	0	0	0	3.66
3-O-Methyl-d-glucose	0	6.32	12.82	7.98
Myo-Inositol	16.36	13.33	21.63	0
Pentadecanal-	0.35	0	0	0
Hexadecanoic acid	2.83	0.5	0	6.18
Phytol	0.65	0	0	0.13
cis-vaccenic acid	0	0	0	10.65
Dansyl glycine	0	0	0	3.69
Aspidofractinine	2.64	0	0	10.01
Squalene	9.61	0.96	3.36	1.68
Nerolidol 2	0.21	0	0	0
Ibogamine-18-carboxylic acid	0	9.32	0	0
Vitamin E	0.4	0	0	0
Stigmasterol	2.32	0	3.65	2.06

detected in Td-Acc.04, Td-Acc.07 and Td-Acc.08 owing to its documented antioxidant and antimicrobial properties (19). Its elevated accumulation may confer enhanced oxidative stress tolerance and pathogen resistance in these genotypes.

Accession Td-Acc.07 exhibits a distinctive chemical profile characterized by the exclusive presence of 2(3H)-furanone, guanosine and ibogamine-18-carboxylic acid. 2(3H)-furanone contributes antimicrobial and antioxidant defences by disrupting microbial growth and communication. Guanosine plays roles in nucleic acid metabolism and stress signalling, supporting cellular repair and adaptation (26), while ibogamine-18-carboxylic acid, a bioactive indole alkaloid confers potent antimicrobial and neuroactive properties (23). This unique metabolite assemblage likely endows Td-Acc.07 with superior microbial resistance, oxidative stress tolerance and ecological fitness due to this the genotype can be suggested as a promising source of pharmacologically relevant compounds within *T. divaricata*.

In addition to cis-vaccenic acid being most predominant and that is reported to exhibit antibacterial, antioxidant and hypolipidemic activities (8), Td-Acc.09 uniquely accumulates several bioactive metabolites including propylene glycol with bactericidal and moisturizing properties (16); methyl salicylate, an ester exhibiting analgesic, anti-inflammatory antipyretic effects that alleviates pain, reduces fever and inflammation by inhibiting prostaglandin synthesis (22) and geraniol, a terpene alcohol, characterized by broad-spectrum antibacterial, anti-inflammatory, antioxidant, insecticidal and anticancer activities, as well as a role as a herbivore-induced volatile mediating plant defence responses (23, 39). Furthermore, p-menthane-1,2,3-triol, a bioactive monoterpenoid with antimicrobial, antioxidant and anti-inflammatory functions and dansyl glycine noted for its antibacterial activity (28) were also exclusively detected in this genotype. The assemblage of these genotype-specific metabolites likely contributes to the enhanced biochemical defence capacity and ecological resilience of Td-Acc.09, thereby defining its unique chemotypic and functional phenotype within *T. divaricata*.

Both squalene and phenol are present across all *T. divaricata* genotypes, which constitute essential bioactive compounds that underpin the species' core biochemical and therapeutic profile. Squalene is noted for its immunomodulatory, anti-senescence, hypolipidemic, antioxidant, anticancer, antibacterial and detoxifying actions (32), while phenol exhibits well-established antioxidant, anti-inflammatory, antimicrobial and anticancer effects (20).

Functional grouping of major floral metabolites in T. divaricata genotypes

Terpenoids and sterols like squalene, caryophyllene, phytol and stigmasterol are found in *Tabernemontana*. Squalene, phytol and stigmasterol are established antioxidants with anti-inflammatory, cytoprotective and membrane-stabilizing effects (31, 32, 36). Caryophyllene is a sesquiterpene with demonstrated antimicrobial, anti-inflammatory and insecticidal properties. Furthermore, via the jasmonic acid signalling pathway it functions as a signalling molecule that induces resistance to microbial diseases in neighbouring plants, thereby serving as a crucial allelochemical in natural plant defense networks (25, 40).

Genotypes with elevated levels notably Td-Acc.09, Td-Acc.08 and Td-Acc.04 may benefit from enhanced oxidative stress tolerance and defence against pathogens or herbivores, suggesting ecological resilience and potential medicinal value related to anti-inflammatory and protective applications.

Aromatic alcohols and aldehydes such as benzene acetaldehyde, benzyl alcohol and phenylethyl alcohol, all of which are prominent floral volatiles with demonstrated roles in pollinator attraction and antimicrobial defense. Phenylethyl alcohol exhibits bactericidal activity against both gram-positive and gram-negative bacteria (18), while benzene acetaldehyde possesses strong antibacterial, antioxidant and anti-inflammatory properties (25). Notably, flies respond to benzyl alcohol facilitating mimicry or targeted pollinator attraction strategies (39). The enrichment of these compounds in Td-Acc.09 suggests an adaptive strategy to optimize pollinator visitation with sweet scented flowers and reproductive success, alongside enhanced inherent protective mechanisms.

Indole alkaloids such as aspidofractinine and ibogamine-18-carboxylic acid are characterized by potent bioactivities including antimicrobial, analgesic, anti-inflammatory and anticancer effects. Ibogamine-18-carboxylic acid was found only in Td-Acc.07 and is unique to this genotype. Due to this unique metabolite, Td-Acc.07 may posess specialized defence potential and unique pharmaceutical prospects (23, 29).

Organic acids and esters like benzoic acid is antimicrobial, antioxidant and also contributes to floral scent and is present in all three fragrant genotypes (28). Hexadecanoic acid (palmitic acid) is a major fatty acid involved in membrane structure and metabolic regulation and have additional bioactivities like antimicrobial, anti-inflammatory and lipid metabolism regulation (30). The widespread occurrence of these compounds, albeit with variations in abundance, supports fundamental defense functions, with certain genotypes such as Td-Acc.04, Td-Acc.07 and Td-Acc.09 showing higher levels of hexadecenoic acid. Stress-related adaptation is crucial as it enables plants to survive and maintain growth under adverse environmental conditions by enhancing their tolerance to biotic and abiotic stresses. This adaptation involves metabolic adjustments that help mitigate damage from factors such as pathogens, drought and temperature extremes, thereby improving plant resilience and fitness.

Carbohydrates and sugar derivatives like 3-O-methyl-d-glucose and guanosine that play key roles in energy metabolism, osmo-protection and nucleic acid synthesis with guanosine additionally involved in signalling and stress responses. (8, 26). Genotypes with elevated sugar alcohols and nucleosides like guanosine in Td-Acc.07, 3-O-methyl-d-glucose common in all except Td-Acc.04 may exhibit improved tolerance to abiotic stresses.

Phenolic compound like catechol is a potent antioxidant and antimicrobial also involved in defence signalling and allelopathy (21). Catechol as a core compound in Td-Acc.07, 08 and 09 genotypes likely support universal defence against microbial challenge and oxidative stress.

Other compounds like 1,2-cyclopentanedione, 2-hydroxy-gamma-butyrolactone and 4H-pyran-4-one often function in aroma or flavour profile formation, some exhibit moderate antimicrobial and antioxidant activity (14, 15). Their presence in genotypes enhances scent complexity which may facilitate both pollinator attraction and ecological communication.

Genotype specialization

Td-Acc.04 stands out for its antioxidant-rich, stress-tolerant profile, making it a promising candidate for applications requiring resilience and health-promoting properties. Td-Acc.07 is notable for the presence of ibogamine-18-carboxylic acid, indicating unique alkaloid biosynthetic capacity with potential pharmacological importance. Td-Acc.08 is characterized by strong ecological and defensive attributes, combining pollinator-attracting scents with antimicrobial and anti-inflammatory compounds, thus offering substantial potential for both ornamental and therapeutic use. Td-Acc.09 exhibits greater abundance of terpenoids (phytol, squalene), fatty acids and floral volatiles (caryophyllene, geraniol, methyl salicylate, benzene acetaldehyde and phenyl ethyl alcohol) suggesting a genotype with sweet scented flowers and potentially optimized for both ecological interaction (pollinator attraction, defence) and bioactive compound yield for medicinal use.

Conclusion

This study provides a comprehensive chemometric and metabolomic characterization of floral volatiles in four genotypes of *T. divaricata* through GC-MS analysis. A total of 30 metabolites selected based on peak area percentage were identified encompassing esters, terpenoids, fatty acid derivatives, lactones and alcohols many of which are known to possess pharmacological and ecological relevance. Multivariate analyses, including PCA and HCA effectively discriminated between genotypes revealing genotype-specific metabolic signatures and underscoring significant chemotypic variation.

In contrast to earlier studies that predominantly focused on vegetative tissues such as leaves and latex which are rich in alkaloids, sterols and long-chain fatty acids, the current floral metabolite profiling revealed a distinct chemical landscape specific to reproductive tissues. The detection of key volatiles such as methyl salicylate and benzene derivatives suggests specialized functional roles in floral scent production, pollinator attraction and potential therapeutic applications. Visualization tools, such as like venn diagram, further corroborated the presence of both conserved and genotype-specific metabolites highlighting the rich chemical diversity inherent within the species.

Collectively, these findings expand the existing metabolic framework of *T. divaricata*, contributing to its chemotaxonomic delineation and offering novel insights into the biochemical roles of floral volatiles. This study lays a foundation for future functional validation and supports the potential utilization of floral metabolites in fragrance-oriented breeding programs, ornamental enhancement and pharmaceutical development.

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Authors' contributions

Conceptualization and methodology were done by SPTS. DLN performed the investigation, software, validation, formal analysis,

writing the review, editing and original draft preparation. PSG collected the resources. All authors have read and accepted the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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