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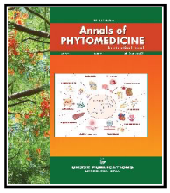

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Unravelling the floral phytochemical profiles in wild type and mutant lines of *Jasminum auriculatum* Vahl.

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Abstract

Jasminum auriculatum Vahl. has long been used in traditional Indian medicine for its therapeutic properties, including wound healing, antioxidant, and antimicrobial properties. This study aimed to scientifically validate the medicinal properties of flowers of *J. auriculatum* using gas chromatography-mass spectrometry (GC-MS) by analysing the bioactive compounds in wildtype (CO.1 Mullai) and two promising mutant lines (COM-PM-1, MM-PM-1), developed through induced mutation. The results revealed the presence of various secondary metabolites, including benzofuran, benzoic acid, phytol, squalene, and 9,12,15-octadecatrienoic acid, known for their antioxidant, antimicrobial, and cytotoxic properties. Additionally, compounds such as 2,5-dimethyl-4-hydroxy-3(2H)-furanone, oxirane, and cyclotrisiloxane which exhibit strong antioxidant, anti-inflammatory, and antibacterial effects were also recorded. The findings of this study support the traditional use of *J. auriculatum* in folk medicine other than the well-established ornamental values and pave the way for further exploration of its bioactive compounds for medicinal and therapeutic applications.

1. Introduction

Plants and herbs with wound healing, antioxidant, and antimicrobial properties have been utilized by traditional healers for centuries in India. However, some have been scientifically studied to confirm their therapeutic potential, while most others remain unexplored. Therefore, it is crucial to establish scientific validation for the medicinal properties of these plants, traditionally used in folk medicine (Arun *et al*., 2016; Deka *et al*., 2021). *J. auriculatum* is widely distributed across India, Nepal, and Sri Lanka, and was explored for its numerous medicinal applications (Arangale *et al*., 2018). Since ancient times, *J. auriculatum* is renowned for its beautiful and fragrant flowers. It is valued as an ornamental plant, with its fresh flowers frequently used in floral arrangements (Deepashree *et al*., 2022). Beyond its ornamental appeal, *J. auriculatum* is also recognized for their pharmaceutical significance, containing important bioactive compounds such as alkaloids, cardiac glycosides, phenols, sterols, tannins, and sesquiterpenes (Kumaresan *et al*., 2019).

In *J. auriculatum*, the roots are particularly effective in treating skin problem such as ringworm, while the flowers are commonly used in traditional medicine and as a flavouring agent in food products such as frozen desserts, beverages, gelatins, and puddings (Mourya *et al*., 2017). The flowers also offer various health benefits, including aphrodisiac, antiseptic, and aromatherapy properties. Jasmine oil, which can be extracted from the flowers, is widely used in the perfumery industry, and the leaves are employed in the treatment of mouth ulcers (Gupta and Chaphalkar, 2016). In addition to these traditional uses, *J. auriculatum* has exhibited a range of pharmacological activities, including wound healing, diuretic, and antilithiatic properties. In recent years, further research has been undertaken to explore its potential in various immune-related pharmacological applications (Gupta and Chaphalkar, 2015).

Advancements in analytical techniques have significantly enhanced our ability to identify and quantify these phytochemicals (Saiharini *et al*., 2022). Gas chromatography-mass spectrometry (GC-MS) stands out as a powerful method for detecting bioactive compounds such as alcohols, ethers, and acids, even in minute quantities (Dhama *et al*., 2022; Sivakumar *et al*., 2022; Sharma *et al*., 2021; Vishnupandi *et al*., 2024). In this study, we employed GC-MS analysis in the flowers of selected putative mutants identification of various bioactive compounds.This approach facilitated an insight into the phytochemical profiles that underpin the traditional medicinal uses of these plants. In line with this, *J. auriculatum* will further be investigated for understanding the various phytochemicals, to evaluate and substantiate its claimed benefits in traditional healing practices.

2. Materials and Methods

2.1 Authentication of plant material

The present study was carried out at the Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University (TNAU), Coimbatore (Latitude of 11000'N, Longitude of 77000'E and an elevation of 412 m above MSL), Tamil Nadu during 2022-2024.Two distinct genotypes, CO.1 Mullai, an improved variety of TNAU and Muthu Mullai, an ecotype of *J. auriculatum* were used in this study for unveiling the various metabolic and volatile compounds present in the control (wild type) and the identified

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promising mutants (Authentication No.: BSI/SRC/5/23/2024-25/Tech./465). The plant authentication was done by the Botanical Survey of India, Coimbatore, Tamil Nadu.

2.2 Metabolite extraction in flowers

Secondary metabolites were extracted from flower samples of the most promising mutant lines of CO.1 Mullai (15 Gy) and Muthu Mullai (15 Gy) and untreated wild type (CO.1 Mullai). The two mutants are designated as COM-PM-1 (CO.1 Mullai-Promising Mutant-1) and MM-PM-1 (Muthu Mullai-Promising Mutant-1). Flowers weighing 50 g were collected, cleaned, and pulverized into a fine powder using liquid nitrogen. The powder was then transferred to the conical flasks and mixed with an equal volume of ethyl acetate. This mixture was stirred at 100 rpm in an orbital shaker for 96 h at 28°C. The mixture was then filtered through Whatman No. 3 filter paper. The filtrate was concentrated using a rotary flash evaporator at 55°C and 80 rpm. The concentrated extract was dissolved in 1 ml of HPLC-grade methanol and filtered through a PVDF hydrophilic membrane (0.22 mm pore size, Himedia) before used for GC-MS analysis. The GC-MS system, equipped with a Thermal Desorber turbo matrix 150 (Perkin Elmer, USA), operated with a 10:1 split ratio, helium carrier gas at 20 psi, and an oven temperature ramp from 50 to 250°C at 10°C per minute. Mass spectrometry was conducted in positive ion mode with electron impact spectra at 70 eV, utilizing a DB-5 column (30 m x 0.25 mm, 0.25 im film thickness).

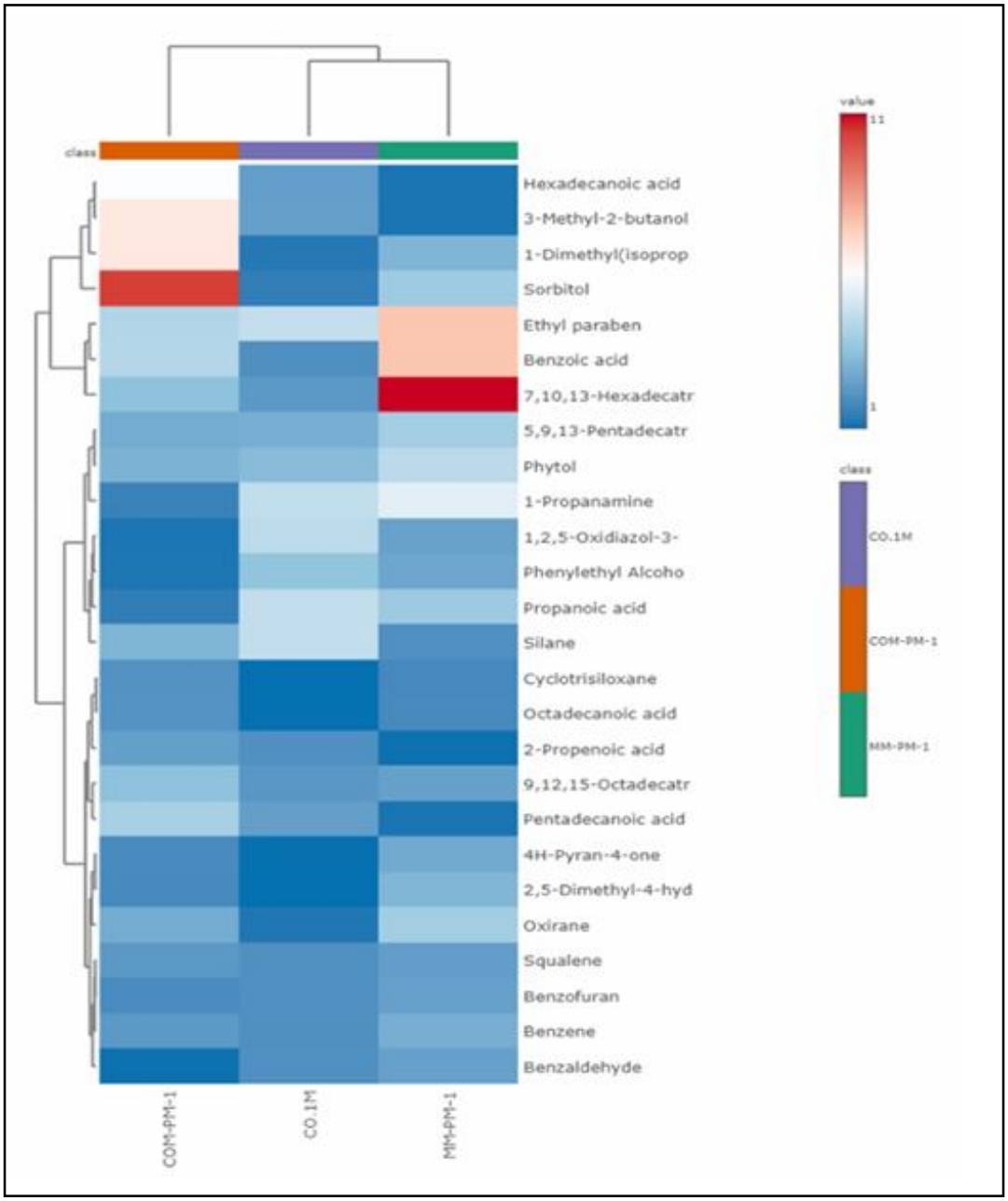

Figure 1: Heat map analysis of metabolites peak area (%) in wild type and mutant lines of *J. auriculatum*.

2.3 Gas chromatography and mass spectroscopy (GC-MS) and data analysis

The solvent free flower samples were dissolved in HPLC-grade methanol. The samples were analyzed using gas chromatography (GC) equipped with mass spectroscopy (MS) (Agilent GC 7890A/ MS 5975C). The column temperature was kept at 60°C (1.36 min), then increased to 325°C and held for 23 min. The injector temperature was set at 280°C injection (split mode100:1; injection volume 1µl; the flow rate of a helium carrier gas set to 1ml/min (with a total run time of 23 min). Mass spectra were set from the range *m/z* 50 to 350. The chromatogram of the sample was confirmed by comparing their mass with spectral database. Compounds detected were identified using the NIST (National Institute of Standards and Technology) mass spectral database. The quantification of common compounds present in both wild type and mutant lines was conducted using gas chromatography-mass spectrometry (GC-MS). The concentration of each compound was determined based on the peak area percentage derived from the chromatograms. For each compound, the peak area was integrated, and the relative abundance was expressed as a percentage of the total peak area in the sample. The higher concentration of common compounds in the mutant lines was confirmed by comparing the peak area percentages between the wild type and mutant lines. All measurements were performed in triplicate to ensure accuracy, and the results were averaged. Statistical analysis was applied to assess significant differences between the wild type and mutant lines.

2.4 Statistical analysis

To identify the differences in the emission of volatile compounds between the flower samples of wild type and mutant lines, all peak area analysis were performed with Metaboanalyst software and hierarchical clustering heat map was performed (Figure 1). A Venn diagram illustrating the significant metabolites along with their interactions was created using j Venn software (Figure 2).

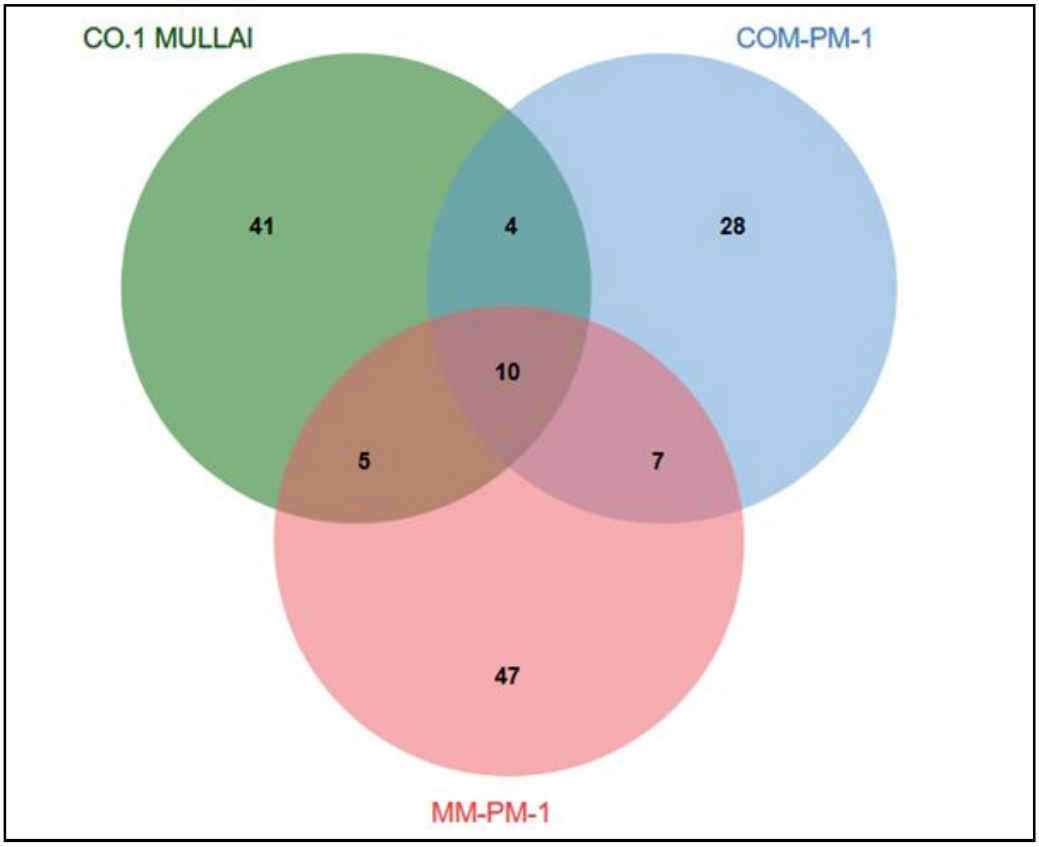

Figure 2: Metabolic divergence: Distinct and shared metabolites between mutant lines and wild type.

3. Results

The presence of various secondary metabolites and phytochemical components was revealed by GC-MS analysis in the floral extracts of promising mutant lines, *viz*., COM-PM-1, MM-PM-1 and CO.1 Mullai (wild type). The identification of these phytochemicals was confirmed through the examination of peak area and properties of the metabolites. These metabolites were confirmed by the NIST (National Institute of Standards and Technology) database.The metabolite profiles of flower extracts are represented in the Figures 3, 4 and 5. The GC-MS analysis of floral extracts from both wild-type CO.1 Mullai and two promising mutant lines, COM-PM-1 and MM-PM-1, revealed the presence of diverse phytochemicals with important biological properties. Among these, the mutant lines consistently exhibited higher peak area percentages for key metabolites compared to the wild type, indicating an enhanced production of bioactive compounds. This increase suggests that the mutant lines possess increased level of antioxidant, antifungal, and antimicrobial activities, which could be of significant interest for further phytochemical exploration.

The common compounds which were present in wild type (CO.1 Mullai) and mutant line (COM-PM-1) included 3-methyl-2-butanol (6.31%), hexadecanoic acid, pentadecanoic acid and 2-propenoic acid (Table 1). It was found that these components were significantly higher in quantity in mutant COM-PM-1, when compared to the wild type. Similarly, the common compounds in wild type (CO.1 Mullai) and mutant line (MM-PM-1) were benzaldehyde, 1-propanamine (4.91%), propanoic acid, phenylethyl alcohol and 1,2,5-oxadiazol-3-amine (Table 2), which were higher in mutant line MM-PM-1 except 1,2,5-oxadiazol-3-amine. In the two mutant lines (COM-PM-1 and MM-PM-1), 2,5-dimethyl-4-hydroxy-3(2h)-furanone, 4h-pyran-4-one, 1-dimethyl(isopropyl)silyloxypropane, sorbitol, oxirane (3.31%), octadecanoic acid and cyclotrisiloxane were present (Table 3). In wild type (CO.1 Mullai) and mutant lines (COM-PM-1 and MM-PM-1), the common metabolites observed were benzo furan, benzene, benzoic acid (7.35%), ethyl paraben (7.35%), silane, 9,12,15-octadecatrienoic acid, 7,10,13-hexadecatrienoic acid, phytol, squalene, 5,9,13-pentadecatrien-2-one (Table 4).

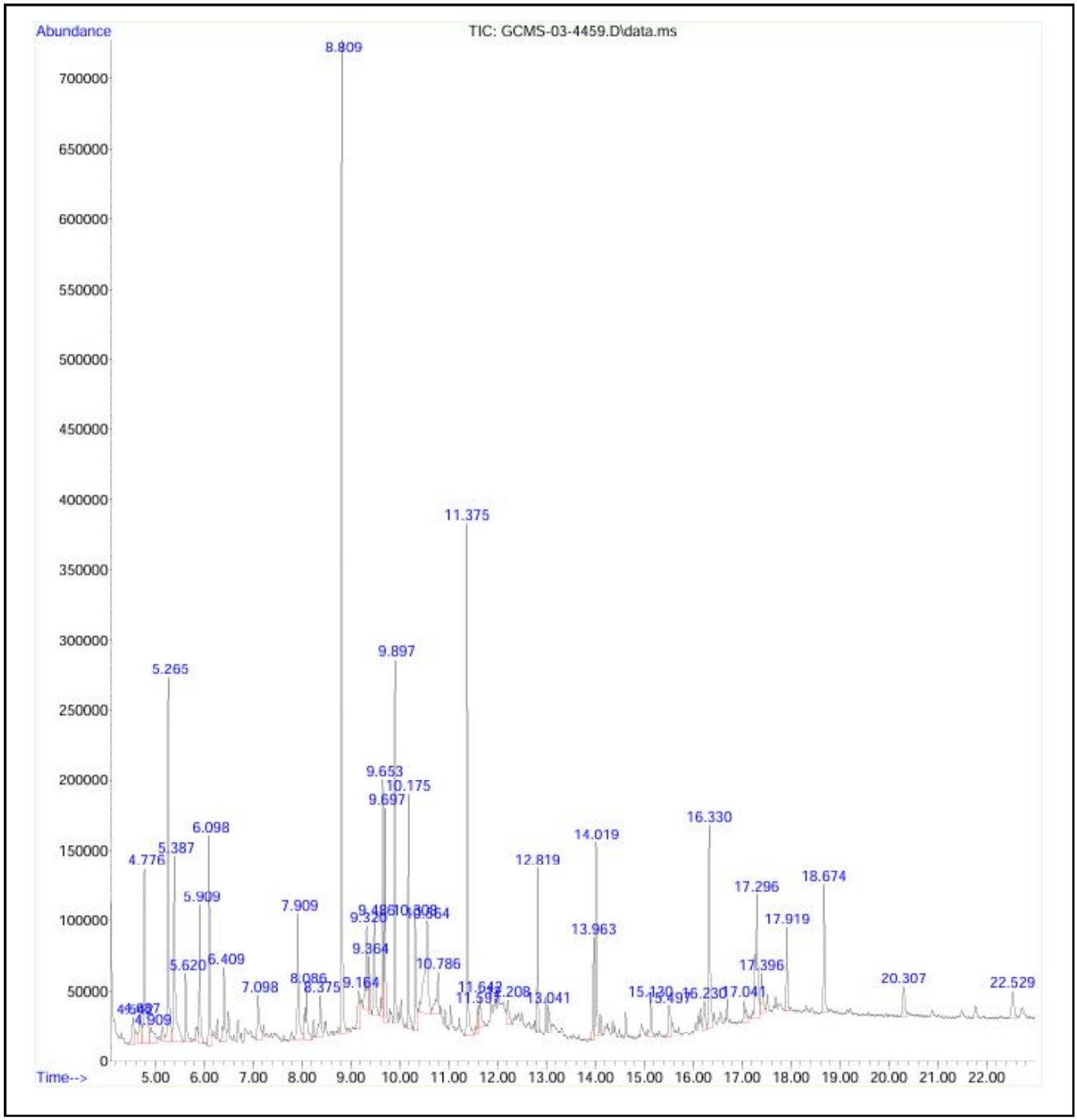

Figure 3: Metabolite profiling of VOCs in flowers of *J. auriculatum* Cv. CO.1 Mullai (Wild type).

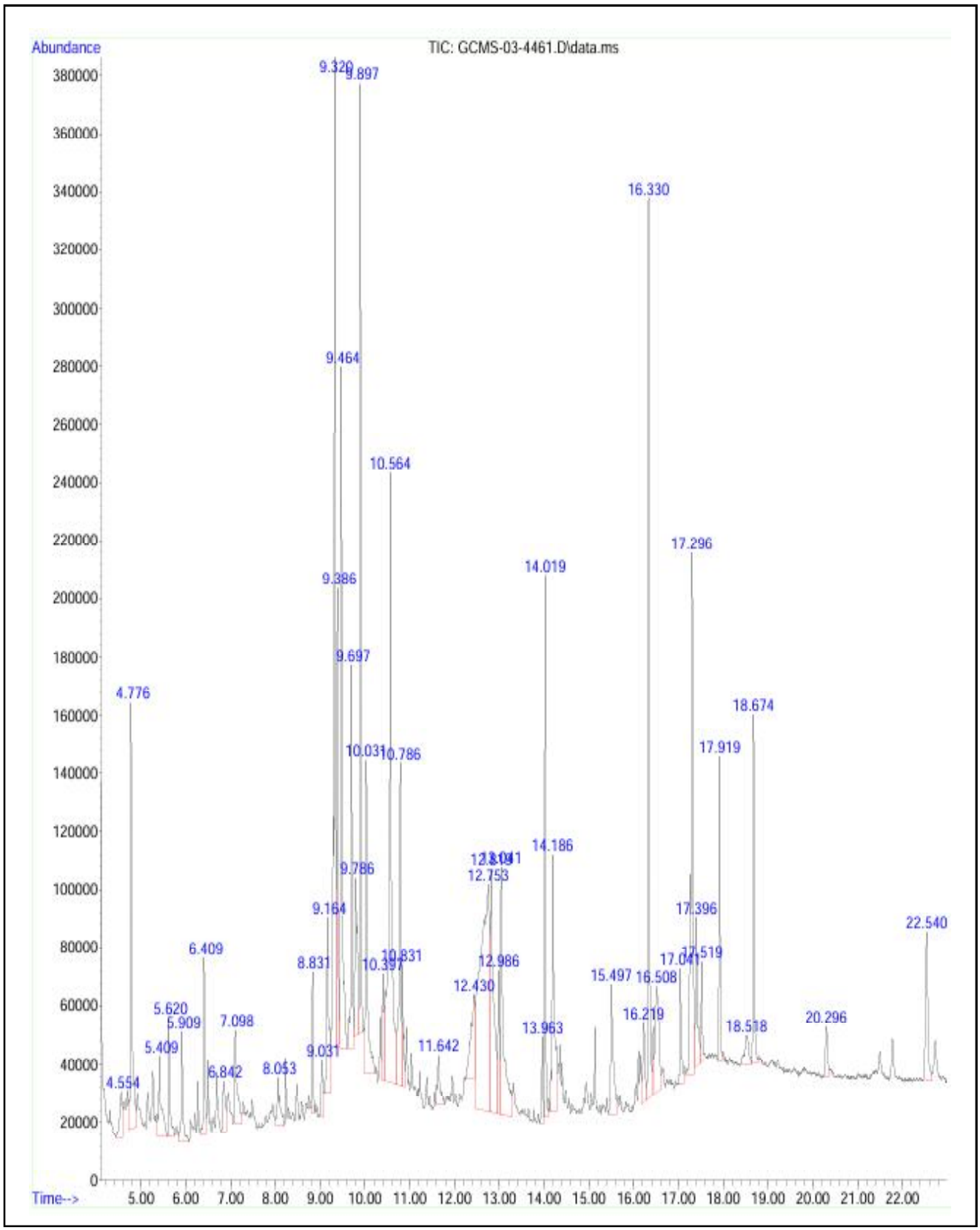

Figure 4: Metabolite profiling of VOCs in flowers of *J. auriculatum* mutant line (COM-PM-1).

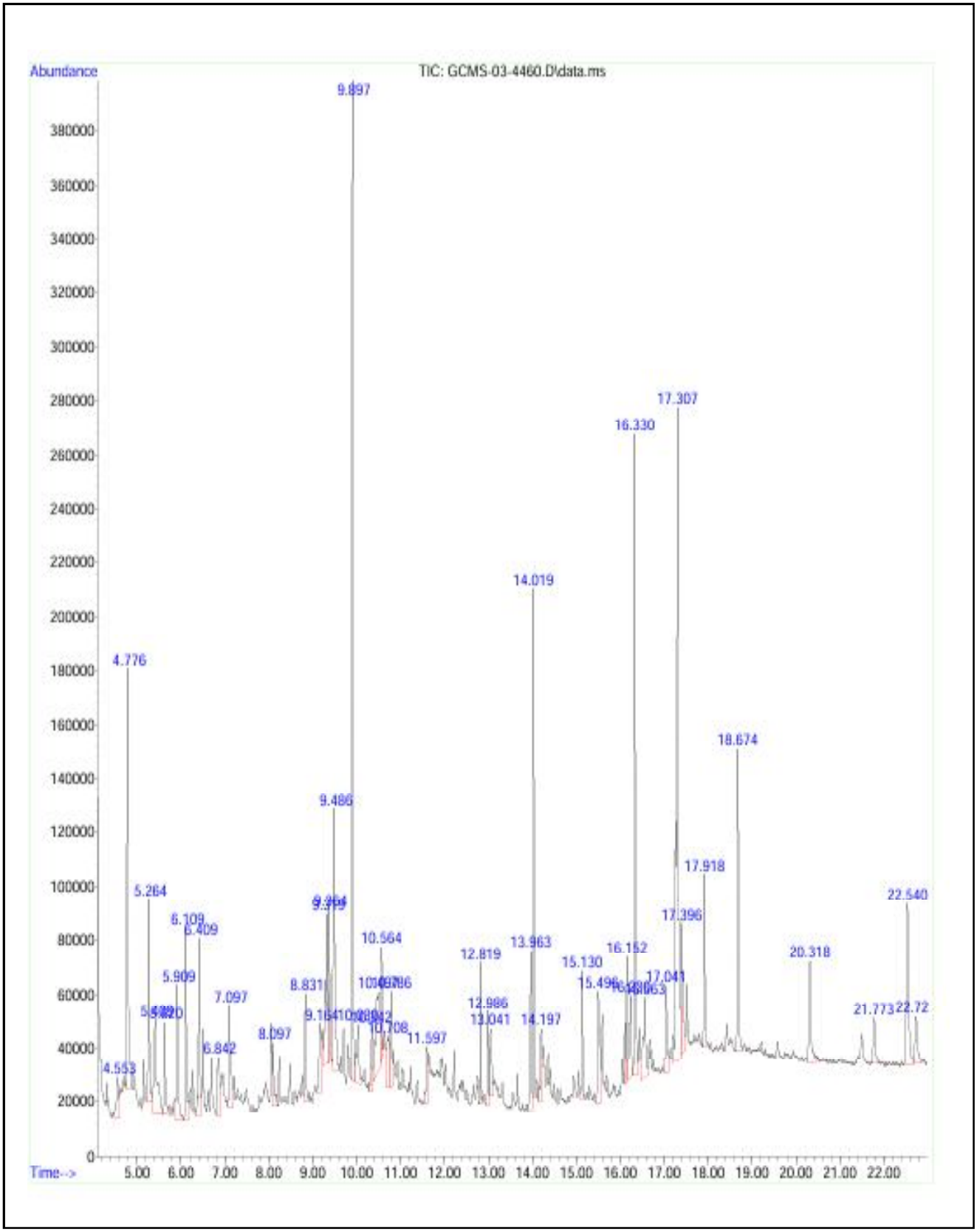

Figure 5: Metabolite profiling of VOCs in flowers of *J. auriculatum* mutant line (MM-PM-1).

Table 1: Phytochemical compounds identified in the flower extracts of Wild type (CO.1 Mullai) and Mutant line (COM-PM-1)

S. No.	Metabolite	Peak area (%)		Class	Properties	Reference
Common compounds in Wild type (CO.1 Mullai) and Mutant line (COM-PM-1)						
		Wild type (CO.1 Mullai)	Mutant line (COM-PM-1)			
1.	3-Methyl-2-butanol	1.78	6.31	Alcohol	Antioxidant activity, Plant metabolite	He *et al.* (2010)
2.	Hexadecanoic acid	1.70	5.58	Fatty acid	Antibacterial activity, Plant metabolite	Purushothaman *et al.* (2024)
3.	Pentadecanoic acid	1.70	3.44	Fatty acid	Antioxidant activity, Plant metabolite	Sharma *et al.* (2018)
4.	2-Propenoic acid	1.25	1.76	Monocarbo-xylic acid	Antibacterial and antioxidant activity	Rani *et al.* (2019)

Table 2: Phytochemical compounds identified in the flower extracts of Wild type (CO.1 Mullai) and Mutant line (MM-PM-1)

S. No.	Metabolites	Peak area (%)		Class	Properties	Reference
Common compounds in Wild type (CO.1 Mullai) and Mutant line (MM-PM-1)						
		Wild type (CO.1 Mullai)	Mutant line (MM-PM-1)			
1.	Benzaldehyde	1.24	1.79	Aldehyde	Antioxidant activity	Wu *et al.* (2022)
2.	1-Propanamine	4.10	4.91	Alkylamine	Antioxidant activity	Deeh *et al.* (2024)
3.	Propanoic acid	4.10	3.21	Carboxylic acid	Antioxidant activity	Al-Huqail *et al.* (2018)
4.	Phenylethyl Alcohol	2.90	1.94	Benzene and its derivatives	Antioxidant activity	Özdemir *et al.* (2022)
5.	1,2,5-Oxadiazol-3-amine	3.95	1.81	Oxadiazole	Antifungal properties	Alfalahi *et al.* (2024)

Table 3: Phytochemical compounds identified in the flower extracts of Mutant lines (COM-PM-1 and MM-PM-1)

S. No.	Metabolites	Peak area (%)		Class	Properties	Reference
Common compounds in Mutant lines (COM-PM-1 and MM-PM-1)						
		Mutant line COM-PM-1	Mutant line MM-PM-1			
1.	2,5-Dimethyl-4-hydroxy-3(2H)- furanone	1.04	2.46	Dihydrofuran	Antioxidant activity	Sakika *et al.* (2022)
2.	4H-Pyran-4-one	1.09	2.03	Pyranone	Antioxidant and anti-inflammatory activity	Premathilaka *et al.* (2016)
3.	1-Dimethyl (isopropyl) silyloxypropane	6.31	2.43	Organometalloid compounds	Antioxidant activity	Kadhim *et al.* (2014)
4.	Sorbitol	10.51	3.21	Monosaccharides and derivatives	Antioxidant activity	Mehmandar *et al.* (2023)
5.	Oxirane	2.10	3.31	Epoxides	Antioxidant, anti-fungal, anti-bacterial and anti-inflammatory activity	Patel *et al.* (2018)
6.	Octadecanoic acid	1.33	1.03	Fatty acid	Antioxidant activity	Yadav *et al.* (2018)
7.	Cyclotrisiloxane	1.33	1.03	Miscellaneous mixed metal	Antioxidant and anti-bacterial activity	Momin *et al.* (2020)

Table 4: Phytochemical compounds identified in the flower extracts of Wild type and Mutants

S. No.	Metabolites	Peak area (%)			Class	Properties	Reference
Common compounds in 3 samples (Wild type, COM-PM-1 and MM-PM-1)							
		Wild type	COM-PM-1	MM-PM-1			
1.	Benzofuran	1.24	1.11	1.79	Benzofuran derivatives	Antioxidant activity	Chand *et al*. (2017)
2.	Benzene	1.24	1.58	2.21	Benzene and its derivatives	Antioxidant activity	Khaksar *et al*. (2017)
3.	Benzoic acid	1.20	3.72	7.35	Benzene and its derivatives	Antioxidant activity	Farghaly *et al*. (2021)
4.	Ethyl paraben	4.13	3.72	7.35	Esters	Antimicrobial activity	Jianmei *et al*. (2015)
5.	Silane	4.09	2.41	1.24	Miscellaneous mixed metal	Cytotoxic activity	Ahmad *et al*. (2016)
6.	9,12,15-Octa decatrienoic acid	1.51	2.84	1.78	Fatty acids	Antioxidant and antimicrobial activity	Emniyet *et al*. (2014)
7.	7,10,13-He-xadecatrienoic acid	1.51	2.84	11.13	Fatty acids	Antioxidant, anti -inflammatory, anti-microbial and activities	Alqahtani *et al*. (2019)
8.	Phytol	2.68	2.38	3.94	Phyto sterols	Cytotoxic activity	Benelli *et al*. (2020)
9.	Squalene	1.25	1.53	1.68	Lipids	Antioxidant and anti-microbial activity	Rameshkumar *et al*. (2018)
10.	5,9,13-Pen-tadecatrien-2-one	2.22	2.10	3.31	Ketone	Antioxidant, anti-inflammatory, antimicrobial and activities	Onyegeme-Okerenta *et al*. (2021)

4. Discussion

In the present study, a significant difference in the key metabolite profiles was observed between the wild type and mutant lines, with the mutant lines exhibiting higher levels of peak area % indicating an enhanced production of phytochemical compounds. Further, the compounds detected in the wild type and mutant lines possess numerous potential phytochemical properties. 3-methyl-2-butanol, an alcohol, hexadecanoic acid and pentadecanoic acid, a fatty acid are known for gaining attention as a potential biomarker for dairy fat intake, contributing to cardiovascular health benefits (He *et al*., 2010; Tamoli *et al*., 2022; Purushothaman *et al*., 2024; Sharma *et al*., 2018), and these compounds also possess antioxidant activity, there by neutralizing the reactive oxygen species (ROS), stabilizing cellular components, and preventing oxidative stress, maintaining the structural integrity of cell membranes, which is critical for plant survival under stress conditions. The compound 2-propenoic acid, a monocarboxylic acid, which has been detected as a common metabolite in wild type (CO.1 Mullai) and mutant line (COM-PM-1), possesses antibacterial and antioxidant activities, protecting plants against microbial infection by disrupting bacterial cell walls and inducing oxidative stress in pathogens. These properties could be useful in developing treatments for skin infections (Rani *et al*., 2019).

The compounds with antioxidant and antifungal properties detected as common metabolites in wild type (CO.1 Mullai) and mutant line (MM-PM-1) included benzaldehyde, an aldehyde (Wu *et al*., 2022), 1-propanamine, an alkylamine (Deeh *et al*., 2024), propanoic acid, a carboxylic acid (Al-Huqail *et al*., 2018), phenylethyl alcohol, benzene and its derivatives (Özdemir *et al*., 2022), with the presence of these metabolites allows plants to neutralize ROS and protect themselves from oxidative stress caused by environmental factors such as UV light, drought, or pathogen invasion. This antioxidant activity is vital for maintaining cellular homeostasis and preventing damage to crucial biomolecules like lipids, proteins, and nucleic acids and it also has sedative effects which is primarily used in aromatherapy for relaxation and stress relief, whereas 1,2,5-oxadiazol-3-amine, an oxadiazole (Alfalahi *et al*., 2024), possesses antifungal property and disrupts fungal cell membranes and inhibits their growth, ensuring that the plant remains free from fungal pathogens.

In the two mutant lines (COM-PM-1 and MM-PM-1), 2,5-dimethyl-4-hydroxy-3(2H)-furanone, adihydrofuran (Sakika *et al*., 2022) and sorbitol, a monosaccharides derivative were detected. Meanwhile, sorbitol has applications as a laxative due to its ability to retain water in the intestines (Mehmandar *et al*., 2023). The compound1-dimethyl(isopropyl)silyloxypropane, a pyranone (Kadhim *et al*., 2014) and octadecanoic acid, a fatty acid plays a role in maintaining skin and are also being explored for anti-inflammatory potential (Yadav *et al*., 2018). The above mentioned metabolites also possess antioxidant activities through diverse mechanisms, including radical scavenging, membrane stabilization, ROS neutralization, and osmotic stress protection, meanwhile, managing oxidative stress, thereby ensuring cellular homeostasis and enhancing survival under stress conditions. 4H-pyran-4-one, a pyranone, possesses antioxidant and anti-inflammatory properties, offering protection against oxidative stress, inflammation besides the ability to scavenge ROS, chelate metals, inhibit pro-inflammatory mediators, and suppress the NF-κB pathway, makes it a valuable compound in both plant defense systems and potential therapeutic applications for humans (Premathilaka *et al*., 2016). Further, oxirane, an epoxide, exhibits antioxidant, antifungal, antibacterial and anti-inflammatory activity (Patel *et al*., 2018), enhances the plant's ability to cope with oxidative damage, pathogens, and inflammation, supporting overall plant vitality and growth. Additionally, cyclotrisiloxane, a miscellaneous mixed metal has moisturizing and skin-protecting properties and is used in formulations for wound healing and skin hydration and it also has antioxidant and antibacterial activitiesas reported by Momin

et al. (2020), providing a strong defence system for plants. It protects against oxidative damage caused by environmental stress (such as UV radiation or pollutants) and combats bacterial infections by disrupting pathogen growth. These activities contribute to overall plant health, resilience, and survival in challenging conditions. The increased concentrations of these compounds in COM-PM-1 and MM-PM-1 highlight their enhanced phytochemical profiles compared to the wild type. This suggests that mutant lines offer a valuable platform for advancing phytochemical research and applications, including researchin pharmaceuticals, cosmetics, and agriculture.

The common compounds which were present in all the investigated samples of *J. auriculatum* include, benzofuran (Chand *et al*., 2017), benzene (Khaksar *et al*., 2017), benzoic acid (Farghaly *et al*., 2021) belonging to the class of benzene and its derivatives are used in various medicinal compounds and have potential therapeutic applications in treating infections and cancer and ethyl paraben, an ester, demonstrate different levels of antioxidant activity, contributing to their therapeutic or preservative uses (Jianmei *et al*., 2015). 9,12,15-octadecatrienoic acid, a fatty acid (Emniyet *et al*., 2014), is crucial for heart health and for reducing inflammation in conditions like arthritis.A class of lipids, squalene supports skin hydration owing to its antiageing properties; it is an immune enhancer exhibiting antioxidant and antimicrobial activities by scavenging the reactive oxygen species; prevents lipid peroxidation, and reduces inflammation, thereby disrupting the microbial cell membranes and inhibits bacterial and fungal growth (Rameshkumar *et al*., 2018). In accordance with the research findings of Benelli *et al*. (2020), phytol, a plant sterol and silane, a miscellaneous mixed metal (Ahmad *et al*., 2016) exhibit cytotoxic activity. 7,10,13-hexadecatrienoic acid, fatty acid (Alqahtani *et al*., 2019) and 5,9,13-pentadecatrien-2-one, belonging to a class of ketone possess antioxidant, anti-inflammatory, antimicrobial and activities (Onyegeme-Okerenta *et al*., 2021). The mutant lines, COM-PM-1 and MM-PM-1, have demonstrated a significant enhancement in phytochemical production compared to the non-mutant or wild-type plants. These lines exhibited increased levels of key bioactive compounds, suggesting that the mutations have positively influenced the metabolic pathways responsible for phytochemical synthesis.

5. Conclusion

This study demonstrates the mutant lines COM-PM-1 and MM-PM -1 present a promising potential as sources of bioactive compounds, particularly for antioxidant and antimicrobial applications compared to the wild type. These findings validate the traditional medicinal uses other than the well-established ornamental values of *J. auriculatum* and pave the way for further exploration of its bioactive compounds for therapeutic applications. Further studies are required for isolation, characterization and biological evaluation of these compounds to fully understand their therapeutic potentials.

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Conflict of interest

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

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