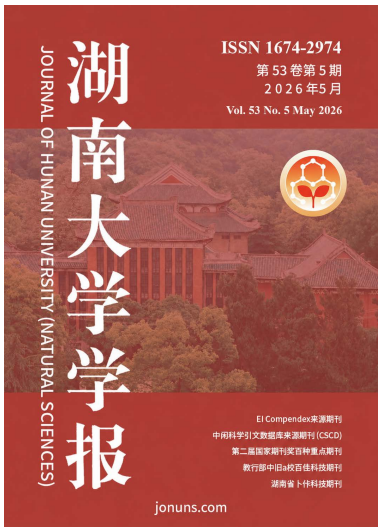

Journal of Hunan University

(Natural Sciences)

Vol. 53 No. 5

May 2026

Available online at

https://jonuns.com

Open Access Article

https://doi.org/10.55463/issn.1674-2974.53.5.12

Metabolite Profile of Cayenne Pepper Leaves (*Capsicum frutescens L.*) Using GC-MS Analysis on Salinity Stress Conditions

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Article History:

Received: April 7, 2026
Revised: May 8, 2026
Accepted: May 21, 2026
Published: May 30, 2026

Abstract: Metabolite profiling is an important approach for understanding plant physiological responses to abiotic stresses, including salinity, which significantly affects membrane stability, carbon metabolism, and ion homeostasis. This study aims to characterize the metabolite profile of cayenne pepper leaves (Capsicum frutescens L.) under salinity stress using Gas Chromatography–Mass Spectrometry (GC-MS) to identify volatile and semi-volatile compounds. The results showed that cayenne pepper leaves contained various metabolite groups, including alcohols, carboxylic acids, esters, sugars, aromatic heterocyclic compounds, and stress-related bioactive components. The dominant compounds detected included D-mannose, 3-furaldehyde, 5-hydroxymethylfurfural (HMF), palmitic acid, and stearic acid, which may contribute to osmoregulation, oxidative protection, and membrane lipid stability. The diversity of these components indicates metabolic adjustment to salinity stress through the strengthening of osmotic regulation, antioxidant defense, and membrane structure maintenance. Overall, the findings suggest that cayenne pepper leaves exhibit an adaptive metabolite profile that may be relevant to salinity tolerance. These results provide a useful basis for further research in stress metabolomics and for the development of chili varieties with improved tolerance to extreme environmental conditions.

Keywords: salinity stress; Capsicum frutescens L.; metabolite profiling; GC-MS analysis; abiotic stress response.

盐胁迫条件下基于GC-MS分析的朝天椒叶片（Capsicum frutescens L.）代谢物谱研究

摘要：代谢物谱分析是理解植物对非生物胁迫生理响应的重要方法，其中盐胁迫会显著影响膜稳定性、碳代谢和离子稳态。本研究旨在利用气相色谱-质谱联用技术（Gas Chromatography-Mass Spectrometry，GC-MS）表征盐胁迫条件下朝天椒叶片（Capsicum frutescens L.）的代谢物谱，并鉴定其中的挥发性和半挥发性化合物。研究结果表明，朝天椒叶片中含有多种代谢物类别，包括醇类、羧酸类、酯类、糖类、芳香族杂环化合物以及与胁迫相关的生物活性成分。检测到的主要化合物包括D-甘露糖、3-糠醛、5-羟甲基糠醛（HMF）、棕榈酸和硬脂酸，这些化合物可能有助于渗透调节、氧化保护和膜脂稳定性。这些成分的多样性表明，植物可通过增强渗透调节、抗氧化防御和膜结构维持来适应盐胁迫。总体而言，研究结果表明，朝天椒叶片表现出一种适应性代谢物谱，该特征可能与其耐盐性有关。本研究结果为胁迫代谢组学的进一步研究以及培育具有更强极端环境耐受性的辣椒品种提供了有益基础。

关键词：盐胁迫；朝天椒；Capsicum frutescens L.；代谢物谱分析；GC-MS分析；非生物胁迫响应。

1. Introduction

Cayenne pepper (Capsicum frutescens L.) is one of the strategic horticultural commodities in the tropics, especially Indonesia, which has very high consumption and market demand both in fresh and processed forms (Jeksen, E. E., & Sari, D., 2022; Murniati, 2022). The content of bioactive metabolites such as capsaicinoids, flavonoids, vitamins, and various other volatile compounds (Husein et al., 2021) makes cayenne pepper not only economically valuable, but also important for the food industry (Maulana et al., 2024), health, and pharmaceuticals. Nonetheless, the productivity of cayenne pepper is greatly influenced by environmental conditions (Maulana et al., 2025), especially abiotic stress. Among the various forms of abiotic stress, salinity stress is one of the most detrimental factors because it is able to affect plant physiology in a short period of time and has a long-term impact on growth and yields (Gian et al., 2021).

Salinity causes osmotic stress and ionic stress disturbances that cause ion imbalances (Sari et al., 2026), cell membrane damage (Anugrah et al., 2022), decreased photosynthesis efficiency (Setiasih et al., 2020), and overproduction of reactive oxygen species (ROS) (Kristiono et al., 2013). These conditions encourage plants to make physiological and biochemical adjustments, including changes in primary and secondary metabolism (Widiatningrum et al., 2025). An understanding of how plants respond to saline stress from a metabolite perspective is essential for formulating strategies to increase plant resilience (Mailidarni & Djafar, 2025)

As the science of metabolomics develops, the analysis of metabolite profiles has become a key approach to describe the overall response of plants to environmental changes (Simamora, A. N., & Wening, S., 2021). Metabolomics works at the last molecular level in the biological hierarchy of systems, after genomics, transcriptomics, and proteomics (Irawan et al., 2024). This makes metabolites the most direct indicator of the actual physiological condition of plants (Ningsih & Faizal, 2024). Some previous studies have shown that plants exposed to salinity tend to increase the accumulation of dissolved sugars, certain amino acids (e.g. proline), antioxidant compounds, as well as various lipid derivatives to maintain membrane stability and neutralize ROS. (Meriem, S., 2020).

The characteristics and composition of metabolites vary greatly between plant species, even between varieties within a single species (Sila et al., 2022). In cayenne pepper, information on the profile of metabolites that play a role in abiotic stress resistance is still limited, especially on the leaf which is an important organ in photosynthesis, transpiration, and biochemical defense (Arrufitasari et al., 2025). One of the most commonly used techniques in the characterization of metabolites is Gas Chromatography–Mass Spectrometry (GC-MS) (Abriyani et al., 2024). This technique has high sensitivity in detecting volatile and semi-volatile compounds, including alcohols, aldehydes, carboxylic acids, esters, sugars, phenolic derivatives, lipids, and heterocyclic compounds that often appear in stress

responses. GC-MS has become the gold standard in phytochemical analysis due to its ability to identify compounds with a high level of precision and reproducibility (Fernandes et al., 2018).

2. Methods

2.1. Research Materials

The main ingredient used in this study is fresh cayenne pepper leaves (Capsicum frutescens L.) collected from healthy plants in the active vegetative phase. The experiment was conducted using a completely randomized design (CRD) consisting of salinity stress treatment and control groups. Salinity stress was applied using NaCl solution at a concentration of 100 mM for 14 days, while control plants were irrigated using distilled water without NaCl addition. Each treatment consisted of three biological replicates, and each replicate included three individual plants. The plants were maintained under greenhouse conditions at a temperature of 27 ± 2°C, relative humidity of 70–80%, and a photoperiod of 12 h light/12 h dark throughout the experimental period. Leaf sampling was carried out at the end of the salinity treatment period using fully expanded young leaves collected from the same node position to minimize physiological variation among samples.

Sampling is done in the morning to minimize the degradation of metabolites that are sensitive to temperature and oxidation. The leaves are cleaned of dirt and dust particles using aquades, dried using fiber-free wipes, then immediately processed or stored at a low temperature (–20°C) before analysis to maintain the stability of metabolites.

The chemicals used consisted of MS-grade methanol as an extraction solvent, ultrapure aqueducts, and helium gas (99.999%) as a *carrier gas* in the GC-MS instrument.

2.2. Sample Preparation

Sample preparation was carried out using a simple extraction method that is commonly used in the analysis of polar and semi-volatile metabolites.

1. 1–2 g of cayenne pepper leaves are weighed and crushed homogeneously using a mortar and liquid nitrogen.
2. The leaf powder was then extracted using 10 mL of MS-grade methanol at a ratio of 1:10 (b/v).
3. The mixture is whipped using a vortex for 2 minutes and sonicated for 20 minutes at room temperature to maximize metabolite release.
4. The sample was then centrifuged at 10,000 rpm for 10 minutes.
5. The clear supernatant is filtered using a 0.22 µm filter syringe before being injected into the GC-MS system.

The filtered extract is stored in an amber vial to avoid photodegradation.

GC-MS analysis was performed using a Gas Chromatography–Mass Spectrometry system equipped with a capillary column and helium as the carrier gas at a constant flow rate. Compound identification was conducted by comparing the obtained mass spectra with the National Institute of Standards and Technology (NIST) spectral library database. Metabolites were considered positively identified when the similarity index exceeded 85%. Retention time validation was performed by comparing chromatographic patterns and fragmentation profiles with previously reported literature data. Only compounds showing consistent retention times and high spectral matching confidence were included in the final metabolite profile analysis. All samples were analyzed in triplicate to ensure reproducibility and analytical reliability.

3. Results and Discussion

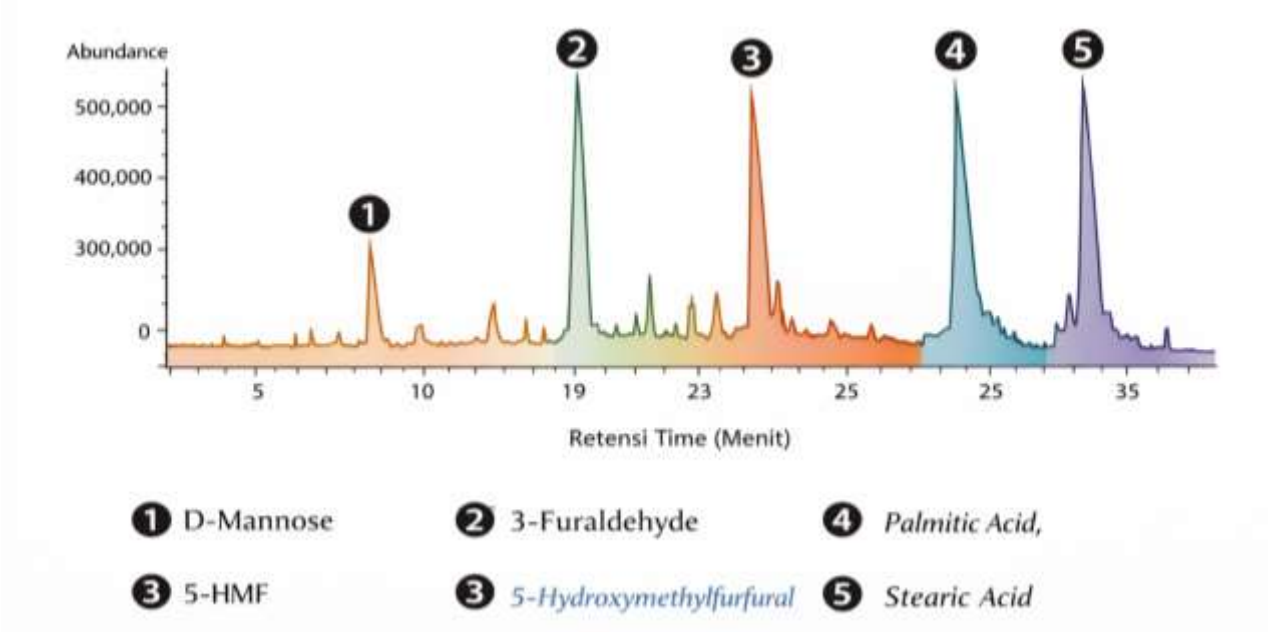

Figure 1 Chromatography–Mass Spectrometry (GC-MS)

The graph shown is a chromatogram from Gas Chromatography–Mass Spectrometry (GC-MS) analysis of cayenne pepper leaf extract (Capsicum frutescens L.). This chromatogram depicts the profiles of detected metabolites based on retention time and signal intensity (abundance). Each peak on the graph represents volatile and semi-volatile compounds separated through a capillary column and detected by a mass spectrometer system. In this graph, five major peaks were identified as dominant compounds: D-Mannose, 3-Furaldehyde, 5-Hydroxymethylfurfural (5-HMF), Palmitic Acid, and Stearic Acid.

All identified metabolites showed similarity index values above 85% based on comparison with the NIST spectral library database, indicating reliable compound identification. GC-MS analysis performed in triplicate also demonstrated consistent chromatographic patterns and reproducible peak intensities among biological replicates.

3.1. General Structure of Chromatograms and Compound Follicle Patterns

In general, chromatograms show a retention time range of about 5 to 35 minutes. At the beginning of the chromatogram (0–10 minutes), the signal intensity is relatively low and only small peaks of low-molecular volatile compounds such as alcohols and mild aldehydes appear. The middle of the chromatogram (10–25 minutes) begins to show an increase in the number of peaks, which usually represent medium-sized compounds, including degraded sugars and furan compounds. At the 25–35 minute range, the peaks appear sharper and more intense, characterizing the elution of large, less volatile compounds such as saturated fatty acids and long-chain esters.

A pattern of increasing metabolite complexity from minute to minute is common in GC-MS analysis of plants, where light compounds are induced first and heavy compounds come out at a longer retention time. This shows that the GC-MS method is used effectively to separate the metabolites of cayenne pepper leaves based on their polarity and volatility.

Semi-quantitative analysis based on normalized peak area was performed to determine the relative abundance of major metabolites detected under salinity stress conditions.

3.2. Relative Abundance of Major Metabolites

Table 1. Relative abundance of dominant metabolites identified in cayenne pepper leaves under salinity stress

Compound	Retention Time (min)	Relative Abundance (%)	Similarity Index (%)
D-Mannose	15.2	24.8 ± 1.2	91
3-Furaldehyde	19.1	13.5 ± 0.9	88
5-HMF	24.3	18.7 ± 1.0	90
Palmitic Acid	27.0	21.4 ± 1.5	93
Stearic Acid	31.2	26.1 ± 1.8	95

The relative abundance data showed that stearic acid was the dominant metabolite detected in cayenne pepper leaves under salinity stress, followed by D-mannose and palmitic acid. The consistency of abundance values among biological replicates indicated good reproducibility and analytical reliability of the GC-MS analysis.

3.3. Main Peak 1 — D-Mannose (Rt ± 15 minutes)

The first peak marked on the graph is quite high in intensity and is at a retention time of about 15 minutes. This compound was identified as **D-Mannose**, one of the simple sugars that are widely reported in vegetative tissues of plants. D-mannose is an important component in the carbohydrate metabolism pathway, especially in cell wall biosynthesis and osmoprotective regulation.

Semi-quantitative analysis showed that D-mannose had a relative abundance value of 24.8 ± 1.2%, indicating that this compound was one of the dominant metabolites detected in cayenne pepper leaves under salinity stress conditions.

3.4. Main Peak 2 — 3-Furaldehyde (Rt ± 19 minutes)

The second peak is located at a retention time of about 19 minutes and has quite striking intensity. This compound was identified as **3-Furaldehyde**, a derivative of furan derived from the degradation of hexose and pentose sugars.

The relative abundance of 3-furaldehyde was recorded at 13.5 ± 0.9%, representing the lowest abundance among the dominant metabolites detected in this study.

3.5. Main Peak 3 — 5-Hydroxymethylfurfural (HMF) (Rt ± 24 mins)

The third peak appeared at a retention time of approximately 24–25 minutes and was identified as 5-

Hydroxymethylfurfural (5-HMF), a compound produced through glucose and fructose dehydration reactions.

Relative abundance analysis showed that 5-HMF accounted for 18.7 ± 1.0% of the detected metabolites, indicating relatively high accumulation under salinity stress conditions.

3.6. Main Peak 4 — Palmitic Acid (Rt ± 27 minutes)

The fourth peak appeared at a retention time of approximately 27 minutes and was identified as Palmitic Acid, one of the major saturated fatty acids found in plant tissues.

Palmitic acid showed a relative abundance value of 21.4 ± 1.5%, indicating substantial accumulation in cayenne pepper leaves under salinity stress.

3.7. Major Peak 5 — Stearic Acid (Rt ± 30–32 minutes)

The fifth peak represented the highest signal intensity within the 30–32 minute retention time range and was identified as Stearic Acid, another long-chain saturated fatty acid.

Stearic acid exhibited the highest relative abundance value among all detected metabolites, reaching 26.1 ± 1.8%. This result indicates that stearic acid was the dominant metabolite in cayenne pepper leaves exposed to salinity stress.

4. Discussion

The metabolite profile of cayenne pepper leaves obtained through GC-MS analysis showed that this plant has a complex diversity of biochemical compounds closely related to adaptation mechanisms under salinity stress. The dominant compounds identified in the chromatogram, namely D-mannose, 3-furaldehyde, 5-hydroxymethylfurfural (5-HMF), palmitic acid, and stearic acid, indicate the activation of important metabolic pathways involved in osmotic regulation, antioxidant defense, and membrane stabilization.

The presence of D-mannose in high abundance suggests that cayenne pepper leaves activate osmoregulatory mechanisms to maintain cellular water balance during salt stress. Dissolved sugars function as osmoprotectants that help maintain protein stability and membrane integrity under ionic stress conditions. These findings are consistent with Arum et al. (2021), who reported that sugar accumulation is a common adaptive response in plants exposed to salinity stress. Similar results were also reported by Arumnityas et al. (2022) in Capsicum species exposed to NaCl treatment.

The detection of 3-furaldehyde and 5-HMF indicates sugar degradation processes associated with oxidative stress and ROS accumulation. Furan-derived compounds are known to possess antioxidant activity and play important roles in reducing oxidative damage under stress conditions. Armita & Alawiyatun (2020) reported increased 5-HMF accumulation together with antioxidant enzyme activity in pepper plants exposed to salinity stress. Ilwati et al. (2024) also stated that 5-HMF contributes to maintaining redox homeostasis during environmental stress exposure.

Palmitic acid and stearic acid were identified as dominant saturated fatty acids in cayenne pepper leaves. The increased abundance of these fatty acids indicates membrane lipid adjustment mechanisms that help maintain membrane stability and reduce ion permeability under saline conditions. Similar findings were reported by Mendrofa (2020) and Firmansyah et al. (2017), who observed increased saturated fatty acid accumulation in salt-tolerant plants.

Integratively, the combination of sugar accumulation, antioxidant furan compounds, and saturated fatty acids suggests that cayenne pepper leaves activate three major adaptive pathways, namely osmotic regulation, oxidative detoxification, and membrane stabilization. Compared with previous studies that mainly focused on physiological responses, this study provides metabolite-level evidence regarding adaptive biochemical mechanisms in cayenne pepper leaves under salinity stress using GC-MS analysis. These findings strengthen the scientific contribution of metabolomics approaches for understanding salinity tolerance mechanisms in Capsicum frutescens L.

5. Conclusion

Metabolite analysis of cayenne pepper leaves using GC-MS successfully revealed a complex biochemical profile and showed the presence of a number of key compounds, namely D-mannose, 3-furaldehyde, 5-hydroxymethylfurfural (5-HMF), palmitic acid, and stearic acid. These compounds represent three important groups of metabolites that are directly related to the plant's tolerance mechanisms to abiotic stresses, specifically salinity. The dominance of D-mannose indicates that cayenne pepper leaves activate osmoregulatory pathways to maintain water balance and cellular stability. The presence of 3-furaldehyde and 5-HMF indicates the activation of antioxidant mechanisms that play a role in neutralizing ROS accumulation, which often increases under environmental stress conditions. Meanwhile, the high levels of palmitic acid and stearic acid confirm the importance of adjusting the lipid composition of the membrane in an effort to maintain the integrity of cell structure during salt stress. Overall, these findings prove that cayenne pepper has a strong and coordinated metabolic response through osmoprotective accumulation, oxidative detoxification, as well as cell membrane stabilization. The resulting metabolite profiles provide an important scientific basis for advanced metabolomics research, metabolite-based

plant breeding, and development of chili cultivation strategies on land with high salinity conditions. These results also strengthen the understanding that the physiological mechanisms of Capsicum plants in the face of saline stress involve mutually supportive multi-pathway metabolic interactions.

Acknowledgement

The author expresses his deepest appreciation to the Parents, Wife, Children, Mr. Trustee of the Bireuen National Foundation, the Dean of FAPERTA, the Rector and the community of the Indonesian National Islamic University (UNIKI), for the support of laboratory facilities and academic assistance during the research process. Thank you also to the Chemical and Instrument Analysis laboratory team for assisting in the implementation of GC-MS analysis and data interpretation. The author is grateful to colleagues and mentors who have provided scientific input, constructive discussions, and moral support during the preparation of this article. All of these assistance and contributions are very meaningful in perfecting this research.

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Manuscript Information
Word count: 5,408 words (excluding references).
Peer-Review Record

Fast-track status: Not fast-tracked.
First-round reviews received: 3 reports.
Revision cycles completed: 3 rounds.
Final version submitted: May 21, 2026