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Metabolite profiling in *Moringa oleifera* leaves under various drying methods and solvents for eco-friendly bio-based natural fertilizer development

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ABSTRACT

This study aimed to identify and compare the chemical and metabolite profiles of the moringa leaves (*Moringa oleifera*) processed through two drying methods: oven drying and sun drying, followed by extraction using three solvents of varying polarities: ethanol, n-hexane, and ethyl acetate. These different treatments significantly influenced the composition of the released phytochemicals, which were crucial for the eco-friendly bio-based natural fertilizer development with elevated nutritional and bioactivity content. The extracts were analyzed using gas chromatography–mass spectrometry (GC–MS) to obtain metabolite fingerprints. Metabolite fingerprint data from all treatments were analyzed using Multivariate Analysis, including principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). The study results indicated that solvent polarity and drying conditions significantly and systematically affected the GC–MS metabolite composition of *M. oleifera* leaf extract. Non-polar n-hexane consistently enriched long-chain aliphatic hydrocarbons, wax-derived lipids, tocopherol-related compounds, and sterols, confirming efficient extraction of hydrophobic metabolites. Ethanol produced the widest chemical diversity; it dominated, consisting of fatty acids, oxygenated lipids, glycosides, sugars and phenolic derivatives, while ethyl acetate selectively extracted semi-polar metabolites including aromatic ketones, lactone-type compounds, phenethyl derivatives, and mid-polarity lipid fragments. Drying treatments also significantly altered chemical profiles. Oven drying effectively preserved thermolabile antioxidants, sterols, and oxygenated lipids, moderate sun drying induced partial oxidation and formation of semi-volatile metabolites. In general, the metabolite indicated that *M. oleifera* leaf extracts – regardless of solvent used – contain metabolite classes relevant for bio-based fertilizer applications. These findings highlight the potential to customize extraction and drying strategies to optimize functional metabolite profiles for specific agricultural applications such as slow-release carbon inputs, microbially responsive amendments, or antioxidant-enriched biofertilizer formulations, and it will form the basis for effective, high-value-added eco-friendly bio-based fertilizer formulations.

Keywords: *Moringa oleifera*, metabolite profile, GC-MS, oven drying, sun drying.

INTRODUCTION

Moringa oleifera Lam. (Moringa) has been globally recognized as a plant with extraordinary nutritional and pharmacological potential, earning it the nickname "miracle tree" (Stohs and Hartman, 2015; Arora and Arora, 2021). Moringa leaves are a rich source of macro nutrients (protein and fiber) and micro nutrients (vitamins and minerals), and contain a wide spectrum of bioactive secondary metabolites (Sahay et al., 2017; Dzuvor et al., 2022). These compounds, including flavonoids (such as quercetin and kaempferol), glucosinolates, and phenolic acids, have been scientifically proven to have strong antioxidant, anti-inflammatory, and chemopreventive properties (Fahey, 2005). Moringa biomass residues also hold promise for application in sustainable agriculture programs, where the use of moringa leaf metabolites as organic/natural fertilizers, biostimulants, and plant nutrient enhancers capable of improving plant growth, crop yields, as well as resistance to biotic/abiotic stresses continues to be developed (Du-Jardin, 2020; Di-Sario et al., 2025). This development aligns with the global need to reduce synthetic chemical usage and promote sustainable agricultural practices (Chen, 2006; Shaikh and Patil, 2023). The functional applications of moringa leaf extracts, whether in terms of nutritional utilization, pharmaceutical use, or agricultural use, are highly dependent on the profiling and abundance of their bioactive metabolites. These metabolite profiles are highly sensitive and can change drastically depending on post-harvest conditions and extraction processes carried out with different solvents.

The drying process is crucial post-harvest for crops. Sun drying is economical and common. However, direct exposure to heat, oxygen, and ultraviolet (UV) radiation can degrade sensitive metabolites. This includes vitamins and phenolic compounds, which can degrade through heat and oxidation (Santos et al.,2012; Noboosse et al., 2017; Razzak et al., 2021). Oven drying uses controlled temperatures and is more energy intensive. It usually offers better metabolite retention (Popova, 2018; Komonsing et al., 2021; Belwal et al., 2022). Variations in the temperature and duration of these drying treatments fundamentally alter the metabolite composition of moringa leaves.

The efficiency of metabolite utilization from moringa leaves is largely determined by the type of solvent used in the extraction process. The polarity of the solvent (e.g., water, methanol, ethanol, hexane) will determine the selectivity of extraction of different metabolite groups. Polar solvents tend to extract sugars and amino acids, while non-polar solvents are more efficient for fats, sterols, and pigments (Dai and Mumper, 2010). The selection of an inappropriate solvent can result in an incomplete metabolite profile, thereby underestimating the functional potential of the biomass. Although there have been separate studies on the influence of one of these factors, there has been no comprehensive and integrated study that systematically compares and deeply maps (profiles) the combined impact of two types of drying treatments (oven and sun) as well as variations in solvent types on the metabolomic spectrum of moringa leaves, specifically for the purpose of developing bio-fertilizers (Chokoe et al., 2025). This study aimed to bridge this knowledge gap by identifying and quantifying variations in the metabolite profile of moringa leaves as a function of two drying treatments and three solvents of varying polarity. A gas chromatography–mass spectrometry (GC–MS)-based metabolomics approach was adopted. GC–MS analysis is a powerful analytical platform for profiling volatile primary and secondary metabolites, such as organic acids, amino acids, sugars, and fatty acids, all of which are key components in plant nutrition and biostimulant function (Sakakibara et al., 2008). Furthermore, to interpret the complex chemical data set, Multivariate Analysis was used. Principal component analysis (PCA) was applied for visualization and identification of natural trends, while partial least squares discriminant analysis (PLS-DA) was used to identify the most significant marker metabolites (biomarkers) that correlate with specific treatment conditions (Yi et al., 2026; Garcia-Perez et al., 2024).

The results of this study are expected to provide a detailed metabolomic map that will directly inform about the most optimal conditions for moringa leaf preparation. This research aimed to identify the treatment that produces the optimal metabolite profile, including beneficial amino acids and sugars for fertilizers. It established a scientific foundation for determining the most efficient drying and extraction conditions to maximize key metabolite retention. The goal was to optimize the process and provide quantified, verified data to support the formulation of highly effective organic fertilizers and natural biostimulants derived from moringa leaves. This will enhance the value of agricultural waste and facilitate the shift towards regenerative agriculture.

MATERIALS AND METHODS

Collection of plant materials

Fresh and fully expanded *Moringa oleifera* leaves were collected from mature trees which have grown naturally in Mandalle District, Pangkep Regency, South Sulawesi, Indonesia. Sampling was carried out in the early morning to minimize metabolic oxidation, and only healthy, disease-free foliage was selected to ensure chemical uniformity across treatments.

Extraction of plant materials

The dried leaf materials obtained from the three post-harvest drying treatments were ground separately into fine powder using a sterile stainless-steel grinder. Each drying treatment consisted of three independent replicates, resulting in a total of nine powdered samples prepared for extraction – A1 (oven-dried for 10 h, n-hexane), A2 (sun-dried for 18 h, h-hexane), A3 (sun-dried for 27 h, n-hexane), B1 (oven-dried for 10 h, ethanol), B2 (sun-dried for 18 h, ethanol), B3 (sun-dried for 27 h, ethanol), C1 (oven-dried for 10 h, ethyl acetate), C2 (sun-dried for 18 h, ethyl acetate), C3 (sun-dried for 27 h, ethyl acetate) – to evaluate the influence of solvent polarity on metabolite recovery, each replicate was extracted using one of three solvents representing non-polar, semi-polar, and polar classes: n-hexane (A), ethanol (B), ethyl acetate (C). Approximately 100 g of each powdered sample was subjected to Soxhlet extraction following the standardized protocol described by (Jensen, 2007) and refined by (Sarker and Nahar, 2012). For each extraction, 300 mL of the designated solvent was used. The Soxhlet apparatus was run at 60–70 °C for 4–6 hours, ensuring exhaustive extraction, as indicated by a clear siphon chamber. The resulting solvent extracts were filtered and concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) at 40 °C to preserve thermolabile compounds. The crude extracts were weighed to determine the extraction yield, following the procedures widely applied in phytochemical investigations (Harborne, 1998). Each extract was stored in amber vials at 4 °C until further analysis.

For the GC–MS analysis, derivatization was performed to enhance the volatility and chromatographic performance of semi-polar compounds. Approximately 1 g of each crude extract was dissolved in 25 mL of its corresponding extraction solvent and heated gently to 60 °C for 60–90 minutes to ensure complete solubilization. The mixture was washed with distilled water to remove hydrophilic impurities, and the organic layer was dried using anhydrous sodium sulfate. The dried extract was then reconstituted in 1.0 mL pyridine, vortexed for homogenization, and allowed to stand for 10 minutes to facilitate derivatization. A 200-µL aliquot was filtered through a 0.22-µm PTFE membrane filter and transferred to GC–MS compatible autosampler vials. This integrated extraction–preparation workflow produced nine derivatized samples, each representing a unique combination of drying treatment and solvent polarity, for subsequent GC–MS profiling.

Gas chromatography-mass spectrometry analysis

All nine prepared extracts representing the combinations of drying treatments and solvent polarity (A1, A2, A3, B1, B2, B3, C1, C2, C3) were analyzed using a GC–MS system (Agilent 7890B GC coupled with a 5977A MSD). Compound separation was performed on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), which is widely used for profiling plant-derived volatile and semi-volatile metabolites due to its low-polarity stationary phase. High-purity helium (99.999%) served as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed with an initial hold at 50 °C for 2 min, followed by a gradual increase to 150 °C at 3 °C/min, and then further raised to 300 °C at 10 °C/min, with a final isothermal hold at 300 °C for 10 min. The injector temperature was maintained at 250 °C, and 1.0 µL of each derivatized sample was introduced in splitless mode to maximize peak sensitivity and metabolite recovery. Mass spectral detection was conducted under electron impact (EI) ionization at 70 eV, and spectra were recorded over an m/z range of 40–550, in accordance with the recommended settings for phytochemical profiling. The resulting chromatograms and spectra were processed using Agilent MassHunter software. Metabolite identification was performed by matching fragmentation patterns and retention indices against the NIST 17 and Wiley digital mass spectral libraries, with a minimum similarity index threshold of 80% considered acceptable for tentative identification. Peak identities showing high similarity scores were further verified by comparing retention behavior and diagnostic ions with the data reported in the literature for structurally related compounds. Relative metabolite abundance was expressed as the percentage of normalized peak area, allowing comparisons across samples and treatments. Each chromatogram was evaluated independently to assess the influence of drying duration and solvent polarity on the qualitative and quantitative distribution of detected metabolites. This analytical workflow ensured comprehensive and reproducible profiling of chemical signatures associated with the nine extract categories.

Statistical analysis by PCA and PLS-DA

GC–MS peak areas were compiled into a data matrix with samples in rows and metabolites in columns, then uploaded to Metabo-Analyst (version 5/6, www.metaboanalyst.ca). Prior to statistical analysis, the features with >20% missing values in any group were removed, and remaining missing values were replaced using the "Replace by a small value" option (half the minimum positive value), as recommended for metabolomics datasets. Peak areas were log-transformed to approximate normality and auto-scaled (mean-centered and divided by the standard deviation of each variable) in the Statistical Analysis (one-factor) module. Unsupervised PCA was first applied to assess overall variation, detect potential outliers, and visualize clustering. Supervised partial least squares–discriminant analysis (PLS-DA) was then used to model class separation among the experimental groups. Metabolites had high variable importance in projection (VIP) scores from the PLS-DA model (VIP > 1.5 or 2, depending on the comparison) were considered key discriminant features and were used to define the metabolite signatures associated with each drying treatment and solvent (Pang et al., 2021).

RESULTS AND DISCUSSION

GC–MS profiling revealed solvent-dependent and drying treatment metabolite diversity

The GC–MS profiling of *Moringa oleifera* leaves extracted demonstrated in Figure 1, sample A1–A3 identified 113 distinct compounds across the three different drying treatments and n-hexane solvents, with profiles overwhelmingly dominated by long-chain aliphatic hydrocarbons (linear and branched alkanes) and a secondary fraction of nonpolar bioactive lipids (tocopherol derivatives, phytosterols, squalane-type molecules) and alkylated aromatics. The alkane-rich signature (C11–C38, numerous methyl-ethyl-substituted homologs) is consistent with the extraction of cuticular waxes and other lipophilic constituents by a nonpolar solvent (Shi et al., 2023).

Short oven drying (A1, 10 h) preserved a broad set of thermally stable hydrophobic metabolites, including sterol and triterpenoid markers, whereas sun drying (A2, 18 h; A3, 27 h) yielded equal or greater diversity of mid-chain and branched alkanes and slightly higher counts of high-molecular-weight hydrocarbons. These observations suggest that the drying regime modulates the partitioning and release of cuticular/waxy fractions: controlled oven drying tends to conserve heavier lipophilic bioactives, while prolonged sun exposure promotes the volatilization of lighter components and the relative enrichment of certain branched alkanes. Such drying-dependent shifts echo recent reports that processing conditions substantially affect leaf lipid and antioxidant composition (Dhakal et al., 2024).

Functionally, the predominance of long-chain alkanes and waxy lipids indicates potential agronomic relevance for bio-based fertilizer development. Plant-derived cuticular waxes and long-chain hydrocarbons can act as slowly available carbon sources and influence soil microbial biomass and enzyme activity when incorporated into soil matrices (Thomas et al., 2021); meanwhile, co-extracted lipophilic antioxidants (tocopherol derivatives) and phytosterols may contribute mild antimicrobial or biostimulant effects that support seedling vigor or stress tolerance. Therefore, n-hexane fractions from oven- and sun-dried *M. oleifera* leaves combine structural carbon (slow-release) with bioactive molecules potentially beneficial in soil amendment contexts (Pareek et al., 2023).

Sample B1–B3 identified 187 distinct compounds across the three different drying treatments and ethanol solvents. The GC–MS profiling revealed a complex metabolome dominated by fatty acids and their esters, long-chain hydrocarbons (cuticular wax components), sterols/triterpenoids, and a complementary fraction of sugars and phenolic derivatives. Oven drying (B1) produced a rich mixture that included numerous high-molecular-weight sterol and triterpenoid markers in addition to abundant fatty-acid derivatives. Sun

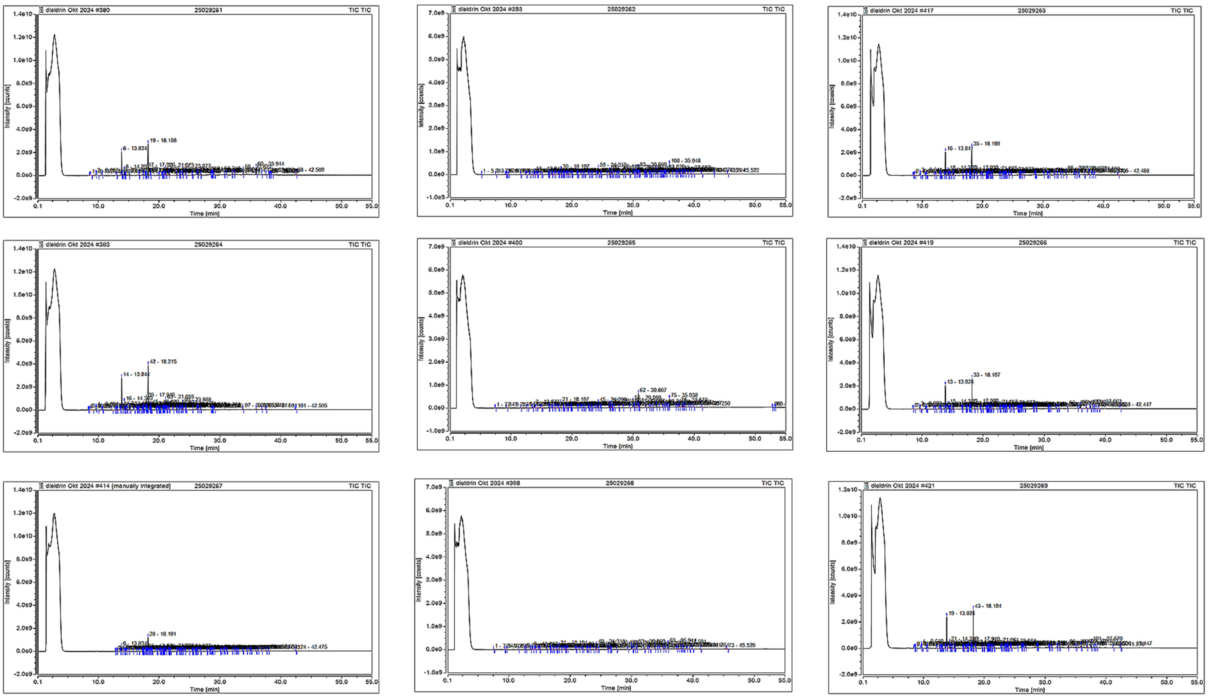


Figure 1. GC-MS metabolite profiles (spectra) of *Moringa oleifera* leaves extracts under different drying treatments and solvents; (a) A1 (oven-dried for 10 h, *n-hexane*), (b) A2 (sun-dried for 18 h, *n-hexane*), (c) A3 (sun-dried for 27 h, *n-hexane*), (d) B1 (oven-dried for 10 h, *ethanol*), (e) B2 (sun-dried for 18 h, *ethanol*), (f) B3 (sun-dried for 27 h, *ethanol*), (g) C1 (oven-dried for 10 h, *ethyl acetate*), (h) C2 (sun-dried for 18 h, *ethyl acetate*), (i) C3 (sun-dried for 27 h, *ethyl acetate*)

drying at 18 h (B2) preserved a broad fatty-acid and terpenoid signature while showing slightly lower overall compound richness compared to B1. Extended sun drying (B3, 27 h) yielded the greatest diversity of lipid-derived metabolites and wax-associated hydrocarbons, consistent with the concentration effects due to water loss and altered matrix extractability.

The predominance of palmitic, linoleic, and linolenic acids and their esters indicates that ethanol effectively extracts both polar and semi-polar lipid pools from *M. oleifera* leaves; these lipids are known contributors to bioactivity and membrane integrity and can serve as labile carbon sources for soil microbiota when applied as amendments. Recent work highlights that ethanol extraction recovers lipidic antioxidants (tocopherols) and sterols from Moringa, supporting the identification of vitamin E-related signals and β-sitosterol in the studied samples (Fitri et al., 2025). Long-chain alkanes and branched hydrocarbons (C11–C38 range) likely reflect cuticular wax components that concentrate during drying; plant cuticular waxes are primarily composed of very-long-chain fatty acids as well as alkanes and have documented persistence and slow turnover in soil matrices, suggesting potential utility as slow-release carbon and physical modifiers when leaf extracts or residues, are used in bio-based fertilizer formulations (Wen et al., 2024). The sterols and triterpenoids identified in the ethanol fractions (e.g., β-sitosterol, β-amyrin, spirostane-type compounds) are consistent with the literature reporting Moringa as a source of membrane sterols and triterpene glycosides; these molecules can function as mild growth stimulants and signaling modulators in soil–plant systems. The coexistence of sugars and glycosides further supports the capacity of the extract to serve as a microbial substrate, potentially enhancing soil microbial biomass and enzymatic activity – effects commonly observed after the application of organic amendments (Sun et al., 2024).

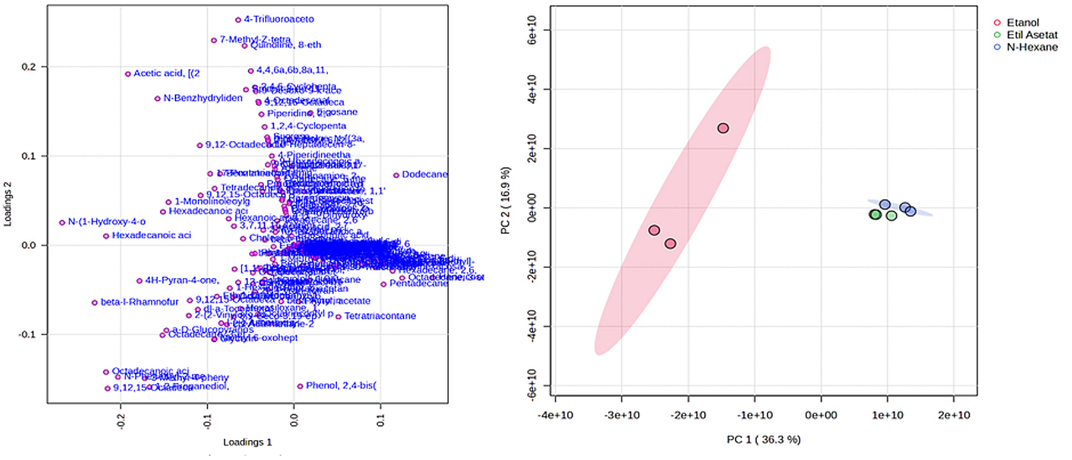
The GC–MS profiling of *Moringa oleifera* leaves extracted with ethyl acetate under three drying regimes (C1: oven-dried 10 h; C2: sun-dried 18 h; C3: sun-dried 27 h) produced a total of 69 unique compound identifications across all samples. Each extract was characterized by a dominance of long-chain aliphatic hydrocarbons (C11–C38), a substantial fraction of fatty acids and their esters, and a recurring presence of sterols, triterpenoids, and phytol-related terpenoids. Minor constituents included substituted phenolics and sugar/ascorbyl esters. Several heavy, highly substituted GC library matches (TMS derivatives and perfluoroesters) were noted and flagged as likely analytical artefacts.

The predominance of long-chain alkanes and methylated homologs is consistent with the extraction of epicuticular wax components: plant cuticular waxes are well established as primary sources of n-alkanes in leaf extracts, and their residues are relatively persistent in soil systems, where they can serve as slowly cycling carbon pools. This transforms the chemical signature of leaf material after drying and can have measurable effects on soil organic matter inputs [27]. The abundant fatty acids and esterified lipids (e.g., palmitic, oleic, linoleic/linolenic derivatives) together with sterols and phytol indicate retention of membrane-derived and chlorophyll-derived lipophilic metabolites in the ethyl acetate fraction. Such lipidic bioactives—particularly tocopherol (Vitamin E) signals and β-sitosterol—are frequently reported in Moringa phytochemical surveys and are associated with antioxidant and potential biostimulant properties (Pop et al., 2022). Functionally, the combined chemical signature suggests clear potential for bio-based fertilizer applications: (i) long-chain hydrocarbons and waxes can contribute structural, slowly degradable carbon; (ii) fatty acids and sugars can serve as labile substrates to stimulate beneficial soil microbiota and increase microbial respiration as well as nutrient cycling; and (iii) sterols, terpenoids and tocopherols may act as mild biostimulants or protective agents that support seedling vigor and stress tolerance. These implications align with broader findings that the organic amendments rich in labile carbon and phytochemicals enhance soil microbial activity and nutrient availability (Wang et al., 2023).

PCA demonstrated clear solvent-based clustering and drying-dependent shifts

PCA of GC–MS metabolite profiles (PC1 = 36.3%, PC2 = 16.9%) revealed a dominant solvent-driven separation: ethanol extracts clustered distinctly on the negative side of PC1, while n-hexane and ethyl acetate extracts grouped on the positive side (Figure 2a). Loadings indicated that this segregation was principally driven by polar/oxygenated metabolites (sugars, ascorbyl esters, and substituted phenolics) that load negatively on PC1 and by long-chain aliphatic hydrocarbons and fatty-acid esters (e.g., dodecane, pentadecane, tritriacontane and related alkyl esters) that load positively. Within-group spread was greater for ethanol extracts, suggesting higher compositional heterogeneity – likely because ethanol (an amphipathic solvent) recovered a broader polarity range of metabolites the abundances of which were sensitive to drying conditions.

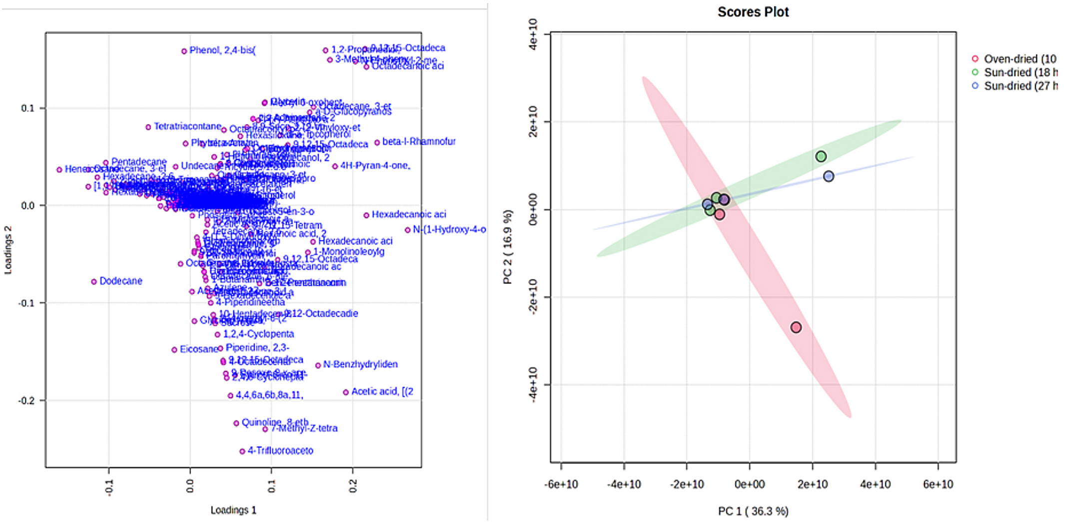
These observations were consistent with the solvent-selectivity effects reported in recent extraction and metabolomics studies (Lee et al., 2024), Ethanol enriches polar glycosides, organic acids, and oxygenated lipids, whereas non-polar solvents concentrate cuticular waxes and



a) Loading Plot

b) Score Plot

Figure 2. Multivariate analysis of metabolite fingerprints of *Moringa oleifera* leaves extracts under different solvents; (a) PCA score plot demonstrated sample classification, (b) loading plot concurrently exhibited the contribution each of metabolites to PCA model



a) Loading Plot

b) Score Plot

Figure 3. Multivariate analysis of metabolite fingerprints of the *Moringa oleifera* leaves extracts under different drying treatments; (a) PCA score plot demonstrated sample classification, (b) loading plot concurrently exhibited the contribution each of metabolites to PCA model

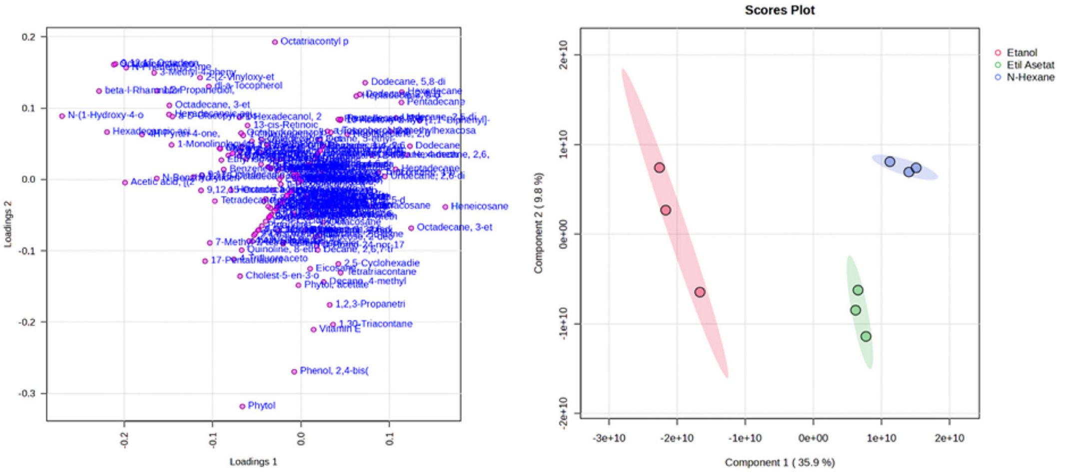
long-chain hydrocarbons. Such solvent-dependent clustering has been repeatedly observed in plant metabolomics and underpins experimental decisions when targeting nutritive against hydrophobic fractions for applications (*e.g.*, biofertilizer formulations where labile C and bioactive polar compounds are desirable vs. waxy, slow-release carbon sources) (Septaningsih et al., 2025).

The PCA model built from Figure 3 explained over 50% of the total variance (PC1 = 36.3%, PC2 = 16.9%). The score plot showed a clear separation among drying treatments. Oven-dried samples clustered toward the positive region of PC1, while sun-dried samples (18 h and 27 h) grouped in the negative PC1 region, forming two distinct yet adjacent clusters. The loading plot indicated that oven-dried samples were associated with metabolites such as long-chain alkanes, oxidized lipid fragments, and heat-derived volatiles, which exhibited high positive loadings. In contrast, compounds such as phytol, sterols, fatty-acyl glycerides, tocopherol derivatives, and other thermolabile metabolites contributed strongly to negative loadings, representing the chemical signatures of sun-dried leaves. These patterns demonstrate that drying temperature and exposure time substantially modify the leaf metabolome, affecting both the abundance and integrity of key bioactive compounds.

The clear clustering along PC1 between oven-dried and sun-dried groups suggests that thermal degradation was the dominant driver of chemical variation. Oven drying often induces substantial breakdown of phenolics, lipids, and volatile constituents, leading to an accumulation of alkanes, short-chain aldehydes, and other oxidation products. Recent studies support this mechanism: high-temperature drying has been shown to rapidly degrade heat-sensitive phytochemicals in aromatic and medicinal leaves, resulting in shifts toward thermally stable or degradation-derived metabolites (Snoussi et al., 2021). For Moringa, recent evaluations demonstrated that sun-drying preserves significantly higher levels of polyphenols, flavonoids, tocopherols, and sterol-related metabolites compared with oven-drying, due to reduced thermal stress (Rohit et al., 2024). These observations align precisely with the PCA loadings in this study, where sun-dried samples retained the profile of complex lipophilic and antioxidant metabolites.

PLS-DA identified discriminant metabolites and validated treatment separation

The supervised PLS-DA model based on GC–MS metabolite profiles from Figure 4 revealed three clearly separated clusters corresponding to ethanol, ethyl acetate, and n-hexane as solvents, demonstrating that solvent polarity strongly shaped the metabolite composition of the *Moringa oleifera* leaves and indicating that solvent polarity significantly influenced the metabolome captured by GC–MS. Similar solvent-driven clustering patterns have been reported in other plant metabolomics studies using GC–MS



a) Loading Plot

b) Score Plot

Figure 4. Multivariate analysis of metabolite fingerprints of the *Moringa oleifera* leaves extracts under different solvents; (a) PLS-DA score plot demonstrated sample classification, (b) loading plot concurrently exhibited the contribution of each metabolite to the PLS-DA model

combined with chemometrics (Leite et al., 2025). Components 1 (35.9%) and 2 (9.8%) together differentiated the samples based on their chemical signatures, with ethanol at the far negative end of Component 1, ethyl acetate in the mid-positive zone, and n-hexane in the highest positive region.

This clear discrimination indicated robust solvent-dependent shifts in metabolite abundance, consistent with the recent multivariate metabolomics studies reporting that extraction polarity is a primary driver of sample segregation in PLS-DA space. Interpretation of the loading plot supports this separation, showed that the n-hexane cluster is associated with metabolites positioned at the far positive side of component 1 – dominated by long-chain alkanes (e.g., heneicosane, octadecane), fatty acids, sterols (e.g., cholest-5-en-3-ol), and hydrophobic terpenoid derivatives. These compounds characteristically accumulated in non-polar fractions due to their high hydrophobicity, as demonstrated in recent plant lipidomics work, where hexane selectively enriched cuticular waxes, saturated fatty acids, and sterols.

The separation suggests that polar and semi-polar metabolites are predominantly extracted by more polar solvents (e.g., ethanol), while non-polar, lipophilic compounds are enriched in non-polar solvent extracts (e.g., n-hexane). This observation aligns with the general principles of phytochemical extraction and has been observed in several studies of medicinal plants (Bhala et al., 2021). This differentiation has strong implications for the design of extraction protocols depending on the target class of bioactive compounds: for example, choosing ethanol (or another polar solvent) when the aim is to recover polar antioxidants or phenolics, whereas n-hexane (or other non-polar solvents) may be preferred for lipids, alkanes, or other hydrophobic constituents. Similar conclusions were drawn in a recent work on solvent-driven variation in extracts of *Moringa oleifera* leaves (Ramanathan et al., 2025).

The Variable Importance in Projection (VIP) plot (Figure 5) highlights the metabolites that contributed most strongly to the discrimination among the ethanol, ethyl acetate, and n-hexane extracts of *Moringa oleifera*. A VIP value greater than 1.0 is typically considered influential, whereas metabolites with VIP > 2.0 are regarded as highly discriminant in PLS-DA modeling. In this dataset, all top-ranked metabolites had VIP values exceeding 2.0, indicated robust separation driven by solvent-dependent chemical composition.

The compound with the highest VIP score—annotated as N-(1-Hydroxy-4-β-L-Rhamnofuranosyl-) – showed markedly higher intensity in the ethanol extract compared to the other solvents. This molecule was likely a polar glycosylated derivative, and its strong association with ethanol was consistent with the well-known ability of polar protic solvents to extract glycosides, sugar-linked phytochemicals, and polar oxygenated metabolites. Previous studies also reported that ethanol efficiently extracts polar phytochemicals from *M. oleifera*, including glycosylated compounds and polar lipids, which display strong discrimination in multivariate models (Enerjiofi et al., 2021). Similarly, several fatty-acid-derived

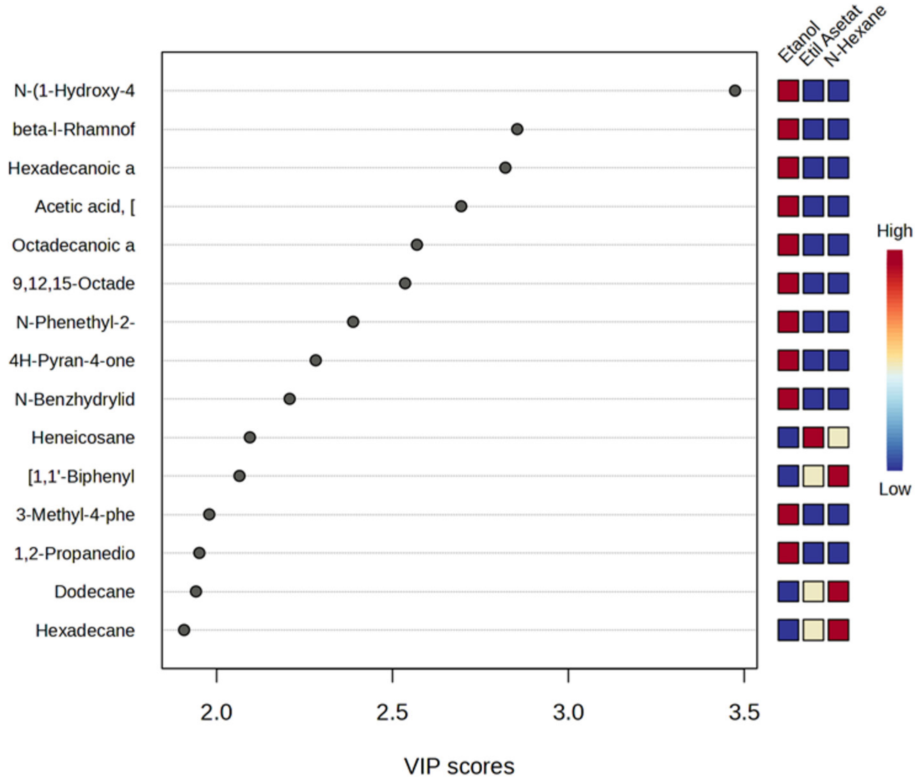
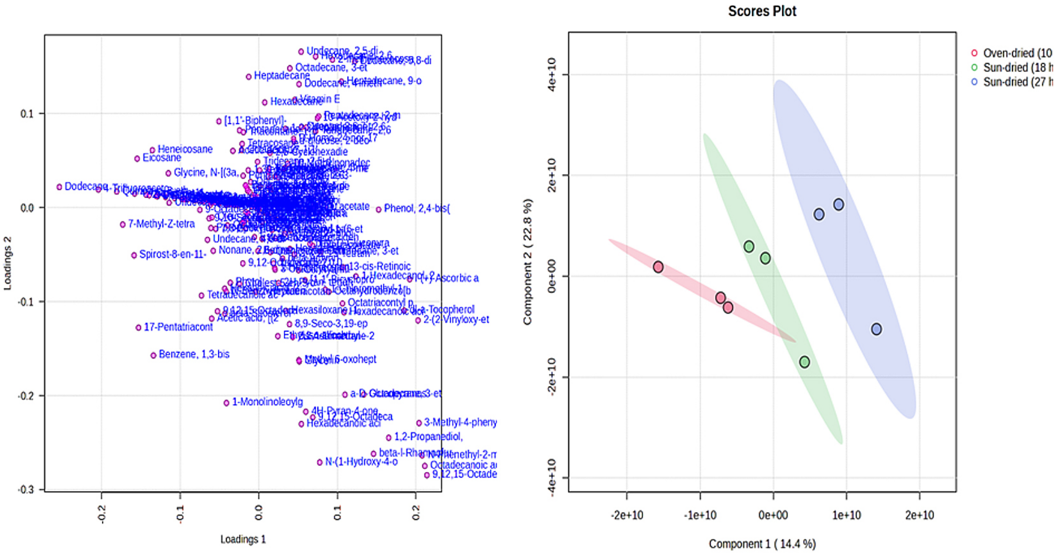


Figure 5. VIP score plot of PLS-DA of metabolite fingerprints from *Moringa oleifera* leaves extracts under different solvents showed the importance of the metabolites obtained in the positive mode and the contribution in the PLS-DA model

metabolites (e.g., hexadecanoic acid, octadecanoic acid, and various polar lipid esters) also showed high VIP values and were enriched in ethanol extracts, indicating the ability of the solvent to extract medium- to high-polarity lipids and oxygenated fatty acids. This aligns with findings in other plant metabolomics studies where ethanol extraction yielded oxygenated lipids and fatty acid derivatives predominantly in polar extracts (e.g., studies using GC–MS for leaf or seed extracts) (Wulandari et al., 2024). The distribution pattern seen in the heatmap – a strong signal in ethyl acetate, moderate in ethanol, and virtually absent in n-hexane – further supports the interpretation of these compounds as selective chemical markers of the ethyl acetate fraction. Similar selectivity has been demonstrated in recent phytochemical studies, where semi-polar extracts yielded phenolic, flavonoid, or alkaloid-like metabolites that were not present in non-polar fractions (Chatepa et al., 2024; Nguyen et al.,2025).

The PLS-DA score plot (Figure 6) revealed a clear separation among oven-dried (10 h), sun-dried (18 h), and extended sun-dried (27 h) samples, indicated that drying conditions significantly modulate the chemical signatures of the *Moringa oleifera* leaves. The oven-dried samples formed a tight cluster, suggesting reduced metabolic variability due to controlled temperature and minimal oxidative exposure. In contrast, prolonged sun drying (27 h) produced the most distinct metabolic profile, consistent with the well-documented impact of sunlight and temperature fluctuations on phytochemical degradation and oxidation in leafy vegetables. This agreed with the recent studies showing that drying intensity strongly influences the retention, transformation, or loss of bioactive metabolites in the *Moringa oleifera* leaves (Rohit et al., 2024).

The PLS-DA loadings plot showed that oven-dried samples clustered with oxygenated fatty acids and low-volatility organic acids – compounds that were better preserved under thermal but light-protected conditions. Moderate sun drying (18 h) aligned with semi-volatile aromatic and ester-type metabolites, reflecting partial oxidation and light-induced transformations. Extended sun drying (27 h) exhibited strong associations with long-chain alkanes (e.g., heneicosane, octadecane, hexadecane) and wax-derived compounds. These non-polar hydrocarbons are known to increase when more unstable metabolites degrade



a) Loading Plot b) Score Plot

Figure 6. Multivariate analysis of metabolite fingerprints of the *Moringa oleifera* leaves extracted under different drying treatments; (a) PLS-DA score plot demonstrated sample classification, (b) loading plot concurrently exhibited the contribution each of metabolites to PLS-DA model

through photolysis or auto-oxidation during prolonged sunlight exposure. Similar metabolite patterns have been reported in drying-related wax composition studies of leafy plant matrices (Khaled et al., 2024). The VIP plot (Figure 7) highlights several metabolites with VIP > 2.0 that strongly contribute to distinguishing oven-dried (10 h), sun-dried (18 h), and extended sun-dried (27 h) treatments. The highest-ranking metabolites – Dodecane and Benzene, 1,3-bis (1,1-dimethylethyl) – were particularly elevated in the extended sun-dried (27 h) samples,

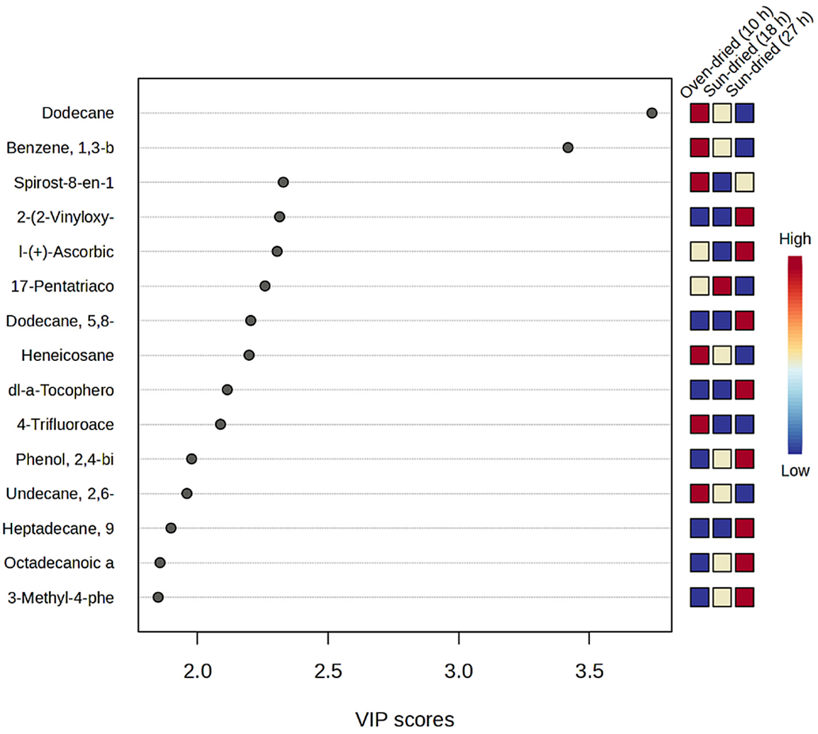


Figure 7. VIP score plot of PLS-DA of metabolite fingerprints from the *Moringa oleifera* leaves extracted under different drying treatments shows the importance of the metabolites obtained in the positive mode and the contribution in the PLS-DA model

suggesting that prolonged exposure to sunlight and heat intensifies the accumulation of non-polar hydrocarbons and aromatic transformation products (Kalalagh et al., 2023). Such increases in hydrocarbon signatures under extended sun exposure have been observed in drying studies where photodegradation preferentially removed labile metabolites while enriching wax-derived alkanes. Compounds such as Spirost-8-en-1-one, 2-(2-Vinyloxy)-l-(+)-Ascorbic derivative, and 17-Pentatriacontene showed stronger intensities in the sun-dried (18 h) samples. These metabolites typically arise from partial oxidation, semi-volatile terpene transformation, and UV-induced rearrangements, reflecting intermediate levels of thermal and photochemical stress. Moderate sun drying exposes leaves to enough heat and UV to induce biochemical transformations, but not as extensively as prolonged drying. This aligns with the studies showing that mid-duration sun drying preserved some semi-polar metabolites while initiating early oxidation steps (Rohit et al., 2024). The VIP profile showed that oven-dried samples were relatively enriched in polar bioactive compounds such as dl-α-Tocopherol (Vitamin E), Phenol derivatives, and oxygenated lipids such as Octadecanoic acid. Controlled oven drying protects antioxidant vitamins and phenolics by limiting photo-oxidation, leading to better preservation of nutrient-related metabolites, which are important for soil microbial stimulation in biofertilizers (Pareek et al., 2023).

The GC–MS profiling of the *Moringa oleifera* leaf extracts under different drying and solvent treatments revealed diverse chemical signatures with distinct ecological implications for bio-based fertilizer applications. On the basis of the integrated metabolome characterization and multivariate analyses (PCA and PLS-DA), key metabolite classes were classified into primary ecological contributors and secondary supporting compounds. Primary ecological metabolites include long-chain aliphatic hydrocarbons, waxy lipids, fatty acids, and their esters, which were consistently abundant across both non-polar and semi-polar solvent extracts. Long-chain hydrocarbons and cuticular wax components (e.g., C11–C38 alkanes and branched homologs) represent relatively recalcitrant carbon pools that contribute slow-release structural carbon, potentially augmenting soil organic carbon and supporting soil microbial processes over extended periods. Organic amendments rich in carbonaceous fractions are widely reported to enhance soil structure and microbial biomass, thereby improving nutrient cycling and soil fertility in agroecosystems. In addition, fatty acids and their esters, particularly those dominant in ethanol extracts (e.g., palmitic, linoleic, and linolenic acids), serve as labile carbon sources that can be readily metabolized by soil microbiota, collectively stimulating microbial activity, respiration, and enzymatic functions that underpin nutrient turnover. Organic carbon inputs from plant-derived lipids and labile compounds have been shown to energize soil microbial communities, thereby enhancing soil organic matter decomposition and nutrient mineralization (Xu et al., 2025).

Secondary metabolites, such as sterols, triterpenoids, tocopherol derivatives, and minor phenolic constituents, while not primary nutrient sources, are considered supporting ecological actors owing to their potential roles in modulating soil biological interactions and stress responses. Sterols and terpenoid-related compounds can function as mild biostimulants or signaling molecules that influence microbial composition and plant stress resilience, albeit indirectly relative to carbon substrate provision. Similar observations have been made in the studies reporting that bio-amendments containing a complex suite of lipophilic and phenolic compounds can alter soil microbial communities and enhance soil fertility beyond simple carbon addition (Li et al., 2025).

The drying and extraction strategies investigated in this study are compatible with adaptation to pilot and industrial scale processing while maintaining considerations of energy efficiency and environmental sustainability. Sun drying and low-temperature oven drying (≤50 °C) represent scalable and practical approaches. Sun drying is inherently energy-efficient and particularly suitable for tropical regions, where solar or solar-assisted drying systems can be implemented with minimal operational energy input. Low-temperature oven drying, as applied here, can be readily translated into industrial tray or conveyor dryers, offering controlled and reproducible processing with moderate energy demand. From an extraction perspective, the use of ethanol and ethyl acetate aligns well with green-chemistry and industrial practices. Ethanol is a renewable, food-grade solvent with established large-scale extraction and solvent-recovery systems, allowing efficient recycling and reduced environmental footprint. Ethyl acetate is similarly regarded as a relatively benign and biodegradable solvent. In contrast, n-hexane is best considered a selective reference solvent in this study for recovering non-polar waxy fractions; its industrial application would require strict solvent-recovery and emission-control measures or substitution with greener alternatives. Importantly, the solvent-dependent metabolite separation observed in PCA and PLS-DA provides a rational basis for process optimization at scale, enabling targeted recovery of either labile bioactive compounds or slow-release carbon fractions depending on the intended fertilizer application.

Despite the comprehensive GC–MS profiling and multivariate analysis, several limitations of this study should be acknowledged. First, metabolite identification was based on GC–MS library matching and therefore represents putative annotations, particularly for complex lipidic, wax-derived, and highly substituted compounds; non-volatile and thermolabile metabolites are likely underrepresented due to the intrinsic analytical scope of GC–MS. Second, while PCA and PLS-DA enabled robust discrimination of drying and solvent effects and supported functional interpretation of metabolite groups, the ecological roles of these metabolites were inferred from chemical composition and literature evidence, rather than direct biological validation. No soil incubation, microbial activity assays, or plant growth experiments were conducted to confirm fertilizer performance. Third, the discussion on scalability, energy demand, and environmental implications of drying and extraction strategies remains conceptual, as no pilot-scale trials or life cycle assessments were performed. Finally, the study focused on leaf material from a single plant source, without evaluating variability associated with genotype, cultivation conditions, or seasonal effects. Future work integrating complementary platforms (e.g., LC–MS-based metabolomics), biological assays, and pilot-scale evaluations will be essential to substantiate and extend the ecological relevance of the present findings.

CONCLUSIONS

This study demonstrated that solvent polarity and drying regimes exerted strong and systematic influences on the GC–MS metabolite composition of *Moringa oleifera* leaves. Non-polar n-hexane consistently enriched long-chain aliphatic hydrocarbons, wax-derived lipids, tocopherol-related molecules, and sterols, confirming efficient extraction of hydrophobic metabolites. Ethanol yielded the broadest chemical diversity, dominated by fatty acids, oxygenated lipids, glycosides, sugars and phenolic derivatives, whereas ethyl acetate selectively recovered semi-polar metabolites including aromatic ketones, lactone-type compounds, phenethyl derivatives, and mid-polarity lipid fragments. These solvent-dependent signatures were strongly supported by PCA and PLS-DA, which produced clear cluster separation driven by hydrophobic vs. polar/oxygenated metabolite loadings.

Drying treatments also significantly altered chemical profiles. Oven drying preserved thermolabile antioxidants, sterols, and oxygenated lipids, while moderate sun drying induced partial oxidation and formation of semi-volatile metabolites. Extended sun drying led to strong enrichment of long-chain alkanes and wax-derived hydrocarbons through photolytic degradation of labile compounds. These trends were confirmed by VIP-ranked discriminant metabolites, which highlighted tocopherol derivatives as markers of oven-dried samples, semi-oxidized terpenoids for moderate sun drying, and dominant non-polar hydrocarbons for prolonged exposure.

Collectively, the chemical signatures indicated that the *M. oleifera* extracts – regardless of solvent – contain metabolite classes relevant for bio-based fertilizer applications. Long-chain hydrocarbons and wax lipids provide slowly decomposing carbon pools, while fatty acids, sugars, and oxygenated lipids provide labile substrates that can stimulate beneficial soil microbiota. Antioxidants, sterols, and terpenoids may further contribute mild biostimulant effects. These findings highlight the potential to customize extraction and drying strategies to optimize functional metabolite profiles for specific agricultural applications such as slow-release carbon inputs, microbially responsive amendments, or antioxidant-enriched biofertilizer formulations.

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