STUDI KOMPARATIF PROFIL METABOLIT SEKUNDER BAYAM MERAH (*Amaranthus tricolor* L.) KONVENSIONAL DAN HIDROPONIK MENGGUNAKAN SPEKTROSKOPI, LC-HRMS, DAN KEMOMETRIK UNTUK IDENTIFIKASI SENYAWA PENANDA KIMIA

DISERTASI

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RINGKASAN

Bayam merah (*Amaranthus tricolor* L.) merupakan sayuran yang kaya metabolit sekunder seperti fenolik, flavonoid, betalain, serta berbagai lipid dan senyawa teroksigenasi lain yang berperan dalam karakter kimia dan potensi bioaktivitasnya, terutama sebagai antioksidan alami. Komposisi metabolit pada tanaman ini tidak bersifat tetap, melainkan dipengaruhi oleh faktor lingkungan, termasuk sistem budidaya. Perbedaan antara sistem konvensional berbasis tanah dan sistem hidroponik terutama terletak pada ketersediaan nutrisi, stabilitas pH, aerasi akar, serta interaksi mikrobiologis media, yang semuanya dapat memengaruhi jalur biosintesis metabolit sekunder. Namun, pendekatan analitik konvensional seperti total fenolik (TPC), total flavonoid (TFC), total betalain, dan ukur aktivitas antioksidan hanya memberikan gambaran agregat tanpa mengungkap komposisi senyawa secara spesifik. Oleh karena itu, penelitian ini bertujuan membandingkan profil kimia bayam merah dari kedua sistem budidaya menggunakan pendekatan multi-platform (UV-Visible, FTIR, dan LC-HRMS) yang dipadukan dengan kemometrik, serta mengidentifikasi kandidat senyawa penanda secara tentatif dan mengeksplorasi indikasi keterkaitan metabolisme secara eksploratif.

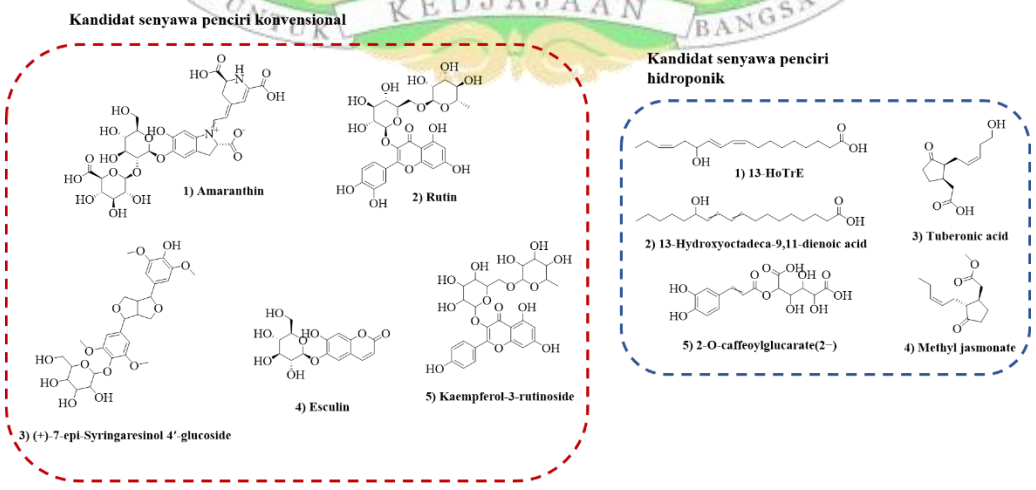
Sampel bayam merah varietas Mira dibudidayakan di Padang, Sumatera Barat, pada sistem konvensional berbasis tanah-kompos dan sistem hidroponik dengan nutrisi AB mix. Panen dilakukan pada umur 25–27 hari, kemudian sampel dikeringkan menggunakan *freeze dryer* dan dihaluskan sebelum analisis. Pemrofilan kimia dilakukan menggunakan spektroskopi UV-Visible (200–800 nm), ATR-FTIR (4000–400 cm^{-1}), serta UHPLC-Orbitrap-HRMS dalam mode ionisasi positif dan negatif. Data dianalisis menggunakan PCA, PLS-DA, OPLS-DA, *volcano plot*, dan VIP score. LC-HRMS menghasilkan 198 anotasi awal yang setelah kurasi menjadi 149 senyawa nonduplikat. Analisis kelas senyawa dan pengayaan jalur dilakukan secara eksploratif. Parameter TPC, TFC, total betalain, dan aktivitas antioksidan DPPH diukur dengan tiga replikasi teknis, sedangkan spektroskopi dan LC-HRMS menggunakan lima replikasi teknis. Integrasi data dilakukan berdasarkan kesesuaian arah perubahan antarplatform dan evaluasi kestabilan data menggunakan RSD, tanpa mengasumsikan korelasi biologis antarulangan.

Secara morfologis, tanaman hidroponik menunjukkan pertumbuhan lebih tinggi dan daun lebih besar dibandingkan konvensional. Spektrum UV-Visible kedua kelompok menunjukkan pola serupa dengan perbedaan intensitas pada 264,5–327,5 nm (fenolik dan flavonoid) serta sekitar 538 nm (betalain), dengan intensitas relatif lebih tinggi pada sampel konvensional. Model PLS-DA UV-Vis menunjukkan pemisahan kelompok dengan $R^2 = 1$, $Q^2 = 1$, dan $p = 0{,}010$. Spektrum FTIR juga menunjukkan kesamaan gugus fungsi utama dengan variasi intensitas pada daerah O–H/N–H, C–H, karbonil, aromatik, dan fingerprint region. Model OPLS-DA FTIR menghasilkan $R^2 = 1$, $Q^2 = 0{,}99$, dan $p = 0{,}05$. Pada LC-HRMS, senyawa yang teridentifikasi didominasi lipid dan lipid-like molecules (47,7%) serta fenilpropanoid dan poliketida (16,8%). Model OPLS-DA LC-HRMS menunjukkan pemisahan yang baik dengan $R^2 = 0{,}982$, $Q^2 = 0{,}972$, dan $p = 0{,}007$. Sebanyak 91 senyawa memiliki VIP > 1, dengan 54 senyawa lebih tinggi pada konvensional dan 39 pada hidroponik. Karena seluruh data berasal dari replikasi teknis, hasil ini terutama mencerminkan konsistensi analitik, bukan variasi biologis populasi.

Hasil kuantitatif menunjukkan pola yang tidak seragam antarparameter. TPC lebih tinggi pada hidroponik (81,66 mg GAE/g berat kering) dibandingkan konvensional (39,51 mg GAE/g berat kering). Sebaliknya, TFC, total betalain, dan aktivitas antioksidan DPPH lebih tinggi pada sampel konvensional, dengan IC_{50} lebih rendah (562,06 µg/mL) dibandingkan hidroponik (709,20 µg/mL). Hal ini menunjukkan bahwa arah perubahan total fenolik tidak diikuti dengan aktivitas antioksidan yang lebih kuat, sehingga komposisi spesifik metabolit kemungkinan lebih menentukan. Analisis pengayaan menunjukkan dominasi *prenol lipids* dan *fatty acyls* dengan signifikansi FDR < 0,001. Pada tingkat jalur, biosintesis flavon dan flavonol menunjukkan sinyal nominal, tetapi tidak signifikan setelah koreksi FDR, sehingga hanya dianggap indikatif secara eksploratif.

Integrasi VIP, *volcano plot*, konsistensi arah perubahan, RSD, dan hasil pengayaan menghasilkan 65 kandidat senyawa penanda tentatif, terdiri dari 36 senyawa dominan pada konvensional dan 29 pada hidroponik. Senyawa kunci pada konvensional meliputi amaranthin, rutin, (+)-7-epi-syringaresinol 4′-glucoside, esculin, dan kaempferol-3-rutinoside, sedangkan hidroponik didominasi 13-HoTrE, 13-hydroxyoctadeca-9,11-dienoic acid, tuberonic acid, methyl jasmonate, dan 2-O-caffeoylglucarate (2−). Pola ini mengindikasikan adanya perbedaan konfigurasi metabolit sekunder antara kedua sistem budidaya, terutama pada kelompok flavonoid, betalain, lipid, dan turunan sinyal lipid.

Secara keseluruhan, pendekatan *multi-platform* yang dikombinasikan dengan kemometrik mampu membedakan profil kimia bayam merah dari sistem konvensional dan hidroponik secara konsisten pada tingkat analitik, serta menghasilkan kandidat senyawa penanda yang dapat digunakan sebagai dasar studi lanjutan. Namun, keterbatasan utama penelitian ini adalah penggunaan replikasi teknis dan belum adanya validasi biologis lintas lokasi dan waktu, sehingga generalisasi pada tingkat populasi masih terbatas. Selain itu, anotasi metabolit masih bersifat tentatif dan interpretasi jalur metabolisme belum menunjukkan bukti fungsional langsung. Penelitian lanjutan diperlukan dengan replikasi biologis, validasi standar autentik, serta analisis kuantitatif tertarget untuk memperkuat temuan dan mendukung pengembangan parameter mutu bahan baku bayam merah berbasis profil metabolit.



Kata kunci: *Amaranthus tricolor* L.; Pemrofilan Metabolit Eksploratif; Kandidat Senyawa Penanda; Analisis Kemometrik

SUMMARY

Red spinach (*Amaranthus tricolor* L.) is a leafy vegetable rich in secondary metabolites such as phenolics, flavonoids, betalains, as well as various lipids and other oxygenated compounds that contribute to its chemical characteristics and potential bioactivity, particularly as a natural antioxidant. The metabolite composition of this plant is not fixed but is influenced by environmental factors, including cultivation systems. The differences between conventional soil-based and hydroponic systems lie primarily in nutrient availability, pH stability, root aeration, and microbial interactions in the growth medium, all of which may affect secondary metabolite biosynthetic pathways. However, conventional analytical approaches such as total phenolic content (TPC), total flavonoid content (TFC), total betalain content, and antioxidant assays only provide aggregate information without revealing specific compound-level composition. Therefore, this study aimed to compare the chemical profiles of red spinach from both cultivation systems using a multi-platform approach (UV-Visible, FTIR, and LC-HRMS) combined with chemometrics, as well as to tentatively identify marker compound candidates and explore possible metabolic associations in an exploratory manner.

Red spinach (cv. Mira) was cultivated in Padang, West Sumatra, under a conventional soil–compost system and a hydroponic system using AB-mix nutrients. Harvesting was conducted at 25–27 days after planting, after which samples were freeze-dried and ground prior to analysis. Chemical profiling was performed using UV-Visible spectroscopy (200–800 nm), ATR-FTIR (4000–400 cm^{-1}), and UHPLC-Orbitrap-HRMS in both positive and negative ionization modes. Data were analyzed using PCA, PLS-DA, OPLS-DA, volcano plots, and VIP scores. LC-HRMS initially generated 198 annotations, which were curated into 149 non-duplicate compounds. Compound class analysis and pathway enrichment were conducted in an exploratory manner. TPC, TFC, total betalain content, and DPPH antioxidant activity were measured in three technical replicates, while spectroscopic and LC-HRMS analyses were performed in five technical replicates. Data integration was based on consistency of directional changes across platforms and assessment of data stability using RSD, without assuming biological correlation among replicates.

Morphologically, hydroponically grown plants showed greater height and larger leaves compared to conventionally grown plants. UV-Visible spectra of both groups exhibited similar patterns with differences in intensity at 264.5–327.5 nm (phenolics and flavonoids) and around 538 nm (betalains), with relatively higher absorbance observed in conventional samples. The UV-Vis PLS-DA model showed clear group separation with $R^2 = 1$, $Q^2 = 1$, and $p = 0.010$. FTIR spectra also showed similar major functional groups with variations in intensity across O–H/N–H, C–H, carbonyl, aromatic, and fingerprint regions. The FTIR OPLS-DA model yielded $R^2 = 1$, $Q^2 = 0.99$, and $p = 0.05$. In LC-HRMS analysis, identified compounds were dominated by lipids and lipid-like molecules (47.7%), followed by phenylpropanoids and polyketides (16.8%). The LC-HRMS OPLS-DA model showed strong separation with $R^2 = 0.982$, $Q^2 = 0.972$, and $p = 0.007$. A total of 91 compounds had VIP > 1, with 54 compounds higher in conventional samples and 39 higher in hydroponic samples.

Since all datasets were derived from technical replicates, these results primarily reflect analytical consistency rather than true biological population variability.

Quantitative results showed non-uniform patterns across parameters. TPC was higher in hydroponic samples (81.66 mg GAE/g DW) compared to conventional samples (39.51 mg GAE/g DW). In contrast, TFC, total betalain content, and DPPH antioxidant activity were higher in conventional samples, with a lower IC_{50} value (562.06 µg/mL) compared to hydroponic samples (709.20 µg/mL). These findings indicate that total phenolic content did not show the same direction of change as DPPH antioxidant activity, suggesting that specific metabolite composition may play a more decisive role. Enrichment analysis indicated a strong representation of prenol lipids and fatty acyls with FDR < 0.001. At the pathway level, flavone and flavonol biosynthesis showed a nominal signal but was not significant after FDR correction, and was therefore considered only as exploratory evidence.

Integration of VIP scores, volcano plot results, directional consistency, RSD values, and enrichment analysis yielded 65 putative marker compounds, consisting of 36 compounds associated with conventional samples and 29 associated with hydroponic samples. Key compounds in conventional samples included amaranthin, rutin, (+)-7-epi-syringaresinol 4′-glucoside, esculin, and kaempferol-3-rutinoside, whereas hydroponic samples were characterized by 13-HoTrE, 13-hydroxyoctadeca-9,11-dienoic acid, tuberonic acid, methyl jasmonate, and 2-O-caffeoylglucarate (2−). These patterns suggest differences in secondary metabolite configurations between the two cultivation systems, particularly in flavonoids, betalains, lipids, and lipid-derived signaling compounds.

Overall, the multi-platform approach combined with chemometric analysis successfully discriminated the chemical profiles of red spinach grown under conventional and hydroponic systems at the analytical level and generated tentative marker compounds for further investigation. However, the main limitation of this study is the exclusive use of technical replicates and the absence of multi-location and time-based biological validation, which limits population-level generalization. In addition, metabolite annotations remain tentative, and pathway interpretations do not yet provide direct functional evidence. Future studies should incorporate biological replicates, authentic standard validation, and targeted quantitative analyses to strengthen these findings and support the development of metabolite-based quality parameters for red spinach raw materials.

Keywords: *Amaranthus tricolor* L.; Exploratory Metabolite Profiling; Putative Biomarkers; Chemometric Analysis