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Metabolomics of leafy vegetables: A systematic review of bioactive effects and factors influencing metabolic variation

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Data analysis.

Conflicts of interest - Chew, FT reports grants from the National University of Singapore, Singapore Ministry of Education Academic Research Fund, Singapore Immunology Network, National Medical Research Council (NMRC) (Singapore), Biomedical Research Council (BMRC) (Singapore), National Research Foundation (NRF) (Singapore), Singapore Food Agency (SFA), Singapore's Economic Development Board (EDB), and the Agency for Science Technology and Research (A*STAR) (Singapore), during the conduct of the study; and consulting fees from Sime Darby Technology Centre; First Resources Ltd; Genting Plantation, Olam International, Musim Mas, and Syngenta Crop Protection, outside the submitted work. The other authors declare no other competing interests.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 3 September 2025 and was last updated on 3 September 2025.

INTRODUCTION

Review question / Objective 1) To examine research trends in leafy vegetable metabolomics and identify the most studied species and families.

2) To comprehensively review the metabolites reported across existing studies.

3) To summarize the reported bioactive effects of leafy vegetables as validated through experimental evidence.

4) To evaluate the intrinsic and extrinsic factors contributing to metabolic variation in leafy vegetables.
5) To identify underexploited metabolites in leafy vegetables.

Rationale Leafy vegetables are a vital component of the human diet, providing essential nutrients, dietary fiber, and a diverse array of bioactive compounds. Beyond their culinary versatility, species such as arugula (Eruca sativa), kale (Brassica oleracea var. acephala), and lettuce (Lactuca sativa) have attracted increasing attention for their potential health-promoting properties. These benefits are largely attributed to their rich metabolite content, encompassing both primary and secondary metabolites, which contribute to nutritional quality, flavor, and functional properties.
Understanding the metabolite composition of leafy vegetables is important across multiple domains, including nutrition, agriculture, and market value. From a nutritional perspective, bioactive compounds such as carotenoids, polyphenols, and glucosinolates are associated with reduced risk of chronic diseases, including cardiovascular disorders and cancer. Agriculturally, metabolites influence plant growth, development, and stress responses. Comprehensive metabolite profiling provides insights into biochemical pathways that regulate flavor, nutritional content, and shelf-life—traits that directly impact commercial value and consumer preference.
Advancements in analytical technologies, particularly liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy, have enabled detailed metabolomic studies across diverse leafy vegetable species and cultivars. In addition, liquid chromatography coupled with UV/diode array detection (LC-UV/DAD) remains widely applied for the targeted analysis of phenolic compounds and other metabolites with characteristic UV absorbance. Together, these technologies allow precise identification and quantification of metabolites, paving the way for metabolite-assisted crop improvement, functional food development, and evidence-based recommendations for dietary intake.
Despite the growing body of research, studies remain scattered across species, platforms, and experimental conditions. Moreover, the extent to which intrinsic factors (such as genotype differences, gene expression, and enzymatic activity) and extrinsic factors (including environmental conditions and post-harvest handling) influence metabolite accumulation and bioactive potential is not comprehensively synthesized. Addressing these gaps is essential to consolidate current knowledge and guide future research.

Condition being studied The condition being studied is the metabolite composition of leafy vegetables under different intrinsic (e.g., genotypic, molecular) and extrinsic (e.g., environmental, post-harvest) influences.

METHODS

Search strategy 1) Scopus database: "(TITLE-ABS-KEY (leafy* AND vegetable* AND metabol*) AND NOT TITLE-ABS-KEY (*soil* OR drug* OR animal* OR arabidopsis* OR bacter* OR root* OR egg* OR obesity* OR fruit* OR expression* OR enzymology* OR diet* OR protein* OR gene*)) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))"
2) Web of Science database: "(TITLE-ABS-KEY (leafy* AND vegetable* AND metabol*) NOT TITLE-ABS-KEY (*soil* OR drug* OR animal* OR arabidopsis* OR bacter* OR root* OR egg* OR obesity* OR fruit* OR expression* OR enzymology* OR diet* OR protein* OR gene*)) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))"
3) BIOSIS database: "(TITLE-ABS-KEY (leafy* AND vegetable* AND metabol*) NOT TITLE-ABS-KEY (*soil* OR drug* OR animal* OR arabidopsis* OR bacter* OR root* OR egg* OR obesity* OR fruit* OR expression* OR enzymology* OR diet* OR protein* OR gene*)) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))".

Participant or population Leafy vegetables comprise leafy greens, Brassica leafy vegetables, leafy aquatic vegetables, and baby leaves, all of which are consumed exclusively for their leaves or aerial parts.

Intervention NA.

Comparator NA.

Study designs to be included This review will include experimental and observational studies reporting metabolite profiling or screening in leafy vegetables. Only research articles with identifiable metabolomics data will be considered, while reviews, meta-analyses, and studies lacking primary data will be excluded.

Eligibility criteria Inclusion Criteria:
1) Studies evaluating metabolites in leafy vegetables.

2) Studies reporting metabolite profiling or screening.
3) The experimental target is clearly defined as part of leafy vegetables.
4) Full-text articles published in English.

Exclusion Criteria:
1) Studies without metabolite profiling or chemical analysis.
2) Review articles, systematic reviews, or meta-analyses.
3) Studies that did not provide, or only partially provided, the analytical methods used.
4) Studies where the aerial parts of leafy vegetables were not collected as samples.
5) Full-text articles that could not be retrieved.

Information sources Electronic databases including Web of Science, Scopus, and BIOSIS will be searched to identify relevant studies. Additional sources may include reference lists of included articles and relevant review papers. Grey literature will not be specifically searched, as the focus is on peer-reviewed journal articles.

Main outcome(s) 1) Comprehensive overview of publication trends in leafy vegetable metabolomics, including yearly frequencies from November 1997 to January 2025, and identification of the most frequently studied species and families.
2) A classification and statistical summary of all reported metabolites, organized into six major groups for primary metabolites (amino acids, carbohydrates, fatty acids, nucleotides, organic acids, and vitamins) and four major groups for secondary metabolites (nitrogen- and sulfur-containing compounds, phenolic compounds, terpenoids, and tetrapyrroles).
3) Synthesis of validated bioactive effects identified across leafy vegetables.
4) Identification of intrinsic factors (e.g., genotype differences, gene expression) and extrinsic factors (e.g., environmental conditions, post-harvest handling) that contribute to metabolic variation.

Additional outcome(s) Highlights of under-explored metabolites in leafy vegetables.

Data management All records and data will be managed systematically throughout the review process. Bibliographic records retrieved from databases will be imported into a reference management software (EndNote) to remove duplicates and organize citations. Screening decisions, eligibility assessments, and data extraction will be recorded in standardized spreadsheets. Extracted data, including species, metabolites, bioactive effects, and regulatory factors, will be stored securely and backed up regularly. Data will be maintained in a structured format to facilitate synthesis, transparency, and potential future updates of the review.

Quality assessment / Risk of bias analysis As this systematic review is primarily descriptive and aims to map metabolites, bioactive effects, and regulatory factors in leafy vegetables, formal quality assessment or risk of bias analysis will not be performed.

Strategy of data synthesis Data from the included studies will be extracted and synthesized narratively. Key information, including species of leafy vegetables, reported metabolites, validated bioactive effects, validation methods, scope of analysis, and factors influencing metabolic variation (intrinsic and extrinsic), will be tabulated and summarized. Trends across studies will be described, and knowledge gaps will be identified. All visual summaries such as tables, charts, or schematic diagrams will be used to illustrate patterns and relationships.

Subgroup analysis Overview of metabolomic studies on leafy vegetables, overview of studied leafy vegetable species and families, overview of reported metabolites across studied leafy vegetables, leafy vegetables with validated bioactive effects, metabolites regulation, and underexploited metabolites in leafy vegetables.

Sensitivity analysis As this systematic review is primarily descriptive and aims to map metabolites, bioactive effects, and regulatory factors in leafy vegetables, sensitivity analysis will not be performed.

Language restriction Yes, only articles published in English will be included in this systematic review.

Country(ies) involved Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore117543, Republic of Singapore.

Other relevant information Meta-data of each included article was recorded, including title, author(s), year of publication, publication journal, scientific name of leafy vegetables, cultivation condition, extraction solvent, and analytical methods.

Keywords Leafy vegetables; metabolomics; metabolites classification; bioactive effects; metabolic variation.

Dissemination plans The findings of this systematic review will be disseminated primarily through publication in a peer-reviewed journal relevant to plant science and metabolomics.

Contributions of each author

Author 1 - Seam Choon Law - Author 1 contributed to conceptualization, literature search, screening, data extraction, data analysis, drafting, and reviewing and editing of the manuscript.
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