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Advanced metabolomics tools for unveiling the phytomedicine potential of spices and their therapeutic applications in pharmacology

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Abstract

Spices are rich in bioactive compounds with pharmacological properties like antioxidant, anti-inflammatory, antimicrobial, anticancer, neuroprotective, and cardioprotective effects. Metabolites are a diverse group of plant compounds including secondary metabolites (*e.g.*, alkaloids, tannins, flavonoids), amino acids, sugars, lipids, organic acids, and other small molecules. Spices are rich sources of secondary metabolites which are bioactive molecules. Plant metabolomics is the complete analysis of metabolites in plant tissues and cells. This field of study is rapidly advancing and is quite intriguing in plant science and systems biology. Through metabolomics, researchers can understand the functional roles of metabolites in plant physiology and development, including stress responses. Studying their metabolic activities provides valuable insights into their chemical composition and biological effects. The immemorial role of advanced metabolomics tools which comprises high-throughput metabolomic techniques like LC-MS, GC-MS, and HPLC coupled with software tools to process the obtained data is monumental. The data obtained could be used to find novel compounds with potential phytomedicinal properties. This can reveal and extend the use of spices for drug development, natural remedies, adjunct therapy, disease prevention, and nutraceuticals. Spices offer potential health benefits and can be incorporated into functional foods or dietary supplements.

1. Introduction

Spices have been an essential part of the human diet for centuries. They are used to add flavor, aroma, and color to food. In addition to enhancing taste, spices have numerous health benefits. They contain antioxidants, antimicrobial agents, and have antidiabetic properties, among others. These benefits are due to the presence of phytochemicals or metabolites, which are small molecules found in spices. Plant metabolomics is the study of all the small molecules or metabolites present in a plant, and it has gained significant interest in recent decades (Gupta *et al.*, 2024). A metabolome is the complete set of low-molecular-weight metabolites in an organism, resulting from gene expression, protein function changes, and environmental factors. It is highly variable and depends on spatial and temporal factors. A plant's metabolomic profile varies based on organ or tissue. Plants produce around 100,000 metabolites, and the model plant alone produces 5,000 (Joshi *et al.*, 2024). Plant species remain incompletely characterized biochemically, with many detected compounds awaiting identification. Metabolomics has expanded since its inception and is now applied to study wild and cultivated plants to understand their metabolism. Early research was on model plants, focusing on primary metabolic pathways, which later expanded to important secondary metabolites (Hong *et al.*, 2016). Plant metabolomics is vital for understanding plant functions and interactions with the environment. It identifies important metabolites and correlations of disease pathogenesis. It also screens secondary metabolites for stress resistance, antimicrobial properties, and quality attributes. Advancements in measuring small molecules detect and quantify numerous metabolites which act as biomarkers to detect dysregulation. Overall, metabolomics comprehensively understands the biochemical composition of medicinal plants and their pharmacological activities (Waris *et al.*, 2022). This review illuminates recent advancements in metabolomic research and its diverse applications in phytomedicine and pharmacology, including the utilization of various software and databases for efficient data analysis.

2. Medicinal spices and herbs in Indian culinary tradition

Nutraceutical and life sciences industries are increasingly exploring the therapeutic potential of herbs and spices, integrating them into fortified foods and organic medicines. Turmeric, scientifically known as *Curcuma longa* L., is a staple ingredient in Asian cuisine renowned for its nutritional and pharmacological value (Dudekula *et al*, 2022). Rosemary (*Rosmarinus officinalis* L.) is widely used in cooking, especially as a seasoning for meat dishes, owing to its aromatic properties and potential health advantages. Yarrow (*Achillea*

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millefolium L.) finds application in salads and herbal teas, with its bitter taste considered refreshing. Red pepper (*Capsicum annuum* L.) is rich in beta-carotene, capsaicin, and vitamins A and C, offering various health benefits, including liver disease prevention. Fenugreek (*Trigonella foenum-graecum* L.) serves as a versatile spice in culinary preparations and is valued in the pharmaceutical and functional food industries for its medicinal properties. Garlic (*Allium sativum* L.) is renowned not only for its flavor-enhancing properties but also for its potential role in preventing various cancers. Onions (*Allium cepa* L.) are used widely in cooking and salads and are recognized for their health benefits attributed to their high content of organosulfur compounds and flavonoids. These herbs and spices not only add flavor to dishes but also contribute to overall health and well-being when incorporated into the diet regularly (Pooja *et al*., 2023; Vasanthkumar, 2023).

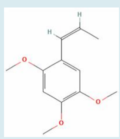
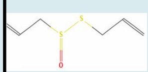
3. Therapeutic potential of spice metabolites

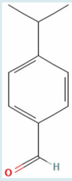
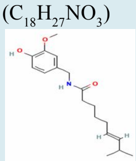
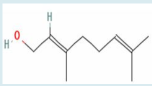
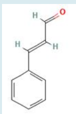
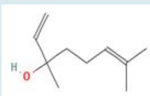
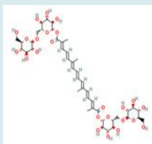
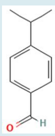
Spices contain a wealth of therapeutic compounds such as thymol, eugenol, curcuminoids, linalool, zingiberene, piperine, alpha crocin, coriandrol, cuminaldehyde, and capsaicin. These compounds offer a diverse range of pharmacological benefits, including antimicrobial, antioxidant, anticarcinogenic, antiemetic, antimutagenic, antihypertensive, antidiabetic, anticonvulsive, antifungal, antiviral, hypolipidemic, chemoprotective, and prebiotic activities. Turmeric (*C. longa*), with its main active component curcumin, offers potent anti-inflammatory effects that can alleviate arthritis symptoms like joint pain and stiffness. It is also renowned for its antimicrobial, antidiabetic, and anticancerous properties (Vali *et al*., 2022). Cinnamon (*Cinnamomum verum* J. Presl), containing cinnamaldehyde, aids in managing diabetes by improving insulin sensitivity and regulating blood sugar levels. Garlic *(A. sativum)*, rich in allicin, contributes to cardiovascular health by reducing blood pressure and cholesterol levels, thus lowering the risk of heart disease. Ginger's (*Zingiber officinale* Roscoe) antiemetic properties, attributed to compounds like gingerol, make it effective against nausea and vomiting, particularly beneficial for pregnant women and those undergoing chemotherapy. Black pepper (*Piper nigrum* L.), containing piperine, enhances digestive function by stimulating enzyme secretion, relieving indigestion, and promoting overall digestive health. Red chilli (*C. annuum*) capsaicin content offers pain relief for conditions like arthritis and migraine headaches, often utilized through topical creams. Cloves (*Syzygium aromaticum* L.), due to eugenol, provide dental health benefits by alleviating toothache and combating oral bacteria, thus contributing to improved oral hygiene (Pooja *et al*., 2023).

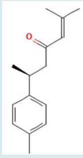
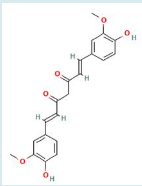
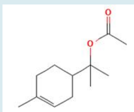
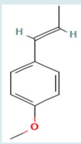
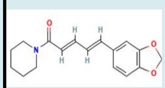
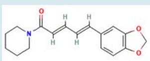
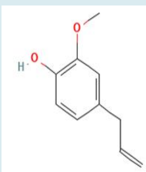
4. Need for metabolomics in spice crops

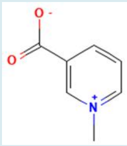
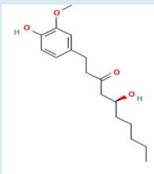
Spices have been traditionally known for their beneficial effects on human health and as preservatives in food (Eneojo and Martins, 2024). Spices are rich in phytochemicals and this expands their use in nutritional and therapeutic areas. Studies in the past decade have revealed the potential of many spices as therapeutics such as the effectiveness of curcuminoids against COVID-19 (Vali *et al.,* 2022). Bioactive compounds of spices with pharmacological activities and traditional uses have been briefed in Table 1. Metabolomic studies of only a handful of spices have been reported so far. This puts us under an obligation to expand metabolomic studies to spice crops. Numerous bioactive compounds with functional properties are yet to be identified and the pharmacological activities of the identified bioactive compounds are yet to be validated. This can be done using integrated omics sciences (Matthews *et al*., 2016).

Table 1: Bioactive compounds and pharmacological activities of spices

S.No.	Crop	Main bioactive compound structure	Traditional uses	Bioactive compounds	Pharmacological activities	References
1.	*Acorus calamus* L. (Acoraceae) Sweet flag	Beta-asarone ($C_{12}H_{16}O_3$) 	Leaves, stems, and rhizomes for flavor and aroma/remedy for digestive disorder, bronchitis, sinusitis	Beta-asarone, fatty acids, sugar, acorenone, *etc*.	Antimicrobial, antioxidant, insecticidal activities, anticonvulsant,. neuroprotective, *etc*.	Zhao *et al*., 2023
2.	*Allium sativum* L. (Amaryllidaceae) Garlic	Allicin ($C_6H_{10}OS_2$) 	Leaf and clove for seasoning and flavoring/remedy for fevers, colic, flatulence, diabetes, rheumatism, intestinal worms, dysentery, liver disorders, high blood pressure and bronchitis	Allicin, alliin, ajoene, diallyl sulfide, diallyl disulfide, diallyl trisulfide, S-allyl-cysteine, *etc*.	Antioxidant, anti-inflammatory, antibacterial, antifungal, immunomodulatory, cardioprotective, anticancer, hepatoprotective, antidiabetic, *etc*.	Tiwari *et al*., 2023
3.	*Allium cepa* L. (Amaryllidaceae) Onion	Diallyl sulfide ($C_6H_{10}S$) 	Leaf and bulb for seasoning and flavoring, as vegetable/ remedy for bruises, colic, colds, fever, earache, bronchitis, intestinal	Flavonoids, organo-sulfur, phytosterols, saponins, *etc*.	Antioxidant, antimicrobial, antidiabetic properties, beneficial against hyper-lipidemia and hypertension, *etc*.	Stoica *et al*., 2023

			parasites, hypertension, jaundice, sores and impotence			
4.	*Nigella sativa* L. (Apiaceae) Black cumin	Cuminaldehyde ($C_{10}H_{12}O$) 	Dried seed for flavor/ used to treat urinary, digestive disorders, inflammation, obesity, and increase breast milk	Caryophyllene, cuminyl acetate, cuminaldehyde, gamma-terpene-7-al, trans-3-caren -2-ol, acetic-acid, methatriene, p-cymene, cuminyl acetate, limonene, *etc*.	Antibacterial, antifungal, anticonvulsant, antihistaminic, antinociceptive, anti-inflammatory, antioxidant, *etc*.	Camlica and Yaldiz, 2019
5.	*Capsicum annuum* L. (Solanaceae) Chilli	Capsaicin ($C_{18}H_{27}NO_3$) 	Fresh and dried fruit as a colorant, flavoring/used as appetizer, weight loss, for inflammation, to cure numbness	Capsaicinoids, carotenoids, flavonoids, steroids, saponins, *etc*.	Antioxidant, cancer chemo-preventive, antidiabetic, gastroprotective, and antimicrobial activities, pain relief, treatment of metabolic syndrome, *etc*.	Mandal *et al*., 2023
6.	*Laurus nobilis L.* (Lauraceae) Bay leaf	Geraniol ($C_{10}H_{18}O$) 	Fresh or dried leaf for aroma and flavor/ used for the treatment of skin rashes, bad mouth odor, earaches, and rheumatism	Saponins, phyto-sterols, fatty acids, monoterpene, sesquiterpene, geraniol, linolol, bornyl acetate, caryophylene oxide, p-coumaric acid, vanillic acid, *etc*.	Antidiarrheic, antitumor, anti-inflammatory, antiarthritic, antitumor, antioxidant, chemo-preventive,gastro protective, antimicrobial, antipyretic, anxiolytic, *etc*.	Awada *et al*., 2023
7.	*Cinnamomum. verum* J. (Lauraceae) Cinnamon	Cinnamaldehyde (C_9H_8O) 	Dried bark for flavor/ used in treating sore throats, cough, indigestion, abdominal cramps, intestinal spasms, nausea, flatulence and diarrhea	Cinnamaldehyde, eugenol, linalool, phenolic acids, tannins, *etc*.	Anti-inflammatory, antimicrobial, antioxidant, antitumor, cardiovascular, cholesterol-and lipid-lowering properties and immunomodulatory effects, hypoglycemic properties, *etc*.	Patel *et al*., 2019
8.	*Coriandrum sativum* L. (Apiaceae) Coriander	Linalool ($C_{10}H_{18}O$) 	Fresh leaves and dried seeds for flavoring and seasoning/remedy for sore throat, allergies, digestion problems, hay fever	Linalool, coriandrin, and α-pinene flavonoids, ferulic acid, salicylic acid, gallic acid, coumarins, tartaric acid, maleic acid, arbutin, *etc*.	Antioxidant, diuretic, antidiabetic, sedative, antimicrobial, anti-convulsant, hypnotic, anthelmintic, antimutagenic, *etc*.	Sharma and Chakraborty, 2019
9.	*Crocus sativus* L. (Iridaceae) Saffron	Crocin ($C_{44}H_{64}O_{24}$) 	Dried stigma for sea-soning and coloring/ remedy for skin disease, asthma, cold and cough	Crocin, kaemferol, safranal, *etc*.	Antioxidant, anti-inflammatory, antimicrobial antidiabetic, aphrodisiac, anxiolytic, antitumor activities, *etc*.	Saadat *et al*., 2024
10.	*Cuminum cyminum* L. (Apiaceae) Cummin	Cuminaldehyde ($C_{10}H_{12}O$) 	Whole seeds and ground powder as perfumery and seasoning/remedy for indigestion, jaundice, diarrhea, cold and flatulence	Cuminaldehyde, coumarin, anthraquinone, flavonoid, glycoside, protein, resin, saponin, tannin, steroid, *etc*.	Antimicrobial, anti-inflammatory, analgesic, antioxidant, hypotensive, anti-osteoporotic, tyrosinase inhibitory effects, *etc*.	Ghalib and Mehrotra, 2022

11.	*Curcuma caesia* R. (Zingiberaceae) Black turmeric	ar-Turmerone ($C_{15}H_{20}O$) 	Rhizome for aroma/ remedy for cough, cold, pneumonia, wounds, toothache, vomiting, allergies, leucoderma, asthma, and menstrual disorders	ar-Turmerone, (Z)-ocimene, ar-curcumene, cineole, elemene, borneol, bornyl acetate, curcumene, *etc*.	Antifungal, smooth muscle relaxant and antiasthmatic activities, analgesic, anthel-mintic, antioxidant, *etc*.	Naikodi *et al*., 2021
12.	*Curcuma longa* L. (Zingiberaceae) Turmeric	Curcumin ($C_{21}H_{20}O_6$) 	Rhizome for flavor and color/remedy for cough, diabetes, wounds, hepatic disorders, rheumatism and sinusitis	Curcumin, demethoxycurcumin, bisdemethoxy-curcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, turmeronols, *etc*.	Anti-inflammatory, antioxidant, anticoagulant, antidiabetic, antimicrobial, antiulcer, wound healing, anticancer, antiarthritis activities, *etc*.	Bhavsar *et al*., 2022
13.	*Elettaria cardamomum* L. (Zingiberaceae)	Cardamom α-Terpinyl acetate 	Dried seed for flavor/ used to treat nausea, indigestion, cold cough, and bad breath	α-Terpinyl acetate, 1,8-cineole, *etc*.	Diuretic, stimulant, stomachic, tonic and antispasmodic, antimicrobial, anti-inflammatory, *etc*.	Ashokkumar *et al*., 2020
14.	*Foeniculum vulgare* M. (Apiaceae) Fennel	Anethole ($C_{10}H_{12}O$) 	Dried seed as flavor and aroma/used to treat bloating, loss of appetite and colic in infants	Trans-anethole fenchoneestragole (methyl chavicol) A-phellandrenesa ponins, flavonoids, cardiac glycosides, sterols, triterpenes, coumarins, volatile oils, *etc*.	Antimicrobial, antifungal, antioxidant, antithrombotic, anti-inflammatory, hepatoprotective, antitumor, activities, *etc*.	Rafieian *et al*., 2023
15.	*Piper longum* L. (Piperaceae) Long pepper	Piperine ($C_{17}H_{19}NO$) 	Dried fruit for seasoning/used to improve appetite and digestion, treat stomachache, heartburn, indigestion, intestinal gas, diarrhea	Piperine, piperlongumine, diaeudesmin pipermonaline, *etc*.	Antioxidant, anti-inflammatory, antimicrobial, antiplatelet, antifertility, antihyper-lipidemic, antiobesity, hepatoprotective, analgesic, larvicidal, radioprotective, cardioprotective, antidepressant and antifungalactivities, *etc*.	Biswas *et al*., 2022
16.	*Piper nigrum* L. (Piperaceae) Black pepper	Piperine ($C_{17}H_{19}NO$) 	Dried fruit for flavor and aroma/remedy for indigestion, bloating, cough and cold, infection	Piperine, volatile oil, oleoresins, alkaloids, *etc*.	Antimicrobial, antioxidant, anticancer, neuro-protective, hypoglycemic, anticonvulsant, analgesic, hypolipidemic andanti-inflammatory activities, *etc*.	Ashokkumar *et al*., 2021
17.	*S. aromaticum* L. (Myrtaceae) Clove	Eugenol ($C_{10}H_{12}O_2$) 	Dried flower bud for aroma and flavor/ remedy for toothache and sore gums, cough and cold, fever, digestive problems, *etc*.	Eugenol, eugenyl acetate, β-caryophyllene, *etc*.	Analgesic, antioxidant, anticancer, antiseptic, antidepressant, antispasmodic, anti-inflammatory, antiviral, antifungal, and antibacterial, *etc*.	Rubika *et al*., 2024

18.	*Trigonella foenum - graecum* L. (Fabaceae) Fenugreek	Trigonelline ($C_7H_7NO_2$) 	Fresh leaves and dried seeds as vegetable flavoring, seasoning/ used for increasing breast milk, treating diabetic wounds, skin irritation, and lowering cholesterol	Trigonelline, isoorientin, orientin, vitexin, isovitexin, *etc*.	Hypoglycemic, antihyper-tensive, antioxidant, immunomodulatory wound healing, hepatoprotective, appetizer, *etc*.	Idris *et al*., 2021
19.	*Z. officinal* R. (Zingiberaceae) Ginger	6-Gingerol ($C_{17}H_{26}O_4$) 	Dried and fresh rhizome for flavoring/ remedy for cough and cold, dyspepsia, flatulence,abdominal discomfort and nausea	Gingerols, shogaols, terpene, *etc*.	Antioxidant, anti-inflammatory, antiemetic, antimicrobial, anticancer, antidiabeti-cactivities, neuroprotection, cardiovascular protection, *etc*.	Yang *et al*., 2024

5. Metabolomic techniques

Metabolome studies are intricate and require various analytical techniques for effective analysis. Due to the complexity of the metabolome, multiple analysis methods are necessary. Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are common techniques used in metabolomic studies. High-throughput metabolomic techniques have enabled simultaneous measurement of steady-state levels of metabolites (Yin *et al*., 2023). Mass spectrometry techniques like LC-MS and GC-MS can identify new metabolic regulations from existing pathways with high sensitivity. This generates extensive data for analyzing metabolites and studying the impact of metabolic regulations on cellular pathways and plants (Manickam *et al*., 2023). Metabolomics uses mass spectrometry or nuclear magnetic resonance to classify metabolites. Preprocessing involves noise reduction, peak detection, and chromatographic alignment. Quality control separates data of different quality and determines variance. Statistical analysis identifies abnormal changes. Significant metabolites are ranked based on a reliable threshold using an appropriate p-value. Pathway analysis identifies pathways that substantially impact biological processes (Chen *et al*., 2022). The metabolomic process or workflow is summarized in Figure 1.

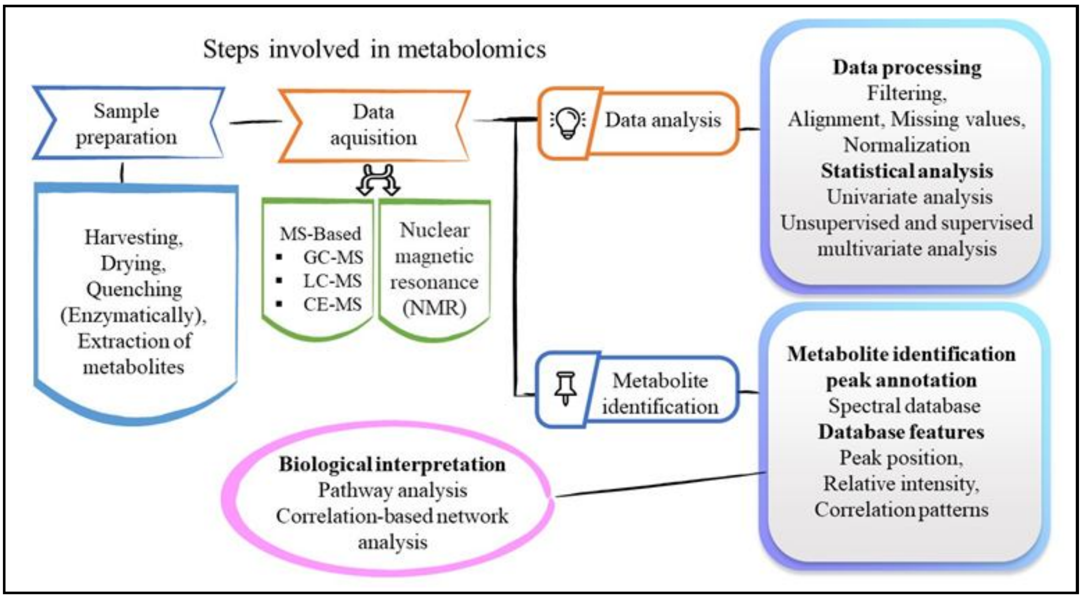


Figure 1: Workflow of metabolomics procedure.

6. Metabolite identification and annotation

Identifying and quantifying metabolites is complex and requires careful data processing. Mass spectrometry provides reliable data in the form of mass-to-charge ratio (m/z) and relative ionized compound intensity. The analysis is improved by combining with chromatographic separation methods. Normalization reduces technical variation and misidentification. Compound identification is done by comparing mass spectrometry data with authentic standard data in libraries or public databases (Chen *et al*., 2022). Standardizing and sharing metabolomic data from plants and crops is crucial for their effective utilization and promotes data transparency for further exploration and analysis (Kisiel *et al*., 2023). Correctly annotating biological features is important to reduce data complexity. Spectra can be compared to databases to identify metabolites. Credentialing omits artifactual features and confirms biological origin, increasing confidence and preventing bioinformatic noise by 15% (Fukushima *et al.*, 2022).

Many metabolome databases have been established and made public. However, data sharing is still delayed, affecting data reanalysis, reusability, and reproducibility. More information sharing is needed (Godzien *et al.*, 2018). Databases such as RIKEN Plant Metabolome Meta-Database store metabolite profiling data in a detailed and structured format that includes information about the sampling and experimental procedures (Figure 2). This database offers intuitive and interactive features, such as metabolite annotations, raw data, and mass spectra. It makes it easy for users to manipulate plant metabolome data in a user-friendly and interactive manner. The database supports raw data in netc. DF format, mass spectra in NIST MSP format, and annotations for metabolites. RIKEN Plant Metabolome Meta-Database stores plant metabolome data in an interactive format, offering features like metabolite annotations, raw data, and mass spectra. The database supports raw data in netc.DF format, mass spectra in NIST MSP format, and annotations for metabolites (Zheng *et al.*, 2024). A summary of the available software tools for data from various instruments is listed in Table 2.

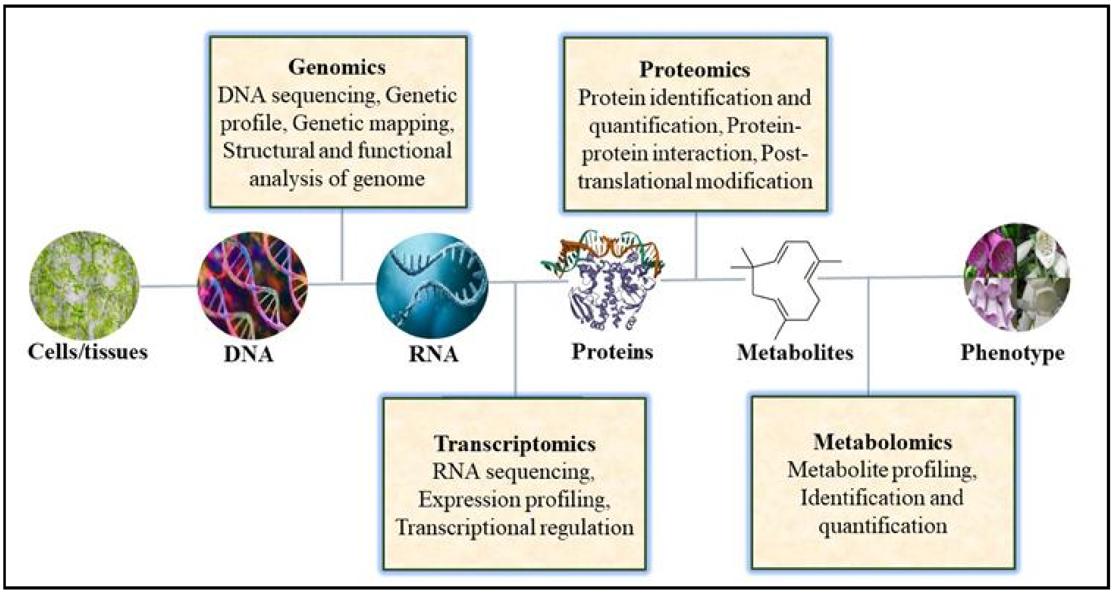


Figure 2: Biological flow of information from genome to metabolome.

Table 2: Software tools available for processing data obtained from various instruments

Instrument data type	Software tools	Purpose	References
MS	KMDA	Post-processing	Peralbo-Molina *et al.*, 2022
NMR	rNMR	Pre-processing	Lewis *et al.*, 2009
	Metabnorm	Post-processing	Misra and van der Hooft, 2016
	mQTL.NMR	Statistical analysis	Hedjazi *et al.*, 2015
MS, NMR	metabolomics	Post-processing, statistical analysis	Emwas *et al.*, 2019
	muma	Post-processing, statistical analysis	Morgan *et al.*, 2019
	MetabolAnalyze	Statistical analysis	Denzer *et al.*, 2022
LC-MS	Open MS-Feature Finder Metabo	Pre-processing	Guo and Huan, 2023
	MS-DIAL	Pre-processing	de Oliveira Costa *et al.*, 2024
	MetAlign	Pre-processing	LaPierre *et al.*, 2020
	mzMatch	Pre-processing	Chokkathukalam *et al.*, 2012
	IDEOM	Pre-processing	Srivastava and Creek, 2020
	Ionwinze	Statistical analysis	Kokubun and D'Costa, 2013
	Galaxy-M	Workflows	Davidson *et al.*, 2016
	MetaboAnalyst 3.0	Workflows	Pang *et al.*, 2022

GC–MS	AMDIS	Pre-processing	Umoh *et al.*, 2023
	MetaboliteDetector	Pre-processing	Au - Hoenigsberger *et al.*, 2018
	MET-IDEA	Pre-processing	Kadir *et al.*, 2020
	metaMS	Pre-processing	Wehrens *et al.*, 2014
	MSeasy	Pre-processing	Nicolè *et al.*, 2012
	SpectConnect	Pre-processing	Vishwanath *et al.*, 2011
LC-MS, GC–MS	MeltDB	Pre-processing	Zulet-Gonzalez *et al.*, 2023
	XCMS	Pre-processing	dos Santos and Canuto, 2023
	MetaboAnalyst 6.0	Pre-processing	Zhao *et al.*, 2024
	metabomxtr	Post-processing	Reisetter *et al.*, 2017
	crmn	Post-processing	Michiel *et al.*, 2020
	XCMS Online	Workflows	Forsberg *et al.*, 2018
	Workflow4metabolomics 3.0	Workflows	Guitton *et al.*, 2017

7. Challenges and future prospects

Metabolomics faces several significant challenges, including the complexity of the plant metabolome, which has a dynamic range, and technical issues with metabolite identification. Additionally, the high cost of analytical platforms and complex data analysis/ interpretation, especially in spices, pose challenges. Researchers may omit essential and biologically meaningful metabolites while setting filtering thresholds for metabolite intensity to reduce noise. Moreover, novel metabolites or metabolites not curated in databases are often ignored by researchers due to a lack of awareness. Data streaming for cloud-based metabolomics can help overcome the challenge of slow data transfer speeds during data file uploads. However, relying on metabolite databases remains a challenge in metabolomics as they are not always comprehensive, and some metabolites cannot be traced to any metabolic pathways. New tools and platforms in metabolomics will help unlock its full potential in crop research. Automated workflows with data upload, processing, identification, and pathway analysis will improve efficiency. Focusing on metabolic pathways, rather than individual metabolites, can help identify the causes of disrupted pathways. Additionally, the metabolomics of spice crops have huge medicinal applications in drug delivery and food fortification, and their additional uses as future prospects further highlight the importance of addressing the challenges in this field.

8. Conclusion

Plant metabolomics is an important technology for understanding plant biochemistry, physiology, and their responses to genetic and environmental changes. However, it is currently limited to only a few plant species and needs to be expanded. Metabolomics can help in drug discovery, identifying gene function and biomarkers of desirable plant traits, and predicting plant responses to stresses. It can also be integrated with other omics for targeted improvements in crop quality and yield, increasing resilience in the dynamic environmental future. The field is rapidly developing and can be a major game changer for global food security and plant-based production.

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Conflict of interest

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

References

Ashokkumar, K.; Murugan, M.; Dhanya, M.K.; Pandian, A. and Warkentin, T. D. (2021). Phytochemistry and therapeutic potential of black pepper *Piper nigrum* L. essential oil and piperine: A review Clinical Phytoscience, **7**(1):52. https://doi.org/10.1186/s40816-021-00292-2.

Awada, F.; Hamade, K.; Kassir, M.; Hammoud, Z.; Mesnard, F.; Rammal, H. and Fliniaux, O. (2023). *Laurus nobilis* leaves and fruits: A review of metabolite composition and interest in human health. Applied Sciences, **13**(7):4606. https://doi.org/10.3390/app13074606.

Bhavsar, S. K.; Humbal, B. R.; Sadariya, K. A. and Baria, T. R. (2022). Evaluation of the *in vitro* antibacterial activity and minimum inhibitory concentration of *Curcuma longa* L., *Ocimum sanctum* L., and *Piper nigrum* L. ethanolic and aqueous extracts. Ann. Phytomed., **11**(1):455-464.

Biswas, P.; Ghorai, M.; Mishra, T.; Gopalakrishnan, A. V.; Roy, D.; Mane, A. B.; Mundhra, A.; Das, N.; Mohture, V. M. and Patil, M. T. (2022). *Piper longum* L.: A comprehensive review on traditional uses, phytochemistry, pharmacology, and health promoting activities. Phytotherapy Research, **36**(12):4425-4476. https://doi.org/10.1002/ptr.7649.

Camlica, M. and Yaldiz, G. (2019). Effect of cultural condition on seed growth and content of essential oil of two populations and one cultivar of genus nigella. Ann. Phytomed., **8**(1):56-62.

Chen, Y.; Li, E. M. and Xu, L. Y. (2022). Guide to metabolomics analysis: A bioinformatics workflow. Metabolites, **12**(4). https://doi.org/10.3390/metabo12040357.

Chokkathukalam, A.; Jankevics, A.; Creek, D. J.; Achcar, F.; Barrett, M. P. and Breitling, R. (2012). mzMatch-ISO: An R tool for the annotation and relative quantification of isotope-labelled mass spectrometry data. Bioinformatics, **29**(2):281-283. https://doi.org/10.1093/bioinformatics/bts674.

Davidson, R. L.; Weber, R. J. M.; Liu, H.; Sharma-Oates, A. and Viant, M. R. (2016). Galaxy-M: A galaxy workflow for processing and analyzing direct infusion and liquid chromatography mass spectrometry-based metabolomics data. Gigascience, **5**(1). https://doi.org/10.1186/s13742-016-0115-8.

De Oliveira Costa, G.; Pontes, C. L. M.; Parize, A. L. and Sandjo, L. P. (2024). Unveiling chemical responses in the kombucha-based fermentation of black tea, banana flower, and grape juice: lc-esims, gnps, ms-dial, and ms-finder-assisted chemical characterization. Food and Function. https://doi.org/10.1039/d3fo04977a.

Denzer, M. L.; Kiyimba, F.; Mafi, G. G. and Ramanathan, R. (2022). Metabolomics of meat color: Practical implications. Current Proteomics, **19**(4):299-307. https://doi.org/10.2174/157016461966621123015 3145.

Dos Santos, E. K. P. and Canuto, G. A. B. (2023). Optimizing XCMS parameters for GC-MS metabolomics data processing: A case study. Metabolomics, **19**(4):26. https://doi.org/10.1007/s11306-023-01992-1.

Dudekula M. V.; Kandasamy V.; Balaraman S. S.; Selvamani S. B.; Muthurajan R.; Adhimoolam K.; Manoharan B. and Natesan S. (2022). Unlocking the genetic diversity of Indian turmeric (*Curcuma longa* L.) germplasm based on rhizome yield traits and curcuminoids. Frontiers in Plant Science. **13**:1036592.

Emwas, A. H.; Roy, R.; McKay, R. T.; Tenori, L.; Saccenti, E.; Gowda, G. N.; Raftery, D.; Alahmari, F.; Jaremko, L. and Jaremko, M. (2019). NMR spectroscopy for metabolomics research. Metabolites, **9**(7):123. https://doi.org/10.3390/metabo9070123.

Eneojo, O. and Martins, E. (2024). Herbs and spices-based value addition for nutritional and healthy living. https://doi.org/10.5772/intechopen.1004345.

Forsberg, E. M.; Huan, T.; Rinehart, D.; Benton, H. P.; Warth, B.; Hilmers, B. and Siuzdak, G. (2018). Data processing, multi-omic pathway mapping, and metabolite activity analysis using XCMS Online. Nature Protocols, **13**(4):633-651. https://doi.org/10.1038/nprot.2017.151.

Fukushima, A.; Takahashi, M.; Nagasaki, H.; Aono, Y.; Kobayashi, M.; Kusano, M.; Saito, K.; Kobayashi, N. and Arita, M. (2022). Development of RIKEN plant metabolome metaDatabase. Plant and Cell Physiology, **63**(3):433-440. https://doi.org/10.1093/pcp/pcab173.

Ghalib, R. and Mehrotra, N. (2022). *In vitro* α-glucosidase inhibitory activity of nanoencapsulated *Cuminum cyminum* L. (Angiosperms: Apiaceae) and it's interaction with acarbose. Ann. Phytomed., **11**(1):465-471.

Godzien, J.; de la Fuente, A. G.; Otero, A. and Barbas, C. (2018). Metabolite annotation and identification. Comprehensive Analytical Chemistry, **82**:415-445. https://doi.org/10.1016/bs.coac.2018.07.004.

Guitton, Y.; Tremblay-Franco, M.; Le Corguillé, G.; Martin, J.-F.; Pétéra, M.; Roger-Mele, P.; Delabrière, A.; Goulitquer, S.; Monsoor, M.; Duperier, C.; Canlet, C.; Servien, R.; Tardivel, P.; Caron, C.; Giacomoni, F. and Thévenot, E. A. (2017). Create, run, share, publish, and reference your LC-MS, FIA–MS, GC–MS, and NMR data analysis workflows with the Workflow4Metabolomics 3.0 Galaxy online infrastructure for metabolomics. The International Journal of Biochemistry and Cell Biology, **93**:89-101. https://doi.org/10.1016/j.biocel.2017.07.002.

Guo, J. and Huan, T. (2023). Mechanistic understanding of the discrepancies between common peak picking algorithms in liquid chromatography-mass spectrometry-based metabolomics. Analytical Chemistry, **95**(14):5894-5902. https://doi.org/10.1021/acs.analchem.2c04887.

Gupta, S.; Singh, A. P.; Singh, G.; Ding, X. and Sharma, A. (2024). Plant based metabolomics: a future prospective and versatile tool for metabolite databases of *Curcuma longa*. Journal of Future Foods, **4**(1):37-47. https://doi.org/10.1016/j.jfutfo.2023.05.003.

Hedjazi, L.; Gauguier, D.; Zalloua, P. A.; Nicholson, J. K.; Dumas, M. E. and Cazier, J. B. (2015). mQTL. NMR: An integrated suite for genetic mapping of quantitative variations of 1H NMR-based metabolic profiles. Analytical Chemistry, **87**(8):4377-4384. https://doi.org/10.1021/acs.analchem.5b00145.

Hoenigsberger, M.; Kopchinskiy, A. G.; Parich, A.; Hiller, K.; Laciny, A.; Zettel, H.; Lim, L. B. L.; Salim, K. A.; Druzhinina, I. S. and Schuhmacher, R. (2018). Isolation of mandibular gland reservoir contents from bornean 'exploding ants' (formicidae) for volatilome analysis by GC-MS and metabolite detector. J. Vis. Exp. (138):e57652. https://doi.org/doi:10.3791/57652.

Hong, J.; Yang, L.; Zhang, D. and Shi, J. (2016). Plant metabolomics: an indispensable system biology tool for plant science. International Journal of Molecular Sciences, **17**(6):767. https://doi.org/10.1263/jbb.100.347.

Idris, S.; Mishra, A. and Khushtar, M. (2021). Phytochemical estimation of germinated *Trigonella foenum-graecum* L. seed extract for better application in phytotherapy. Ann. Phytomed., **10**(2):213-222.

Joshi, R.; Sharma, S. and Kumar, D. (2024). Advances of ion mobility platform for plant metabolomics. Critical reviews in analytical chemistry, **54**(1):175-191. https://doi.org/10.1080/10408347.2022.2070000.

Kadir, F. A. A.; Azizan, K. A. and Othman, R. (2020). Datasets of essential oils from naturally formed and synthetically induced *Aquilaria malaccensis* agarwoods. Data in Brief, **28**:104987. https://doi.org/10.1016/j.dib.2019.104987.

Kisiel, A.; Krzemińska, A.; Cembrowska-Lech, D. and Miller, T. (2023). Data Science and Plant Metabolomics. Metabolites, **13**(3):454. https://doi.org/10.3390/metabo13030454.

Kokubun, T. and D'Costa, L. (2013). Direct and unbiased information recovery from liquid chromatography-mass spectrometry raw data for phenotype-differentiating metabolites based on screening window coefficient of ion currents. Analytical Chemistry, **85**(18):8684-8691. https://doi.org/10.1021/ac401545b.

Kumar, S.; Kalita, S.; Basumatary, I. B.; Kumar, S.; Ray, S. and Mukherjee, A. (2024). Recent advances in therapeutic and biological activities of *Aloe vera*. Biocatalysis and Agricultural Biotechnology, **57**:103084. https://doi.org/10.1016/j.bcab.2024.103084.

LaPierre, N.; Alser, M.; Eskin, E.; Koslicki, D. and Mangul, S. (2020). Metalign: efficient alignment-based metagenomic profiling *via* containment min hash. Genome Biology, **21**(1):242. https://doi.org/10.1186/s13059-020-02159-0.

Lewis, I. A.; Schommer, S. C. and Markley, J. L. (2009). rNMR: Open source software for identifying and quantifying metabolites in NMR spectra. Magnetic Resonance in Chemistry, **47**(S1):S123-S126. https://doi.org/10.1002/mrc.2526.

Mandal, S. K.; Rath, S. K.; Logesh, R.; Mishra, S. K.; Devkota, H. P. and Das, N. (2023). *Capsicum annuum* L. and its bioactive constituents: A critical review of a traditional culinary spice in terms of its modern pharmacological potentials with toxicological issues. Phytotherapy Research, **37**(3):965-1002. https://doi.org/10.1002/ptr.7660.

Manickam, S.; Rajagopalan, V. R.; Kambale, R.; Rajasekaran, R.; Kanagarajan, S. and Muthurajan, R. (2023). Plant metabolomics: current initiatives and future prospects. Current Issues in Molecular Biology, **45**(11):8894-8906. https://doi.org/10.3390/cimb45110558.

Matthews, H.; Hanison, J. and Nirmalan, N. (2016). "Omics"-informed drug and biomarker discovery: Opportunities, challenges and future perspectives. Proteomes, **4**(3):28. https://doi.org/10.3390/proteomes 4030028.

Michiel, B.; Ramon, B.; Serwet, D.; Ed, H. J.; Oussoren, E.; Ans, T. v. d. P.; Margreet, A. E. M. W.; Robert, M. W. H.; Henk, J. B.; Marcel, J. T. R. and George, J. G. R. (2020). Screening for inborn errors of metabolism using untargeted metabolomics and out-of-batch controls. bioRxiv: 2020.2004. 2014.040469. https://doi.org/10.1101/2020.04.14.0 40469.

Misra, B. B. and van der Hooft, J. J. (2016). Updates in metabolomics tools and resources: 2014-2015. Electrophoresis, **37**(1):86-110. https://doi.org/10.1002/elps.201500417.

Morgan, M. A.; Griffith, C. M.; Dinges, M. M.; Lyon, Y. A.; Julian, R. R. and Larive, C. K. (2019). Evaluating sub-lethal stress from Roundup® exposure in *Artemia franciscana* using 1H NMR and GC-MS. Aquatic Toxicology, 212: 77-87. https://doi.org/10.1016/j.aquatox.2019. 04.023.

Naikodi, M. A. R.; Chaithra, D.; Venkatesham, B.; Siddiqui, J. I. and Husain, M. (2021). Phytonanotechnological perspectives and biological activities in *Curcuma species*. Ann. Phytomed., **10**(2):82-89.

Nicolè, F.; Guitton, Y.; Courtois, E. A.; Moja, S.; Legendre, L. and Hossaert-McKey, M. (2012). MSeasy: Unsupervised and untargeted GC-MS data processing. Bioinformatics, **28**(17):2278-2280. https://doi.org/ 10.1093/bioinformatics/bts427

Pang, Z.; Zhou, G.; Ewald, J.; Chang, L.; Hacariz, O.; Basu, N. and Xia, J. (2022). Using MetaboAnalyst 5.0 for LC–HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. Nature Protocols, **17**(8):1735-1761. https://doi.org/10.1038/ s41596-022-00710-w

Patel, V.; Mehrotra, N. and Khan, S. A. (2019). *Ex vivo* study of *Cinnamomum zeylanicum* Blume on antioxidative system and structural modifications of erythrocytes under hyperglycemic conditions. Ann. Phytomed., **8**(2):150-155.

Peralbo-Molina, Á.; Solà-Santos, P.; Perera-Lluna, A. and Chicano-Gálvez, E. (2022). Data processing and analysis in mass spectrometry-based metabolomics. In mass spectrometry for metabolomics (pp: 207-239). Springer. https://doi.org/10.1007/978-1-0716-2699-3_20.

Pooja, U. K., Vasanthkumar, S. S. and Prathiksha, S. R. (2023). Biological active compounds in herbs and spices. In B. Pavan Kumar Naik, P. Sudheer Kumar Reddy, and G. Jyothi (Eds.), Advances in Horticulture and Allied Sciences (Vol. 2, pp. 1-14). Royal Book Publishing. ISBN: 9789395423861.

Rafieian, F.; Amani, R.; Rezaei, A.; Karaça, A. C. and Jafari, S. M. (2023). Exploring fennel (*Foeniculum vulgare*): composition, functional properties, potential health benefits, and safety. Critical Reviews in Food Science and Nutrition: pp:1-18. https://doi.org/10.1080/ 10408398.2023.2176817.

Reisetter, A. C.; Muehlbauer, M. J.; Bain, J. R.; Nodzenski, M.; Stevens, R. D.; Ilkayeva, O.; Metzger, B. E.; Newgard, C. B.; Lowe, W. L. and Scholtens, D. M. (2017). Mixture model normalization for non-targeted gas chromatography/ mass spectrometry metabolomics data. BMC Bioinformatics, **18**(1):84. https://doi.org/10.1186/s12859-017-1501-7.

Rubika, R.; Vasanthkumar, S. S.; Pooja, U. K.; Kumaresan, M.; Gowshika, R.; and Priya, L. (2023). Package of practice for cultivation of clove. In Naik, S., Sravanthi, B. and Ramala, S. K. (Eds.), Horticulture Science (Vol 2 pp. 144-154). Royal Book Publishing. ISBN: 978-81-19821-12-9.

Saadat, S.; Ghasemi, Z.; Memarzia, A.; Behrouz, S.; Aslani, M. R. and Boskabady, M. H. (2024). An overview of pharmacological effects of *Crocus sativus* and its constituents. Iran J. Basic Med. Sci., **27**(4):391-417. https://doi.org/10.22038/ijbms.2023.73410.15950.

Sharma, S. and Chakraborty, D. (2019). Antimicrobial and antioxidant activity of *Coriandrum sativum* L. Ann. Phytomed., **8**(1):135-139.

Srivastava, A. and Creek, D. J. (2020). Using the IDEOM workflow for LCMS-based metabolomics studies of drug mechanisms. Computational Methods and Data Analysis for Metabolomics, pp:419-445. https://doi.org/10.1007/978-1-0716-0239-3_21.

Stoica, F.; Rau, R. N.; Vele'cu, I. D.; Stănciuc, N. and Râpeanu, G. (2023). A comprehensive review on bioactive compounds, health benefits, and potential food applications of onion (*Allium cepa* L.) skin waste. Trends in Food Science and Technology, **141**:104173. https://doi.org/10.1016/j.tifs.2023.104173.

Tiwari, P.; Srivastava, R. and Mishra, M. K. (2023). Pharmacognostic investigation and biological evaluation of *Allium sativum* L. Ann. Phytomed., **12**(1):1-7.

Umoh, S. D.; Bojase, G.; Masesane, I. B.; Majinda, R. T.and Sichilongo, K. F. (2023). Untargeted GC-MS metabolomics to identify and classify bioactive compounds in *Combretum platypetalum* subsp. *oatesii* (Rolfe) Exell (Combretaceae). Phytochemical Analysis, **34**(1):127-138.

Vali, D. M., K. Venkatesan, N. Senthil, B. S. Selvi, and M. Raveendran. (2022). Comparative analysis of *Curcuma species* essential oil through gas chromatography-mass spectrometry. Medicinal Plants-International Journal of Phytomedicines and Related Industries, **14**(4):632-636.

Vali, D. M., K. Venkatesan, N. Senthil, B. S. Selvi, M. Raveendran, and S. Divya. (2022). Efficiency of curcuminoids against COVID-19 proteins and human ACE-2 receptor: A molecular docking study. Medicinal Plants-International Journal of Phytomedicines and Related Industries, **14**(4):644-647.

Vasanthkumar, S. S. (2023). Medicinal plants in traditional and modern folk medicine. In B. Pavan Kumar Naik, P. Sudheer Kumar Reddy, and G. Jyothi (Eds.), Advances in Horticulture and Allied Sciences (Vol. 2, pp:138-155). Royal Book Publishing. ISBN: 978939 5423861.

Vishwanath, V.; Sulyok, M.; Weingart, G.; Kluger, B.; Täubel, M.; Mayer, S.; Schuhmacher, R. and Krska, R. (2011). Evaluation of settled floor dust for the presence of microbial metabolites and volatile anthropogenic chemicals in indoor environments by LC-MS/MS and GC-MS methods. Talanta, **85**(4):2027-2038. https://doi.org/ 10.1016/j.talanta.2011.07.043.

Waris, M.; Kocak, E.; Gonulalan, E. M.; Demirezer, L. O.; Kýr, S. and Nemutlu, E. (2022). Metabolomics analysis insight into medicinal plant science. Trends in Analytical Chemistry, **157**:116795. https://doi.org/ 10.1016/j.trac.2022.116795.

Wehrens, R.; Weingart, G.and Mattivi, F. (2014). MetaMS: An open-source pipeline for GC-MS-based untargeted metabolomics. Journal of Chromatography B, **966**:109-116. https://doi.org/10.1016/ j.jchromb.2014.02.051.

Yang, Z.; Guo, Z.; Yan, J.and Xie, J. (2024). Nutritional components, phytochemical compositions, biological properties, and potential food applications of ginger (*Zingiber officinale*): A comprehensive review. Journal of Food Composition and Analysis, **128**:106057. https://doi.org/10.1016/j.jfca.2024.106057.

Yin, Z.; Huang, W.; Fernie, A. R. and Yan, S. (2023). Mass spectrometry imaging techniques: A versatile toolbox for plant metabolomics. Trends in Plant Science, **28**(2): 250-251. https://doi.org/10.1016/j.tplants. 2022.10.009.

Zhao, T.; Zhao, Y.; Chen, H.; Sun, W. and Guan, Y. (2024). A GC-MS-based untargeted metabolomics approach for comprehensive metabolic profiling of mycophenolate mofetil-induced toxicity in mice. Frontiers in Molecular Biosciences, **11**:1332090. https://doi.org/10.3389/fmolb.2024.1332090.

Zhao, Y.; Li, J.; Cao, G.; Zhao, D.; Li, G.; Zhang, H. and Yan, M. (2023). Ethnic, botanic, phytochemistry and pharmacology of the acorus genus: A review. Molecules, **28**(20):7117. https://doi.org/10.3390/molecules28207117.

Zheng, S.; Qin, W.; Chen, T.; Ouyang, R.; Wang, X.; Li, Q.; Zhao, Y.; Liu, X.; Wang, D. and Zhou, L. (2024). Strategy for comprehensive detection and annotation of gut microbiota-related metabolites based on liquid chromatography-high-resolution mass spectrometry. Analytical Chemistry. https://doi.org/10.1021/acs.analchem.3c05219.

Zulet-Gonzalez, A.; Gorzolka, K.; Döll, S.; Gil-Monreal, M.; Royuela, M. and Zabalza, A. (2023). Unravelling the phytotoxic effects of glyphosate on sensitive and resistant *Amaranthus palmeri* populations by GC-MS and LC-MS metabolic profiling. Plants, **12**(6):1345. https://doi.org/10.3390/plants12061345.

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