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# Assessing the chemical composition and ecological relevance of root exudates in legume species

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Global food security and environmental sustainability are closely linked to positive plant-soil feedback, for example through reduced dependence on synthetic agrochemicals. Beyond their nutritional value as protein- and lipid-rich crops, legumes have an essential ecological function by enhancing soil fertility and consequently reducing the use of synthetic fertilizers. Legume root systems exude a diverse array of secondary metabolites (SM) into the soil. These exudates affect the rhizosphere microbiome (structure and functions) as well as nutrient availability to the plant and stimulate microbial activity in the soil. Thereby they modulate ecological interactions, which may result in positive plant-soil feedback that can contribute to improve soil health and resilience of plant production systems. Considering the importance of legume root exudate and SMs to food production and environment protection, this review fills a gap in the literature summarizing the state-of-the-art regarding the elucidation of their chemodiversity and functional significance. For that, we have compiled the literature on legume root exudates of the last 25 years (from 1999 to 2024) resulting in a comprehensive list of secondary metabolites. We also discussed how they are accessed and their biological relevance in root symbiosis. We found that root exudates were characterized in 22 legume species, which led to the identification of 92 SM. The most reported metabolites are shikimate and phenylpropanoid derivatives, especially flavonoids. Based on the information organized in this review, we observed that the rapid progress of metabolomics and high-resolution analytical techniques provided invaluable data on legume exudate chemical diversity, although other chemical classes remain underrepresented. Besides, this knowledge remains fragmented across few legume taxa, with most research concentrated on a limited number of model and crop legume species. We argue that expanding the research beyond cultivated species, and combining metabolomics strategies to access their chemical diversity with ecological functions will strengthen the knowledge on their regulatory mechanisms and their influence on rhizosphere microbiome.

Ultimately, by addressing legume root exudate chemistry and ecological functions, this comprehensive review expands our current understanding and brings new insights on how such metabolites contribute to plant performance, enhance soil health and promote sustainable agricultural practices.

KEYWORDS

exudates, Fabaceae, legumes, metabolomics, plant interactions, secondary metabolites

# 1 Introduction

To ensure global food security, we must increase crop production in a sustainable manner in order to feed the growing human population while minimizing its environmental footprint (Wang et al., 2025). However, the sustainable provision of sufficient food is hampered by various environmental and social factors, including climate change, leading to an increasing frequency of extreme weather events, changes in dietary habits and a high level of food waste (Preece and Peñuelas, 2020).

Agricultural intensification has boosted crop productivity, but often at the expense of soil fertility and health, as well as biodiversity, largely due to the high reliance on synthetic fertilizers and pesticides (de Graaff et al., 2019). These practices have been threatening long-term ecosystem stability by contributing to greenhouse gas emissions and environmental pollution, thereby exacerbating climate change. Therefore, strategies are required to restore and maintain soil fertility and health through practices that improve nutrient cycling and reduced dependence on agrochemicals.

The incorporation of legumes in crop rotation enhances plant diversity and thus microbial diversity in the soil and this contributes to soil fertility (Kokkini et al., 2025; Walia et al., 2025). Legumes establish symbiotic relationships with nitrogen-fixing bacteria, resulting in improved soil organic matter and structure. In addition, the deep roots of legumes and their residues introduce both carbon and nitrogen into the soil, thereby reducing the dependence on synthetic nitrogen fertilizers. Important agricultural legume crops, such as soybeans and alfalfa, fix between 40 and 90 teragrams nitrogen (N) per year (Taylor et al., 2020).

Legume-based rotations benefit subsequent non-legume crops through soil "legacy effects", as legumes positively shape soil microbial community structure (Schaedel et al., 2021) thereby reducing disease pressure caused by soil-borne pathogens (Wei et al., 2014). The cultivation of legumes contributes in crop rotation thus sustaining sustainable agriculture and food security (Egli et al., 2021) by increasing the diversity of soil microbiota, and enhancing ecosystem resilience (Wagg et al., 2021; Yang et al., 2025; Stagnari et al., 2017; Telles et al., 2023). Consequently, legumes represent both a cornerstone of global food security and a driver of ecosystem services that support more resilient and environmentally sustainable agriculture.

New strategies are needed to strengthen the capacity of plants to cope both abiotic and biotic stressors. To meet these challenges, it is necessary to focus not only on improving important aboveground production traits, such as yield, taste and flavor, but also to belowground traits, including root architecture and root exudation patterns (Griffin and Jungers, 2025). Even though belowground plant traits are vital for nutrient cycling, soil formation/stability, and ecosystem functioning, they remain understudied, which limits our understanding of their broader ecological importance (van Dam and Bouwmeester, 2016). The interaction between plants and the surrounding soil microbiome and environment occurs through root exudation. From a functional-metabolite perspective, exuded compounds can be evaluated according to their ecological effects, including signaling, defense, chelation, and nutrient mobilization (Ma et al., 2022). For example, isoflavonoids released by legumes function as key signals to induce symbiotic nitrogen-fixing relationships with rhizobia. In addition, they may suppress fungal pathogens in the soil, contributing to the maintenance of a beneficial rhizosphere microbial community (Yang et al., 2025). Increasing our knowledge of root exudate metabolic profiles is therefore important to understand how they may contribute to sustainable food security under global change conditions.

Root exudates comprise organic molecules, including organic acids, sugars, amino acids, phenolic compounds, volatile organic compounds (VOCs), and phytohormones (van Dam and Bouwmeester, 2016; Massalha et al., 2017; Sharma et al., 2023), which in turn acts as signaling molecules in plant-environment, plant-plant, and plant-microbe interactions (Yang et al., 2025). The quantity of root exudation depends mainly on the plant species, age, cultivar type, plant root metabolic attributes, root system architecture, and environmental conditions encountered during plant growth (Cesari et al., 2019). Root exudate composition changes in response to abiotic and biotic stress, thereby mediating the recruitment and assembly of microorganisms by multiple mechanisms (Vives-Peris et al., 2020; Yang et al., 2025). Therefore, these metabolites are increasingly recognized as key drivers of soil health and plant resilience, linking legume cultivation to both agricultural sustainability and ecological restoration.

Considering the role of legumes in improving soil and plant health, information about the chemical composition and functional characterization of legume root exudates, with an emphasis on bioactive SMs, is crucial for the development and implementation of effective, sustainable, and resilient agricultural systems.

# 2 Role and relevance of root exudates

Most terrestrial plants including crops release root exudates (Bais et al., 2006; Rasmann and Hiltpold, 2022; Robert et al., 2025).

Root exudates are defined by van Dam and Bouwmeester (2016) as "the total of molecules actively or passively released by plant roots into the soil or any other medium surrounding the roots". Therefore, root exudation mediates belowground interactions between plants, their environment, and dynamic ecosystems, which in turn affects plant growth performance (Oburger and Jones, 2018; Rasmann and Hiltpold, 2022; Yang et al., 2025).

These exudates are a complex mixture of primary and secondary metabolites (SM) and, based on their molecular weight, the organic molecules exuded by roots can be categorized into three groups: (i) low-molecular-weight compounds, including sugars, amino acids, small peptides, phytohormones, organic acids, volatile organic compounds (VOCs), and SM such as phytosiderophores, phenolics, and alkaloids; (ii) high-molecular-weight compounds, comprising mucilage (polysaccharides and polyuronic acids) and proteins, and (iii) extracellular enzymes (Ma et al., 2022). While high-molecular-weight compounds such as proteins and mucilage constitute essential components of root exudates, the diversity is considerably greater among low-molecular-weight metabolites (Balyan and Pandey, 2024).

Root exudation occurs via various mechanisms, summarized in the Figure 1, including passive release through cell sloughing and mucilage secretion at the root tip, as well as active transport across cell membranes by special transporter proteins, such as those that mediate the secretion of siderophores for the chelation and uptake of metal ions (Badri and Vivanco, 2009; Nozoye et al., 2010; Ma et al., 2022). The majority of root exudates are primary metabolites, which are released predominantly passively via diffusion at the root tips. Root tips are the part of the plant that explores the soil and are therefore crucial for the root response to environmental

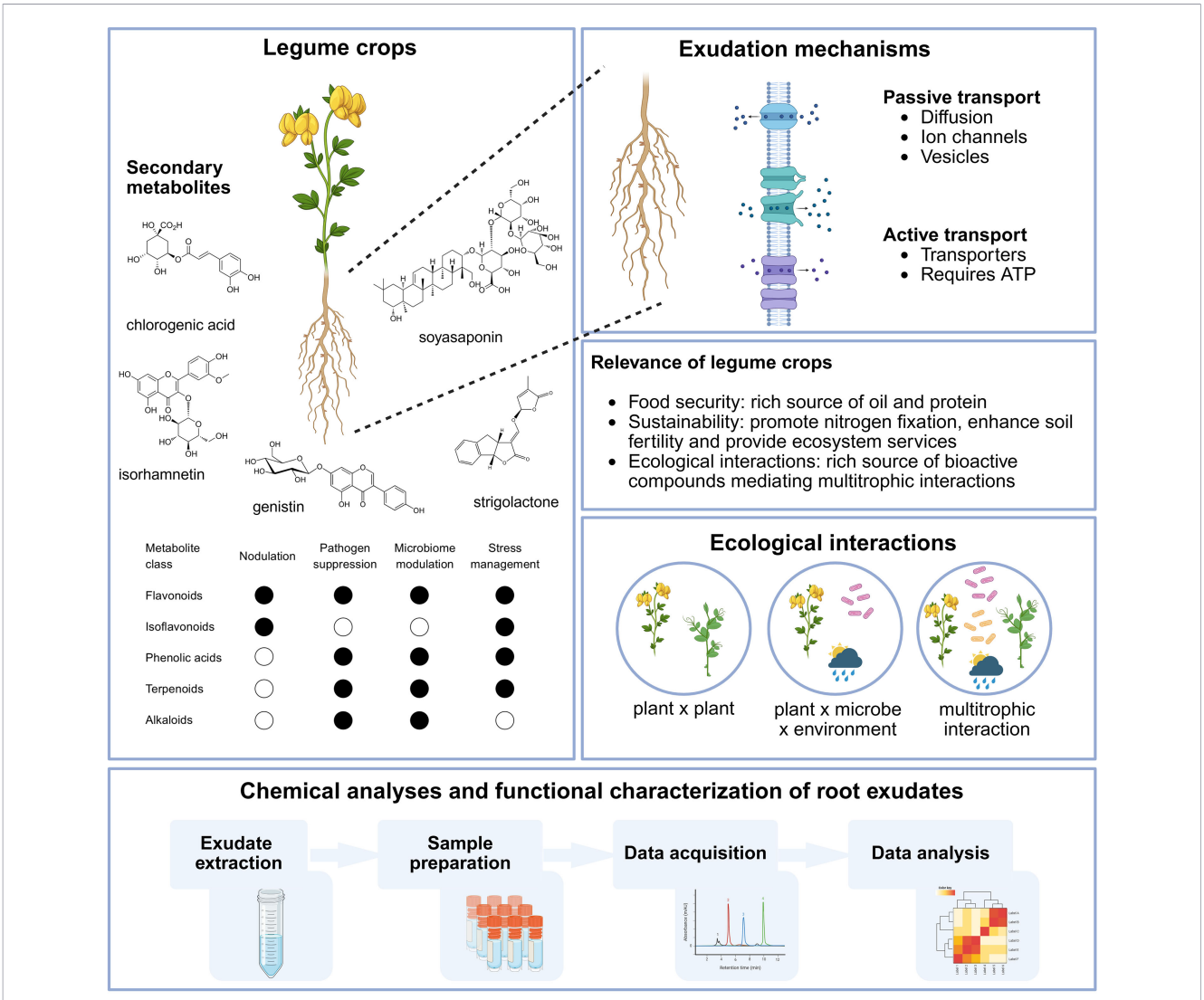


FIGURE 1
Legume crops are essential in food security, providing plant-based proteins as food, feed and fodder, as well as essential ecosystem services such as increasing soil health. Their root exudates are a rich source of primary and secondary metabolites, which mediate ecological interactions between plants, microorganisms, and the soil. The metabolic composition of root exudates is determined by plant physiological processes as well as abiotic and biotic factors. The chemical and functional characterization of secondary metabolites in root exudates provides invaluable information for understanding the mechanisms underlying these interactions. For this purpose, cutting-edge analytical technologies, ranging from sample extraction to data analysis, have been increasingly utilized to elucidate these chemical interactions. This figure was created with BioRender (https://app.biorender.com/) and is licensed for publication.

stimuli (Canarini et al., 2019). Other compounds, such as SM, proteins, and polysaccharides, are generally secreted into the rhizosphere by an active mechanism via different membrane-bound proteins. Plants regulate the abundance of these metabolites either through source-sink dynamics or via the expression and modulation of specific efflux carriers (Afridi et al., 2024; Chen and Liu, 2024).

By shaping the local flux of root exudates, plants can alter the concentration of common metabolites in the rhizosphere, where these compounds function as integrators of plant nutritional status and as indicators of nutrient availability in the immediate environment (Canarini et al., 2019). The production of root exudates is specific to plant species and their genotype, even though there are some similarities between plant species (McLaughlin et al., 2023; Iannucci et al., 2021). Exudation is a dynamic process, meaning that the composition of root exudate profiles is influenced by the plant's stage of development, its nutrient status, the soil environment, its root traits (*e.g.*, root architecture), and its root metabolic attributes (Cesari et al., 2019; Ritter et al., 2025). Besides, root exudation is an important component for carbon input into the soil, accounting for 5% to 25% of the total photosynthetically fixed carbon released into the rhizosphere (Jones et al., 2009). Therefore, root exudates contribute to the global carbon-nitrogen cycle, shaping physiochemical properties of soil.

Root exudates also act as nutrients and signals to root-associated microorganisms, as well as to enhance nutrient availability for plant uptake. To this end, the plant uses root exudates to alter the rhizosphere environment. This includes solubilizing recalcitrant nutrients such as phosphorus, facilitating root penetration through compacted soils via mucilage sheaths, and helping roots navigate physical barriers (Falik et al., 2005; Novoplansky, 2019). In addition, root exudates act as core components in ecological signaling. The Box 1 summarizes the main roles and mechanisms of root exudates and their effects on the microbial community. Overall, root exudates integrate nutrient acquisition and ecological signaling, providing a critical link between plant physiology, rhizosphere ecology, and soil environment (Lyu and Smith, 2022; Ma et al., 2022).

In general, root exudates contribute to shape soil ecology by chelating micronutrients and mobilizing phosphorus and iron, thereby modifying nutrient availability in the rhizosphere. Low molecular weight organic compounds released by root exudates, including organic acids (such as citric acid, malic acid, and oxalic acid), phenols, and flavonoids, promote iron uptake mainly through chelation by mobilizing insoluble iron or by acidifying rhizosphere (Ma et al., 2022).

Shikimate- and phenylpropanoid-derived metabolites such as phenolic acids (*e.g.*, ferulic, coumaric, caffeic, and salicylic acids), flavonoids, lignin monomers, and tannins, are among the most frequently reported compounds mediating plant-microbial interactions in soil ecology through root exudation (Tingting et al., 2025). They act as powerful chemical signals that recruit and structure microbial communities, including beneficial bacteria and mycorrhizal fungi, while suppressing pathogens through antimicrobial or allelopathic effects (Sugiyama and Yazaki, 2014).

In plant defense against pathogenic microorganisms, phenolic acids (such as ferulic, *p*-coumaric and caffeic acids) act as signaling molecules leading finally to the up-regulation of defense-related genes. High levels of phenolic acid often stimulate the production of salicylic acid and hydrogen peroxide. Both are key, interconnected signals in plants, that are crucial for the establishment of systemic acquired resistance (Cao et al., 2024). They also trigger the Mitogen-Activated Protein Kinase (MAPK) signaling pathway, which promotes the synthesis of protective compounds such as flavonoids, further enhancing plant resistance. At the same time, they can directly suppress pathogen growth by damaging their cell membranes and disrupting their metabolism (Tingting et al., 2025).

In agricultural systems, the strategic use of such metabolites or systems able to release them offers promising routes to enhance crop productivity, resilience, and protection. Biological nitrification inhibition mediated by root-exuded secondary metabolites, for instance, is a biological alternative to reduce the nitrification process, which is the primary driver of N loss in many agroecosystems. Species such as *Brachiaria humidicola*, *Thinopyrum intermedium*, sorghum (*Sorghum bicolor*), and pearl millet (*Pennisetum glaucum*) release compounds, including brachialactone, sorgoleone, vanillic acid, and coumarins, which can suppress nitrifying microorganisms by 60–90% under controlled and field conditions (Ahmed et al., 2026). Indeed, breeding or engineering crops with optimized production or exudation of specific compounds improve nutrient acquisition efficiency and foster beneficial soil microbiomes, reducing dependence on synthetic fertilizers. These metabolites can also strengthen plant defense by priming immune responses and directly inhibiting pests and pathogens, while also shaping the rhizosphere microbiome toward disease-suppressive and plant-protective functions, thereby supporting more sustainable plant protection strategies (Berendsen et al., 2012).

The multifunctional nature of root exudates sets the stage for their diverse ecological functions, ranging from mediating plant-plant interactions to shaping complex microbial communities, responding to, or alleviating environmental stress factors. In the following sections we will discuss those interactions with focus on legume species.

## 3 Legume crops: classification and importance to global food security

Legumes are angiosperms belonging to the family Fabaceae Lindl., *nom. cons.*, also referred to as Leguminosae Juss., *nom. cons.* Fabaceae is the third largest family of flowering plants, with approximately 20,000 species, surpassed in size only by Asteraceae and Orchidaceae (APG IV, 2016; LPGW, 2017). The family has a cosmopolitan distribution, with its primary diversity hotspot located in tropical regions, particularly in South America, followed by Africa and temperate Asia (LPGW, 2017; Siddiqui, 2025). Legume plants have undergone several domestication events throughout human history (*e.g.* Rendón-Anaya et al., 2017; Zheng et al., 2024).

**BOX 1** Main functional roles and mechanisms of root exudates in structuring microbial communities.

1. Recruitment and assembly of beneficial microbes and microbiomes.
   - Mechanisms: Root exudates contain sugars, amino acids, organic acids, phenolics that act as chemo-attractants; Specific compounds guide chemotaxis, colonization, and biofilm formation; They increase exudation of selective signals ("cry-for-help") that attract plant growth-promoting and mutualistic microorganisms.
   - Effects on the microbial community: Enhance growth and establishment of beneficial microbes such as *Bacillus*, *Pseudomonas*, rhizobia, mycorrhizal fungi; Enhance root colonization efficiency, improve nutrient mobilization, increase hormone-related functions, and greater plant stress tolerance.
   - References: Liu et al., 2024; Yang et al., 2025.
2. Modulation of microbial signaling networks.
   - Mechanisms: Root exudates alter quorum-sensing (QS) dynamics; Microbial QS molecules influence community behavior and biofilms; Exudates modulate inter-microbial communication.
   - Effects on the microbial community: Increase the cooperative interactions among beneficial microbes (biofilm formation, coordinated metabolite production); Stronger signaling networks support community resilience and plant growth.
   - References: Upadhyay et al., 2022; Ahmed et al., 2026.
3. Suppression and modulation of pathogenic microorganisms.
   - Mechanisms: Exudation of antimicrobial secondary metabolites that control pathogens directly or indirectly; Altered exudate profiles in response to pathogen attack can recruit antagonists that suppress pathogens; Competition for exudate-derived nutrients reduces resources available to pathogens.
   - Effects on the microbial community: Decrease prevalence and virulence of soilborne pathogens; Pathogen suppression mediated by recruited beneficial taxa that antagonizes pathogens.
   - References: Berendsen et al., 2012; Sugiyama and Yazaki, 2014; Yuan et al., 2018; Tingting et al., 2025.
4. Biocontrol promotion and defensive plant-microbial feedback.
   - Mechanisms: Recruitment of microbial biocontrol agents that produce antibiotics, siderophores, and volatiles; Plant-microbe feedback loops modify subsequent exudation patterns.
   - Effects on the microbial community: Stimulation of microbes that induce systemic resistance; Strengthen the plants immunity against pathogens; Enhancing the efficacy of biocontrol agents through modified exudate patterns.
   - References: Upadhyay et al., 2022; Yang et al., 2025.

Most cultivated legumes belong to the subfamily Papilionoideae, one of the six currently recognized subfamilies within the Fabaceae family, alongside Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, and Duparquetioideae (LPGW, 2017; Choi et al., 2022).

After cereals, legumes represent the second most important crop, contributing substantially to global food security due to their high protein content. They provide pulses, oil seeds, forage, and fresh green pods, serving as vital sources of protein, lipids, and micronutrients for humans and livestock (FAO, 2025). Soybean [*Glycine max* (L.) Merr.], a major warm-season crop, is a leading global source of vegetable oil and protein for human and animal diets. Other important warm-season legumes, including common bean (*Phaseolus vulgaris* L.), peanut (*Arachis hypogaea* L.), cowpea [*Vigna unguiculata* (L.) Walp], and lupines (*Lupinus* spp. L.), along with cool-season species such as lentil [*Vicia lens* (L.) Coss. & Germ.], chickpea (*Cicer arietinum* L.), fava bean (*Vicia faba* L.), and peas (*Lathyrus oleraceus* Lam., formerly known as *Pisum sativum* L.) are central to food and nutrition security, poverty alleviation, and sustainable agriculture. Collectively known as pulses, these crops are defined as the dried, edible seeds of certain leguminous plants, characterized by high protein and fiber content, as well as low fat levels. In contrast, forage legumes such as alfalfa (*Medicago sativa* L.) and *Lotus japonicus* (Regel) K. Larsen are designated as oil crops due to their elevated fat content (Bueno and Lopes, 2020).

Besides legume crops, wild legumes, perennial species, and tropical leguminous trees contribute to a plethora of rhizosphere processes and symbiotic interactions. Wild legumes support diverse rhizobia with unique exudation traits that influence soil microbes and nutrient cycling. Symbiotic associations in wild and tree legumes contribute to nitrogen fixation and soil fertility beyond agricultural systems (Zahran, 2001). Perennial legumes influence soil structure, organic matter, and belowground functional processes through persistent root-microbe interactions (Drinkwater et al., 2021). Finally, leguminous trees in tropical forests mediate soil nitrogen, plant neighbor diversity, and belowground interactions (Xu et al., 2020).

# 4 Ecological functions of legume root exudates and multitrophic interactions

## 4.1 Plant-plant interaction

Plant-plant interactions influence the structure of plant communities, the distribution of resources, and the resilience of ecosystems. Legume root exudates mediate complex exchanges with neighboring plants, influencing growth, competition, and defense. For example, intercropping peanut with maize enhances agricultural productivity and positively modulates secondary metabolism, particularly through the secretion of isoflavonoids (Jiang et al., 2022). Similarly, in banana-legume intercropping systems, such as with *Desmodium uncinatum* (Jacq.) DC. and *Mucuna pruriens* (L.) DC., the secondary metabolites released into the rhizosphere modify soil chemistry, suppress soil-borne pathogens, and contribute to sustainable disease management (Were et al., 2022). Under competitive conditions, the co-cultivation of legumes such as alfalfa and clover with durum wheat results in a significant increase in the production and exudation of flavonoids (Leoni et al., 2021).

The plant-plant interaction is also well studied in weed (parasitic) control, in which specific allelopathic compounds have been described. For example, trigoxazonane from *Trigonella foenum-graecum* L. inhibits *Orobanche crenata* Vell. seed germination (Evidente et al., 2007); vestitol contributes to defense against *Striga hermonthica* (Delile) Benth. parasitism in *Lotus japonicus* (Ueda and Sugimoto, 2010); and *C*-glycosylflavonoids

from *D. uncinatum* prevent striga parasitism of maize (Hooper et al., 2010). The chemical characterization of biologically active fractions from *D. uncinatum* root exudates, using mass spectrometry and NMR spectroscopy, has elucidated the structure of these compounds (Tsanuo et al., 2003).

## 4.2 Legume-microbe interactions mediated by root exudates

In general, root exudates are a nutrient source for soil microbes (Ma et al., 2022), but they also contain compounds governing the intricate interaction between plants and soil microbes. Legumes form a symbiosis with specific beneficial microbes, which supply the plant with nutrients such as nitrogen (N) and phosphorus, but also help to cope with abiotic and biotic stressors (Lamichhane et al., 2023; Lamichhane et al., 2024). Legume root exudates therefore differ from other plant systems due to their ability to promote a symbiotic relationship with N-fixing bacteria, enabling the biological N-fixation by converting atmospheric $N_2$ into ammonia ($NH_3$), a form that can be utilized by plants. Metabolites released by roots act as signals in the interaction with microbes, such as in recognition of certain bacteria able to fix nitrogen (collectively referred as rhizobia). Rhizobia (*e.g.*, genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*) are well-known symbiotic partners of legumes, forming nitrogen-fixing nodules in plant roots (Masson-Boivin et al., 2009). Root-associated arbuscular mycorrhizal fungi are also symbionts of legumes, promoting the nodulation and $N_2$-fixation (Li et al., 2025). In this process, various steps occur prior to the establishment of a beneficial symbiotic partnership between the legume and the symbionts.

Legume attracts rhizobia over short distance from the soil by specific root exudates such as flavonoids, thereby influencing the assembly and structure of the entire root-microbiome (Bais et al., 2004; Tsiknia et al., 2021; Hartman et al., 2023). In that sense, unlike bulk exudates such as sugars and organic acids, legume common compounds such as the isoflavones naringenin, daidzein, genistein, which activate rhizobial nodulation genes, can be considered key compounds that even in lower concentrations can trigger a significant ecological effect. For instance, young roots of *Eperua falcata* Aubl., a tropical rainforest tree, exudes the flavonoid liquiritigenin, modulating microbial communities by inhibiting nitrate-consuming microbes and promoting beneficial symbionts, thereby optimizing nitrogen uptake (Michalet et al., 2013).

Rhizobia, in turn, secrete specific signaling molecules, known as Nod factors (lipochitooligosaccharides molecules), which are fundamental for inducing all early responses required for symbiosis establishment. Research on *Lotus japonicus* shows that Nod factor production is induced in symbionts primarily when host-specific (iso)flavonoids are secreted from starved roots; then symbiont-derived Nod factor signals contribute to microbiota homeostasis indirectly, via the host. The symbiont modulates the remaining members of the community by inducing Nod factor-dependent signaling in the host, which in turn ensures a diverse and interconnected bacterial microbiome, likely via changes in the exudate profile (Tao et al., 2024). This signaling pathway provides a mechanism framework in which a defined plant metabolite triggers a measurable and reproducible microbial genetic response, which represents a causal metabolite–microbe interaction in plant systems.

In soybeans, inoculation with a *Bradyrhizobium diazoefficiens noeI* mutant deficient (that does not form nodules) reduces flavonoid exudation, particularly daidzein, thereby altering the root microbiome (Liu et al., 2021). Comparable results were found in pea, where higher flavonoid exudation occurs in symbiosis with the wild-type of the rhizobia *Rhizobium leguminosarum* compared to the interaction with the *noeI* mutant, which affects microbial recruitment and symbiosis efficiency (Pini et al., 2017). This underlines the importance of specific root exudates for the legume-symbiont interaction (Liu et al., 2021).

Specific phytohormones, such as strigolactones exuded for example by *L. japonicus* or *P. sativum*, act as a signal to promote the symbiosis with arbuscular mycorrhizal fungi and interaction with non-mycorrhizal microbes including rhizobia (Kim et al., 2022; Klein et al., 2024; Mcadam et al., 2017). Primary metabolites also shape microbial behavior: fumarate in intercropping of maize with pigeon pea exudates induce chemotaxis and biofilm formation in *Rhizobium* sp. IC3109 (Vora et al., 2021).

The great variety of compounds in roots exudates can interfere with, or modulate quorum sensing signals (QS), thereby influencing QS–regulated behaviors such as biofilm formation, N-fixation, synthesis of degradative enzymes, exopolysaccharides, toxins, and nutrient metabolism in rhizosphere microbes (Hassan and Mathesius, 2012). Microbes, thereby, can manipulate root exudation to improve the nutritional environment and facilitate their own establishment in the root system (Stringlis et al., 2018). QS and metabolite exchange among rhizosphere bacteria, such as *Pseudomonas, Bacillus*, and *Streptomyces* species, can regulate biofilm formation, nutrient mobilization, and antagonistic activity against pathogens, ultimately shaping microbial community structure and ecosystem functionality (Venturi and Keel, 2016). For example, *Pseudomonas aeruginosa* P4 alters peanut root exudates to increase the production of organic acids, amino acids, and phenolics, thereby enhancing its colonization and the recruitment of nodulating *Bradyrhizobium* strains (Gupta et al., 2020).

In soils contaminated with persistent pollutants such as phytotoxic polychlorinated biphenyls (PCBs), legumes like clover and alfalfa contribute to rhizoremediation by sustaining the degradative potential of the soil microbiome. PCBs are phytotoxic, dramatically impairing plant physiology and metabolic functions. Therefore, plants also employ the 'cry-for-help' strategy to recruit degradative microbes and fuel their catabolic activities in the rhizosphere, ultimately alleviating pollutant-induced stress (Rolli et al., 2021). In the clover rhizosphere cultivated in a site polluted by metals and organic pollutants, co-occurrence network and correlation analyses showed that Rhizobiales and Micromonosporales were associated with cadmium removal. At the same time, Rhizobiales, Burkholderiales, and Xanthomonadales were identified as the leading players for PCB clean-up (Wang et al., 2023).

Interestingly, flavonoids are recognized as inducers of the bacterial degradative operon *bph* due to their structural similarities with the PCB backbone (Ghitti et al., 2022), although other exudate chemical families may also be involved (Musilova et al., 2016; Rolli et al., 2024).

In addition, legumes release a wide array of root exudates, that regulate interactions with soil microbes as well as with neighboring plants and environmental stressors (Si et al., 2025). This ability enhances soil fertility and also increases carbon sequestration by stabilizing organic carbon through root biomass and microbial activity. Ultimately, this process reduces the need for synthetic fertilizers, which is crucial for sustainable cropping systems (Srivastava et al., 2025). In intercropping systems, ethylene signaling has also been shown to mediate legume–microbe–environment feedback: in peanut-cassava associations, cyanide released by cassava induces ethylene production in peanut roots, which reconfigures the rhizosphere microbiota by promoting keystone taxa such as *Catenulispora* sp., ultimately improving nutrient availability and seed production (Chen et al., 2020). Overall, legume root exudates orchestrate the assembly of microbial communities, symbiotic efficiency, and ecosystem functions. They recruit a broad spectrum of bacterial genera, including *Nitrobacter*, *Agrobacterium*, *Azospirillum*, *Citrobacter*, *Ochrobactrum*, *Paracoccus*, *Pseudomonas*, *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, and *Stenotrophomonas*, which contribute to nutrient cycling, plant growth promotion, pathogen suppression, and rhizoremediation.

Beyond those examples, other experimental approaches have strengthened causal inference in studies of legume root exudates. Gnotobiotic systems and synthetic community (SynCom) experiments enable testing of microbial responses to salicylic acid under sterile and environmentally controlled conditions (Bai et al., 2015; Lebeis et al., 2015). In parallel, the use of plant mutants impaired in flavonoid biosynthesis has revealed altered nodulation efficiency and changes in microbial colonization patterns, demonstrating that modification of specific metabolic pathways can directly influence microbial behaviors (Subramanian et al., 2006). Stable isotope tracing approaches allow tracking of plant-derived carbon into defined microbial taxa, confirming active metabolic utilization rather than simple co-occurrence (Fan et al., 2022). Together, these controlled experimental strategies illustrate how functional causality are established in legume metabolite–microbe interactions.

## 4.3 Legume-environment-microbe interactions mediated by root exudates

As the richest source of organic molecules in the soil environment, plant root exudates not only modulate belowground interactions, which reflect the selection of beneficial or pathogenic microorganisms, but also improve soil fitness through the adjustment of physical and chemical conditions (Massalha et al., 2017). However, plants are continuously exposed to various environmental stress factors, including heat, cold, drought, salinity, waterlogging, heavy metal toxicity, nutrient deficiency, and oxidative stress, compromising symbioses and triggering the adaptive recruitment of beneficial microbes (Yang et al., 2025). Nitrogen availability, water limitation, and salinity dynamically modulate root exudate pattern, impacting the structure and function of associated microbiomes (Chen et al., 2020).

Under drought, the complex alterations in the composition of plant root exudates depend on the level of the stress factor, growth conditions, and the species. For instance, Cesari et al. (2019) found that restrictive water conditions alter root exudation of peanut (*Arachis hypogaea* cv. Granoleico) increased the amount of naringenin, oleic fatty acid, citric acid, and lactic acid secreted, while stimulating terpene secretion, which are essential for the recruitment and colonization of rhizobia, such as *Bradyrhizobium* sp. and *Azospirillum brasilense* (Cesari et al., 2019). Drought also triggers the release of soluble sugars, amino acids, and organic acids, providing osmotic adjustment and creating conditions that support the growth of drought-tolerant microorganisms (Williamsde Vries, 2020). Under this condition, however, greater chemotaxis was observed when simple and double inoculation with *Bradyrhizobium* SEMIA6144 and *Azospirillum brasilense* Az39 was applied, suggesting that this could be related to the molecules exuded by the root under water deficit.

Salinity leads to decreased plant productivity and loss of soil fertility, reducing the nodulation and nitrogen fixation, deduced by the disruption of molecular communication required for symbiosis establishment. For instance, the early events of *Mesorhizobium*-chickpea symbiosis were affected by salinity due to substantial changes in the composition of phenolic compounds of chickpea (*C. arietinum*) root exudates with negative effects on *Mesorhizobium*-chickpea symbiosis (Ben Gaied et al., 2024a). Although the inoculation with halotolerant endophytes partially rescued the *Mesorhizobium*-chickpea symbiosis, this study highlights the fragility of plant-microbe positive interactions under abiotic stresses (Ben Gaied et al., 2024a).

Decreased nodulation and N supply have been observed in grain legumes under high-salinity conditions, resulting from disruptions in molecular signaling or failures in the infection process. The study by Ben Gaied et al. (2024a) further showed the presence of 13 phenolic compounds (such as gallic acid, chlorogenic acid, 1,3-di-*O*-caffeoylquinic acid, rutin, and quercetin-3-*O*-galactoside) in root exudates collected under control conditions. In contrast, only 7 of these compounds were identified in reduced concentrations in the exudates exposed to salt stress.

The legume-*Rhizobium* symbiosis is also affected by heat stressor, either directly by limiting the microsymbiont growth and/or indirectly by modulating the physiological and biochemical state of the host plant. Ben Gaied et al. (2024b) showed the potential of non-rhizobial endophytes from root nodules of wild legumes grown in arid regions to improve the symbiotic performance of rhizobia lacking 1-aminocyclopropane-1-carboxylate deaminase (ACC, a key enzyme involved in the ethylene modulation and in the nodulation process) in *Pisum sativum* (pea) under heat stressor. Their findings show that the phenolic content of pea root exudates increased under heat, while the content of the phytohormone indoleacetic acid (IAA) remained

unaffected, resulting biofilm formation in consortia containing rhizobia and non-rhizobial endophytes and indicating synergistic bacterial interactions.

The effect of elevated temperature in legume roots exudates was also investigated in alfalfa (*M. sativa*), when combined with cadmium contamination and arbuscular mycorrhizal fungi colonization (*Funneliformis mosseae*) (Ding et al., 2024). The presence of *F. mosseae* in elevated temperature and cadmium contamination led to a significant increase in total soluble sugars, mannose, rhamnose, caffeic acid, and total flavonoids in alfalfa roots. Additionally, the presence of *F. mosseae* also decrease glucose, chlorogenic acid, *p*-coumaric acid, ferulic acid, kaempferol, apigenin, and tricin under elevated temperature and cadmium contamination, which was reflected in significant decrease in root exudation. Through these interactions, root exudates integrate plant metabolism, microbial community dynamics, and environmental adaptation, highlighting the multifaceted functions of the legume holobiont in agrosystem functioning.

## 5 The chemistry of legume root exudates with a focus on secondary metabolism

Although the plant metabolome includes products of primary and secondary metabolism (SM) (Pang et al., 2021), in the context of root exudates, SM are of particular interest because they act as chemical mediators of plant-plant, plant-microbe, and plant-environmental interactions (Rizaludin et al., 2021; Wang et al., 2022). SM are specialized compounds involved in plant defense against pathogens and herbivores by shaping microbial communities in the rhizosphere and influencing interactions between plants through allelopathy (Liu and Smith, 2022). Given the variability of Fabaceae family, the plant domestication shapes the SM release in the rhizosphere and the ecological consequences. The domesticated plants have often less genetic variability, reflecting in the reduced metabolic diversity, compared with their wild relatives, and simplifies or shifted exudate compositions (Ku et al., 2020; Pueppke et al., 1998). As a consequence, modifications in ecological functions of root exudates occur, including microbial recruitment, nutrient mobilization, and defense against pathogens, reconfiguring beneficial plant-microbe associations (Ku et al., 2025). In legumes, changes in exuded flavonoids and other signaling compounds affect symbiotic communication with rhizobia and the broader rhizosphere community, leading to differences in N-fixation efficiency and ecosystem functioning relative to wild progenitors (Ku et al., 2020).

Aiming to summarize the knowledge on secondary metabolites in the root exudates of legumes, this review encompasses research articles published over the last 25 years. A total of 92 SM were identified in 22 legume root exudates included in this review (Table 1). The great majority of the studied species belong to the subfamily Papilionoideae, which includes pulses and oil crops. Peanut, soybean, and peas are the three main pulse species studied regarding root exudates and secondary metabolism, while alfalfa and *L. japonicus* are the most studied oil/forage species (Figure 2). Interestingly, the tropical tree species *E. falcata* is the only member of the subfamily Detarioideae whose root exudates have been studied, with samples collected *in situ*. From the root exudates of this species, the flavanones liquiritigenin and liquiritigenin glycoside, the chalcones isoliquiritigenin and isoliquiritigenin glycoside, and the aurone sulfuretin were identified by HPLC-DAD-ESI-QTOF (Michalet et al., 2013).

Most of the compounds identified in these studies belong to the superclass of flavonoids and cinnamic acid derivatives, both of which are biosynthesized by the shikimic acid biosynthetic pathway (Figure 3). Indeed, flavonoids constitute a major group of SM that have been widely studied in plant root exudates. Compounds belonging to this class are phenylpropanoids containing a diphenyl propane (C6-C3-C6) backbone in which two aromatic rings (A and B) are linked through the central three-carbon chain (C ring). Hydroxylation, methylation, glycosylation, or acylation of the A and B rings confer distinct biological activities (Wang et al., 2022). Based on the saturation and oxidation of the C ring, the majority of these compounds are allocated within six major categories including flavonols, flavones, isoflavones, anthocyanins, flavanones, and flavanols. Examples of such classes identified in legume root exudates can be found in Figures 4, 5.

Flavonoids can be secreted into the rhizosphere either actively, via ATP-dependent ABC transporters, or passively through root cell degradation. One of their functions in legumes is to induce nodule formation by activating bacterial nod*D* genes, which then trigger other nod genes to establish symbiosis with Rhizobia. Apart from their function in inducing nodulation, they promote phosphorus and iron acquisition, auxin biosynthesis and localization, pollination facilitation, UV and antioxidant protection (Kumar et al., 2024). In particular, isoflavonoids are among the most characteristic flavonoids in legume species. They are formed by isoflavone synthase (only found in legumes), a cytochrome P450 enzyme that converts flavanones (*e.g.*, naringenin) into isoflavones such as daidzein and genistein (Wang et al., 2022). In legumes, the active forms of vacuolar isoflavonoids, the aglycones, can be rapidly secreted via apoplastic $\beta$-glucosidase to promote legume–rhizobia symbiosis and strengthen plant defenses against pathogens, and to induce rhizobial nod genes that initiate root hair curling, infection thread formation, and nodule development in cortical cells (Kumar et al., 2024).

Terpenoids and derivatives are the second major class of compounds studied in legume root exudates. Saponins and oleanane derivatives stand out among the identified compounds, since they also constitute chemical markers in legume species. Saponins are amphiphilic molecules with polar sugar moieties attached to a nonpolar fat-soluble pentacyclic aglycone (fat-soluble). They can be classified into triterpenoidal, steroidal, or steroid-alkaloidal glycosides. In legumes, they are oleanane triterpenoids, biosynthesized by the mevalonate pathway (Bueno and Lopes, 2020). The soyasaponins, ubiquitously present in legume plants and in their root exudates, consist of a soyasapogenol (aglycone) backbone linked to oligosaccharide moieties. Soyasaponins are typically classified into four subgroups based on their aglycone structures: groups A, B, and E (glycosides of

TABLE 1 Secondary metabolites, source, and analytical techniques used in the analysis of legume root exudates.

| N. | Compound | Classification | Plant species | Data acquisition | References |
|---|---|---|---|---|---|
| 1 | 2-Hydroxydaidzein | Isoflavones | *T. alexandrinum* | LC-MS | Heuermann et al., 2023 |
| 2 | 6”-Malonylgenistin | Isoflavones | *T. alexandrinum* | LC-MS | Heuermann et al., 2023 |
| 3 | Apigeninidin | Isoflavones | *M. sativa* | LC-MS | Fagorzi et al., 2021 |
| 4 | Biochanin A | Isoflavones | *A. hypogaea, C. arietinum*, G. max, M. polymorpha*, M. sativa*, T. alexandrinum*, T. incarnatum*, T. subterraneum** | LC-MS; GC-MS | Leoni et al., 2021; Jiang et al., 2022; Heuermann et al., 2023; Fujimatsu et al., 2024; Qiu et al., 2024 |
| 5 | Calycosin | Isoflavones | *C. arietinum** | LC-MS | Fujimatsu et al., 2024 |
| 6 | Daidzein | Isoflavones | *A. hypogaea, C. arietinum*, G. max*, M. polymorpha*, M. sativa*, P. vulgaris*, T. alexandrinum*, T. incarnatum*, T. subterraneum** | LC-MS; GC-MS | Leoni et al., 2021; Liu et al., 2021; Jiang et al., 2022; Heuermann et al., 2023; Paniagua-López et al., 2023; Fujimatsu et al., 2024 |
| 7 | Daidzin | Isoflavones | *G. max, M. sativa, T. incarnatum, T. subterraneum* | LC-MS | Leoni et al., 2021; Qiu et al., 2024 |
| 8 | Formononetin | Isoflavones | *A. hypogaea, C. arietinum*, M. polymorpha*, M. sativa*, T. alexandrinum*, T. incarnatum*, T. subterraneum** | LC-MS; GC-MS | Leoni et al., 2021; Jiang et al., 2022; Heuermann et al., 2023; Fujimatsu et al., 2024 |
| 9 | Genistin | Isoflavones | *M. polymorpha*, M. sativa*, T. alexandrinum*, T. incarnatum*, T. subterraneum*, V. villosa, V. unguiculata* | LC-MS; GC-MS | Leoni et al., 2021; Heuermann et al., 2023; Seitz et al., 2023 |
| 10 | Methylbiochanin A | Isoflavones | *C. arietinum** | LC-MS | Fujimatsu et al., 2024 |
| 11 | Methylenedioxyrobol | Isoflavones | *C. arietinum** | LC-MS | Fujimatsu et al., 2024 |
| 12 | Pratensein | Isoflavones | *C. arietinum*, T. alexandrinum* | LC-MS | Heuermann et al., 2023; Fujimatsu et al., 2024 |
| 13 | Pseudobaptigenin | Isoflavones | *C. arietinum** | LC-MS | Fujimatsu et al., 2024 |
| 14 | 5,7,2’,4’-Tetrahydroxy-6-3-methylbut-2-enyl-isoflavanone | Isoflavones | *D. uncinatum* | NMR; LC-MS | Tsanuo et al., 2003 |
| 15 | 4”,5”-Dihydro-5,2’,4’-trihydroxy-5”-isopropenylfurano-2”,3”;7,6-isoflavanone | Isoflavones | *D. uncinatum* | NMR; LC-MS | Tsanuo et al., 2003 |
| 16 | 4”,5”-Dihydro-2’-methoxy-5,4’-dihydroxy-5”-isopropenylfurano-2”,3”,7,6 isoflavanone | Isoflavones | *D. uncinatum* | NMR; LC-MS | Tsanuo et al., 2003 |
| 17 | Vestitol | Isoflavones | *L. japonicus* | NMR; LC-MS | Ueda and Sugimoto, 2010 |
| 18 | Astragalin | Flavonols | *M. polymorpha*, M. sativa*, T. incarnatum*, T. subterraneum** | LC-MS | Leoni et al., 2021 |
| 19 | Hyperoside | Flavonols | *C. arietinum*, L. pedunculatus*, M. polymorpha*, M. sativa*, P. sativum, T. incarnatum*, T. subterraneum** | CE-UV; GC-MS; LC-MS | Steele et al., 1999; Leoni et al., 2021; Ben Gaied et al., 2024a |
| 20 | Isorhamnetin | Flavonols | *T. alexandrinum** | LC-MS | Heuermann et al., 2023 |
| 21 | Isorhamnetin-3,7-di-*O*-glucoside | Flavonols | *P. sativum, V. villosa* | LC-MS | Seitz et al., 2023, 2024 |
| 22 | Kaempferol | Flavonols | *L. japonicus*, M. polymorpha*, M. sativa*, R. pseudoacacia*, T. alexandrinum*, T. incarnatum, T. subterraneum* | LC-MS | Leoni et al., 2021; Heuermann et al., 2023; Li et al., 2024; Salomonsen et al., 2024 |
| 23 | Kaempferol glycoside | Flavonols | *P. sativum, T. alexandrinum, V. villosa* | LC-MS | Heuermann et al., 2023; Seitz et al., 2023, 2024 |
| 24 | Methoxy-quercetin | Flavonols | *L. pedunculatus** | CE-UV; GC-MS; LC-MS; LC-UV | Steele et al., 1999 |

*(Continued)*

TABLE 1 Continued

| N. | Compound | Classification | Plant species | Data acquisition | References |
|---|---|---|---|---|---|
| 25 | Morin | Flavonols | *G. max** | LC-MS | Liu et al., 2021 |
| 26 | Myricetin | Flavonols | *L. japonicus** | LC-MS | Salomonsen et al., 2024 |
| 27 | Nicotiflorin | Flavonols | *M. polymorpha**, *M. sativa**, *T. incarnatum**, *T. subterraneum** | LC-MS | Leoni et al., 2021 |
| 28 | Quercetin | Flavonols | *A. hypogaea*, *D. uncinatum**, *L. japonicus**, *L. pedunculatus**, *M. pruriens*, *P. sativum** | CE-UV; GC-MS; LC-MS; LC-UV | Steele et al., 1999; Jiang et al., 2022; Were et al., 2022; Ben Gaied et al., 2024b; Salomonsen et al., 2024 |
| 29 | Quercetin glycoside | Flavonols | *L. pedunculatus**, *M. polymorpha**, *M. sativa**, *P. sativum*, *T. incarnatum**, *T. subterraneum**, *V. villosa* | CE-UV; GC-MS; LC-MS; LC-UV | Steele et al., 1999; Leoni et al., 2021; Seitz et al., 2023 |
| 30 | Rhamnetin | Flavonols | *L. pedunculatus** | CE-UV; GC-MS; LC-MS | Steele et al., 1999 |
| 31 | Rutin | Flavonols | *A. hypogaea**, *C. arietinum**, *M. polymorpha**, *M. sativa**, *P. sativum**, *T. incarnatum**, *T. subterraneum** | LC-MS | Cesari et al., 2019; Leoni et al., 2021; Ben Gaied et al., 2024b, 2024a |
| 32 | 7, 4′- dihydroxyflavone | Flavones | *G. max** | LC-MS | Liu et al., 2021 |
| 33 | Apigenin | Flavones | *A. hypogaea**, *G. max**, *L. japonicus**, *M. polymorpha*, *M. sativa*, *P. vulgaris**, *R. pseudoacacia**, *T. incarnatum*, *T. subterraneum* | LC-MS | Cesari et al., 2019; Fagorzi et al., 2021; Leoni et al., 2021; Liu et al., 2021; Paniagua-López et al., 2023; Li et al., 2024; Salomonsen et al., 2024 |
| 34 | Apigenin glycoside | Flavones | *P. sativum**, *T. alexandrinum* | LC-MS | Heuermann et al., 2023; Seitz et al., 2023; Ben Gaied et al., 2024b |
| 35 | Apiin | Flavones | *V. unguiculata* | LC-MS | Seitz et al., 2023 |
| 36 | Chrysin | Flavones | *A. hypogaea**, *G. max** | LC-MS | Cesari et al., 2019; Liu et al., 2021 |
| 37 | Diosmetin | Flavones | *T. alexandrinum** | LC-MS | Heuermann et al., 2023 |
| 38 | Diosmin | Flavones | *V. villosa* | LC-MS | Seitz et al., 2023, 2024 |
| 39 | Fortunellin | Flavones | *V. villosa* | LC-MS | Seitz et al., 2023 |
| 40 | Isoschaftoside | Flavones | *D. uncinatum* | LC-MS | Hooper et al., 2010 |
| 41 | Luteolin | Flavones | *A. hypogaea**, *G. max** | LC-MS | Cesari et al., 2019; Liu et al., 2021 |
| 42 | Saponarin | Flavones | *V. unguiculata* | LC-MS | Seitz et al., 2023 |
| 43 | Tricin | Flavones | *M. sativa** | LC-UV | Ding et al., 2024 |
| 44 | 7-hydroxyflavanone | Flavanones | *M. sativa** | LC-MS | Pino et al., 2016 |
| 45 | Hesperetin | Flavanones | *G. max**, *P. vulgaris**, *P. sativum** | LC-MS; GC-MS | Pini et al., 2017; Liu et al., 2021; Paniagua-López et al., 2023 |
| 46 | Liquiritigenin | Flavanones | *E. falcata**, *R. pseudoacacia**, *M. sativa* | LC-MS | Michalet et al., 2013; Fagorzi et al., 2021; Li et al., 2024 |
| 47 | Liquiritigenin glycoside | Flavanones | *E. falcata** | LC-MS | Michalet et al., 2013 |
| 48 | Naringenin | Flavanones | *A. hypogaea**, *C. arietinum**, *G. max**, *L. japonicus**, *L. pedunculatus**, *P. vulgaris**, *M. polymorpha**, *M. sativa**, *R. pseudoacacia**, *T. incarnatum**, *T. subterraneum** | CE-UV; GC-MS; LC-MS; LC-UV | Steele et al., 1999; Cesari et al., 2019; Leoni et al., 2021; Liu et al., 2021; Paniagua-López et al., 2023; Fujimatsu et al., 2024; Li et al., 2024; Salomonsen et al., 2024 |
| 49 | Naringin | Flavanones | *A. hypogaea**, *P. sativum** | LC-MS | Cesari et al., 2019; Ben Gaied et al., 2024b |
| 50 | Isoliquiritigenin | Chalcones | *E. falcata**, *G. max** | LC-MS | Michalet et al., 2013; Liu et al., 2021 |
| 51 | Isoliquiritigenin glycoside | Chalcones | *E. falcata** | LC-MS | Michalet et al., 2013 |
| 52 | Kuwanon Y | Chalcones | *P. sativum* | LC-MS | Seitz et al., 2023 |
| 53 | Cyanidin 3,5-diglucoside | Anthocyanidin | *P. sativum*, *V. villosa*, *V. unguiculata* | LC-MS | Seitz et al., 2023, 2024 |
| 54 | Sulfuretin | Aurones | *E. falcata** | LC-MS | Michalet et al., 2013 |

*(Continued)*

TABLE 1 Continued

| N. | Compound | Classification | Plant species | Data acquisition | References |
|---|---|---|---|---|---|
| 55 | Taxifolin | Dihydroflavonols | *A. hypogaea* | GC-MS | Jiang et al., 2022 |
| 56 | Epicatechin | Flavan-3-ol | *L. japonicus*, L. pedunculatus*, P. sativum** | CE-UV; GC-MS; LC-MS | Steele et al., 1999; Ben Gaied et al., 2024b; Salomonsen et al., 2024 |
| 57 | Coumestrol | Coumestan | *G. max** | LC-MS | Liu et al., 2021; Qiu et al., 2024 |
| 58 | Trifoliol | Coumestan | *T. alexandrinum* | LC-MS | Heuermann et al., 2023 |
| 59 | 6aR,11aR-trifolirhizin | Pterocarpans | *T. pratense** | LC-MS | Liu et al., 2013 |
| 60 | 4-methoxymaackiain | Pterocarpans | *T. alexandrinum* | LC-MS | Heuermann et al., 2023 |
| 61 | Maackiain | Pterocarpans | *C. arietinum*, T. alexandrinum, T. pratense** | LC-MS | Liu et al., 2013; Heuermann et al., 2023; Fujimatsu et al., 2024 |
| 62 | Medicarpin | Pterocarpans | *C. arietinum*, M. polymorpha*, M. sativa*, T. alexandrinum, T. incarnatum*, T. subterraneum** | LC-MS | Leoni et al., 2021; Heuermann et al., 2023; Fujimatsu et al., 2024 |
| 63 | Trifolirhizin-6'-*O*-malonate | Pterocarpans | *T. alexandrinum* | LC-MS | Heuermann et al., 2023 |
| 64 | Grossamide | Neolignans | *P. sativum, T. pratense, V. villosa* | LC-MS | Seitz et al., 2023 |
| 65 | Salviolinic acid | Neolignans | *P. sativum** | LC-MS | Ben Gaied et al., 2024b |
| 66 | Magnoshinin | Lignans | *T. alexandrinum* | LC-MS | Heuermann et al., 2023 |
| 67 | 1,3-di-*O*-caffeoylquinic acid | Phenylpropanoids | *C. arietinum*, P. sativum** | LC-MS | Ben Gaied et al., 2024a, 2024b |
| 68 | 5-Hydroxyferulic acid | Phenylpropanoids | *L. japonicus** | LC-MS | Shimamura et al., 2022 |
| 69 | Caffeic acid | Phenylpropanoids | *L. japonicus*, M. sativa*, P. sativum** | LC-MS; LC-UV | Shimamura et al., 2022; Ben Gaied et al., 2024b; Ding et al., 2024 |
| 70 | Chlorogenic acid | Phenylpropanoids | *C. arietinum*, P. sativum** | LC-MS | Ben Gaied et al., 2024b, 2024a |
| 71 | Cinnamic acid | Phenylpropanoids | *D. uncinatum*, L. japonicus*, M. pruriens, P. sativum** | LC-MS | Shimamura et al., 2022; Were et al., 2022; Ben Gaied et al., 2024b; Salomonsen et al., 2024 |
| 72 | Ferulic acid | Phenylpropanoids | *L. japonicus*, M. sativa** | LC-MS | Ding et al., 2024; Salomonsen et al., 2024 |
| 73 | *p*-Coumaric acid | Phenylpropanoids | *D. uncinatum*, L. japonicus*, M. sativa*, M. pruriens* | LC-MS; LC-UV | Shimamura et al., 2022; Were et al., 2022; Ding et al., 2024; Salomonsen et al., 2024 |
| 74 | Phloretic acid | Phenylpropanoids | *L. japonicus** | LC-MS | Shimamura et al., 2022 |
| 75 | Rosmarinic acid | Phenylpropanoids | *P. sativum** | LC-MS | Ben Gaied et al., 2024b |
| 76 | Sinapic acid | Phenylpropanoids | *L. japonicus** | LC-MS | Shimamura et al., 2022 |
| 77 | Indole-3-acetic acid | Indole alkaloids | *A. hypogaea** | LC-MS | Cesari et al., 2019 |
| 78 | Riboflavin | Pteridine alkaloids | *L. japonicus** | LC-MS | Salomonsen et al., 2024 |
| 79 | Strigolactone | Apocarotenoids | *L. japonicus** | LC-MS | Mori et al., 2020 |
| 80 | Benzoic acid | Phenolic acids | *D. uncinatum*, M. pruriens* | LC-UV | Were et al., 2022 |
| 81 | Gallic acid | Phenolic acids | *C. arietinum*, P. sativum** | LC-MS | Ben Gaied et al., 2024a, 2024b |
| 82 | *p*-Hydroxybenzoic acid | Phenolic acids | *M. sativa*, D. uncinatum*, M. pruriens* | LC-UV | Were et al., 2022; Ding et al., 2024 |
| 83 | Protocatechuic acid | Phenolic acids | *P. sativum** | LC-MS | Ben Gaied et al., 2024b |
| 84 | Quinic acid | Phenolic acids | *P. sativum** | LC-MS | Ben Gaied et al., 2024b |
| 85 | Salicylic acid | Phenolic acids | *L. japonicus** | LC-MS | Salomonsen et al., 2024 |
| 86 | Syringic acid | Phenolic acids | *P. sativum** | LC-MS | Ben Gaied et al., 2024b |
| 87 | Vanillin | Phenolic acids | *D. uncinatum*, M. pruriens* | LC-UV | Were et al., 2022 |
| 88 | Soyasaponin Ba | Saponins | *V. unguiculata* | LC-MS | Seitz et al., 2023 |
| 89 | Soyasaponin Bb | Saponins | *G. max*, T. alexandrinum, T. incarnatum, T. pratense, V. villosa, V. unguiculata* | LC-MS | Fujimatsu et al., 2020; Heuermann et al., 2023; Seitz et al., 2023, 2024 |
| 90 | Soyasaponin Bb' | Saponins | *G. max** | LC-MS | Fujimatsu et al., 2020 |

*(Continued)*

TABLE 1 Continued

| N. | Compound | Classification | Plant species | Data acquisition | References |
|---|---|---|---|---|---|
| 91 | Soyasaponin Be | Saponins | *G. max** | LC-MS | Fujimatsu et al., 2020 |
| 92 | Soyasapogenol B | Triterpenoid | *G. max**, *V. villosa*, *V. unguiculata* | LC-MS | Fujimatsu et al., 2020; Seitz et al., 2023 |

(*) Represents the identification of secondary metabolites confirmed by the authentic standard.

soyasapogenols A, B, and E, respectively), and the DDMP soyasaponins — the latter being soyasapogenol B glycosides with a DDMP moiety (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) linked at the C-22 position (Tsuno et al., 2018). In the reviewed literature, four soyasaponins from group B and soyasapogenol B were detected in the root exudates of *Vigna unguiculata, Vicia villosa* Roth, *Trifolium incarnatum* L., *T. pratense* L., *T. alexandrinum* L., and *Glycine max* (Table 1).

Among the secondary metabolites identified or annotated in the root exudates of the 22 legume species, particular attention should be given to salicylic acid (SA, a phenolic acid), indole-3-acetic acid (IAA, an indole alkaloid), and strigolactones (apocarotenoids). These phytohormones mediate biological and functional interactions between the plant and its rhizosphere. SA contributes to plant defense by activating systemic acquired resistance against pathogens and by modulating growth, photosynthesis, and responses to biotic and abiotic stressors (Chen and Liu, 2024). IAA, the principal auxin in plants, regulates cell elongation and division, tissue differentiation, and plant growth. In fungi, it affects cell expansion, disturbs cell division, and in some species, induces spore germination (Korenblum et al., 2022). Strigolactones modulate symbiosis with arbuscular mycorrhizal fungi by stimulating hyphal branching and root colonization, thereby enhancing fungal nutrient acquisition, particularly phosphorus. They also function as plant hormones, influence plant-plant interactions and regulate plant-microbe symbiotic relationships (Adedeji and Babalola, 2020; Singh et al., 2023; Maitra et al., 2024).

One the key challenges in the studies on organic compounds in the rhizosphere is the distinguishing the biosynthetic source, in reason to the plant root exudates and microbial products often produce the same classes of metabolites, such as sugars, organic acids, and secondary metabolites (Salem et al., 2022). The stable isotope labeling techniques allow the tracing of plant-derived carbon in the rhizosphere to differentiate the metabolite (Fan et al., 2022). Another approach is spatial metabolomics, which employs mass spectrometry combined with different ionization sources, such as matrix-assisted laser desorption/ionization imaging (MALDI-imaging), laser ablation electrospray ionization (LAESI), and live single-cell mass spectrometry (LSC-MS) (Lohse et al., 2021). These techniques enable metabolite analysis at high spatial resolution, including the single-cell level, facilitating the discrimination of plant-specific metabolites from those produced by associated microbiomes.

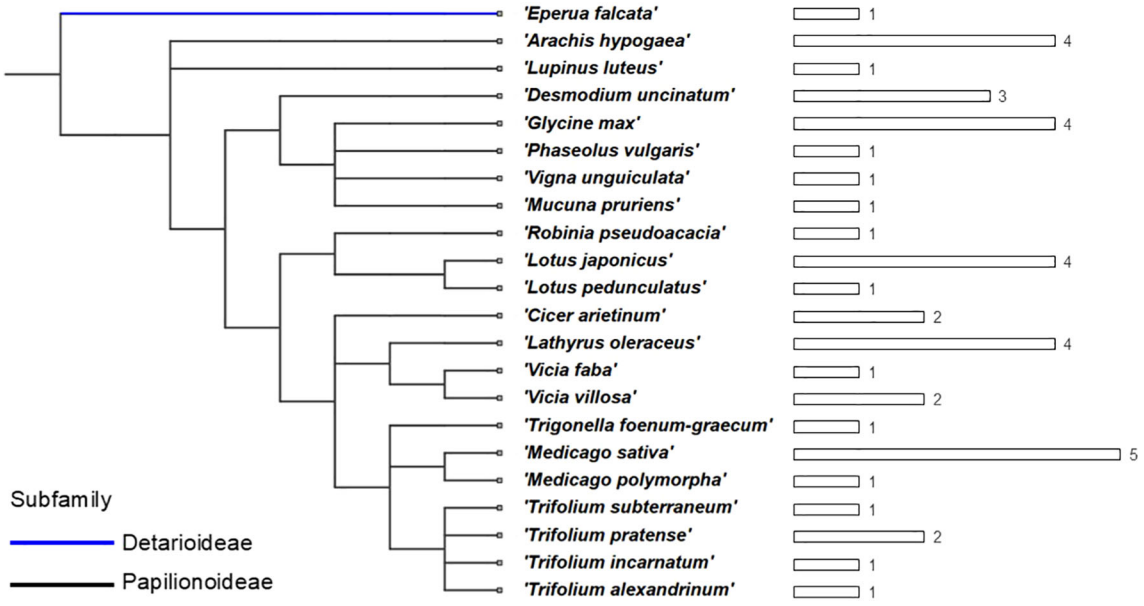


FIGURE 2
Phylogenetic tree containing Fabaceae species that have been studied for secondary metabolites in root exudates in the last 25 years. The bar graph shows the number of studies carried out for each species that had roots exudates characterized to date. The tree was plotted using the NCBI Taxonomy/Common Tree tool https://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi and edited using iTOL version 7.2.1. The observed pattern shows that only 22 legume species, restricted to a few taxa, had their root exudates characterized according to their secondary metabolites profile so far.

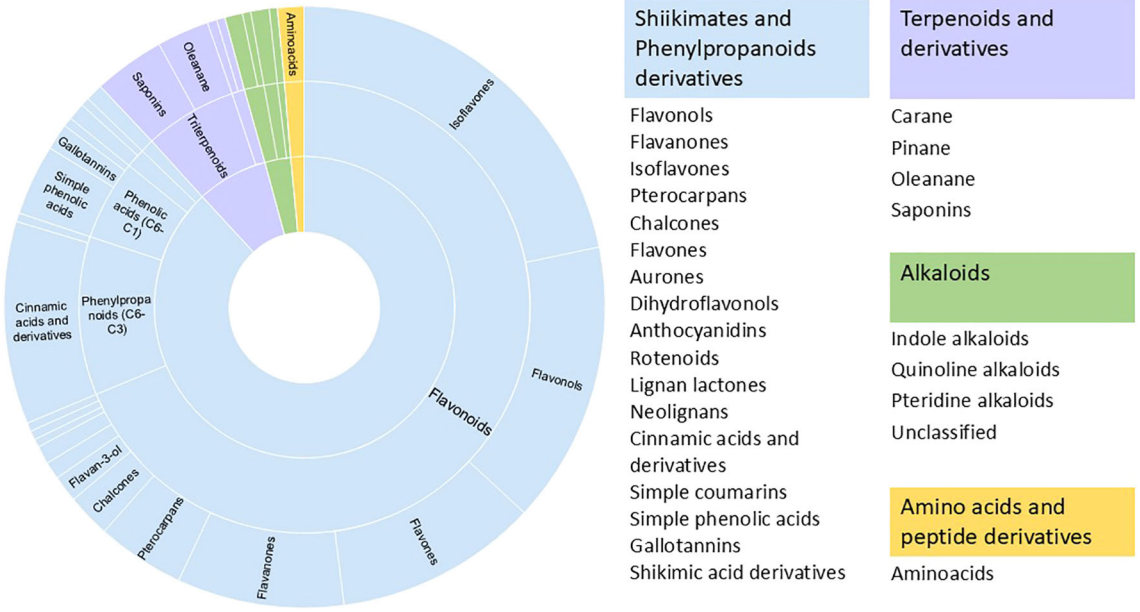


FIGURE 3
Biosynthetic pathways, super-classes, and classes of secondary metabolites characterized or annotated in legume roots exudates studied over the last 25 years. The classifications were performed according to NPClassifier (https://npclassifier.ucsd.edu/). In legume species research on root exudates, the great majority of compounds that are found are assigned to the shikimic acid pathway (shikimate- and phenylpropanoid-derived compounds). This aligns with major ecological functions such as recruiting beneficial microbes, shaping healthy soil microbiomes as well as interference with microbial communication and signaling.

# 6 Metabolomic approaches for the study of root exudates

The chemical complexity of root exudates, combined with the low concentration of their constituents presents a major analytical challenge for the accurate identification and quantification of these metabolites. However, advances in omics sciences, particularly metabolomics have been enabling the analyses and compound annotation, further contributing for the understanding on how legumes modulate their root exudation in response to environmental cues. By definition, metabolomics provides a snapshot of the metabolic state in the living organisms, allowing the characterization and quantification of metabolites in cells, tissues, and biological fluids under specific conditions (Fiehn, 2001, Brunetti et al., 2018; Bueno and Lopes, 2020).

Essentially, two complementary approaches are generally employed in metabolomics. By untargeted metabolomics, an exploratory approach is used aiming at detecting a broad spectrum of metabolites without prior knowledge, which is quite helpful in identifying novel compounds and global shifts in root exudation. By targeted metabolomics, the focus is on detecting and quantifying predefined metabolite classes or compounds (Bueno and Lopes, 2020). Choosing the appropriate approach is the starting point that defines the next steps in metabolomics investigations.

A major challenge in metabolomics is that no single extraction or detection method can capture the full chemical diversity of the metabolome. This limitation has led to the development of multiple protocols, with researchers often combining methods to increase coverage (Döll et al., 2024; Salomonsen et al., 2024).

The metabolomic analysis of legume root exudates - from sample collection to metabolite detection, data visualization, and final interpretation - requires a carefully designed workflow tailored to the specific research question. Recent studies on legume species have applied such metabolomic approaches to compare, for example, plant growth conditions (Salomonsen et al., 2024), different species (Seitz et al., 2023), and metabolic responses to nitrogen sources (Li et al., 2024), among others. The interpretation of metabolomics data is essential, when comparing results across different growing systems, collection process or analytical platforms, as the observed profiles reflects methodological factors as much as biological differences (Oburger and Jones, 2018). In the following sections, we highlight the approaches described in the literature for assessing secondary metabolites in legume root exudates using metabolomics approaches.

## 6.1 Collection and sample preparation

Sampling root exudates remains methodologically challenging and is a topic of ongoing debate (P'etriacq et al., 2017; Oburger and Jones, 2018; McLaughlin et al., 2023; Döll et al., 2024). Key issues include the use of sterile versus non-sterile systems and determining the origin of metabolites, whether they are produced by the plant or by associated microbes. In practice, a functional approach is often most useful, as it acknowledges the limitations of each method while allowing for the testing of hypotheses regarding exudate function in ecological and agricultural contexts.

The collection of root exudates is a decisive step in metabolomic studies, as sampling conditions significantly impact the metabolite profiles observed. Growth systems, whether hydroponic, pot-based,

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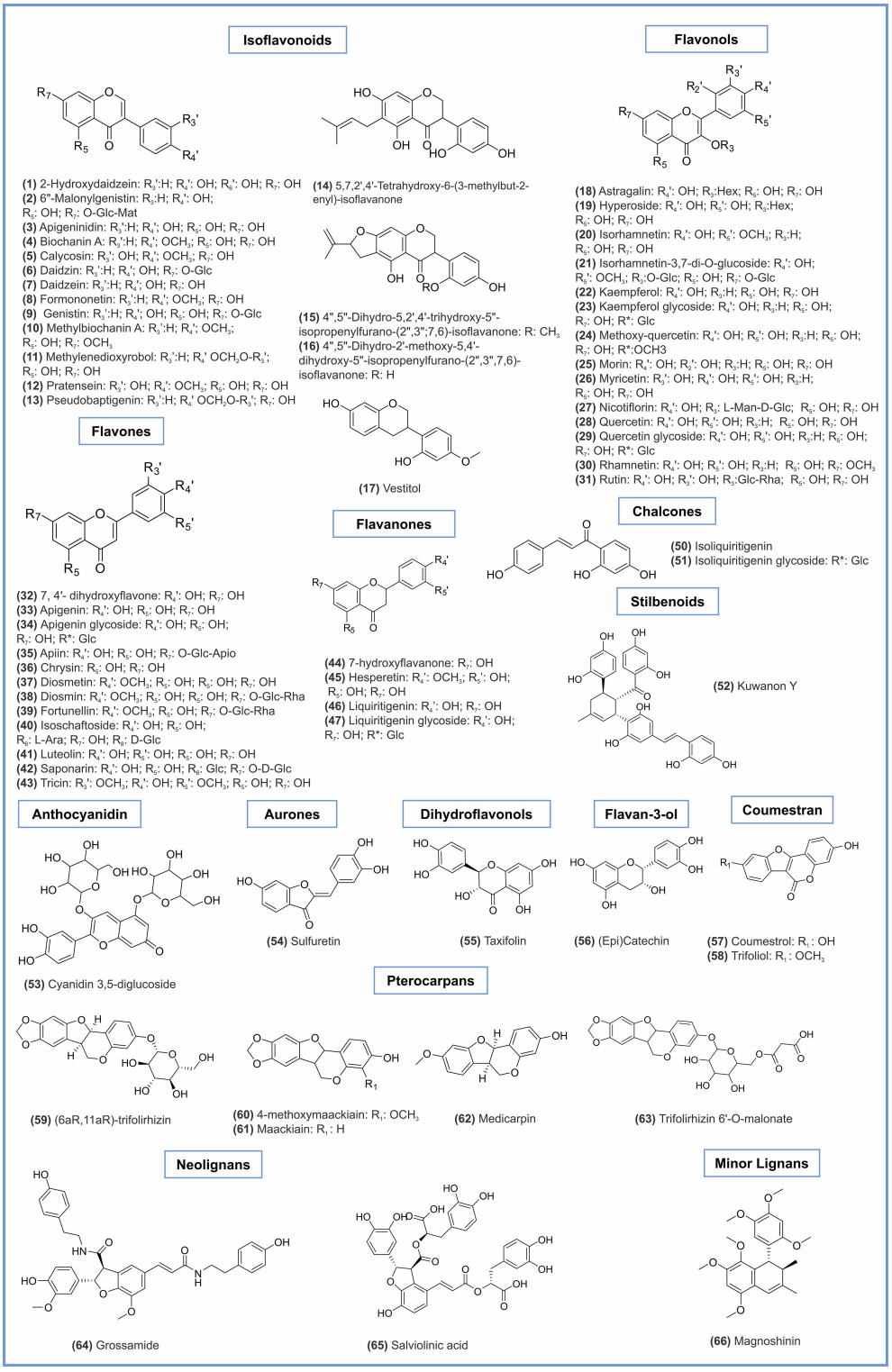


FIGURE 4
Chemical structures of the main classes of secondary metabolites described in legume root exudates, including isoflavonoids, flavonols, flavones, flavanones, chalcones, stilbenoids, anthocyanidins, aurones, dihydroflavonols, flavan-3-ol, coumestrans, pterocarpans, neolignans and minor lignans.

or field-grown, each come with their own biases and advantages. For studies focusing on secondary metabolites, hydroponic systems are the most employed, followed by soil-based approaches in pots and field conditions. At the same time, growth media are the least frequently used.

Indeed, soil-grown versus hydroponically grown crops have been shown to release distinct classes of secondary metabolites and different concentrations, underscoring the importance of sampling context (Heuermann et al., 2023). The same has been observed in hydroponic-hybrid and plate-grown

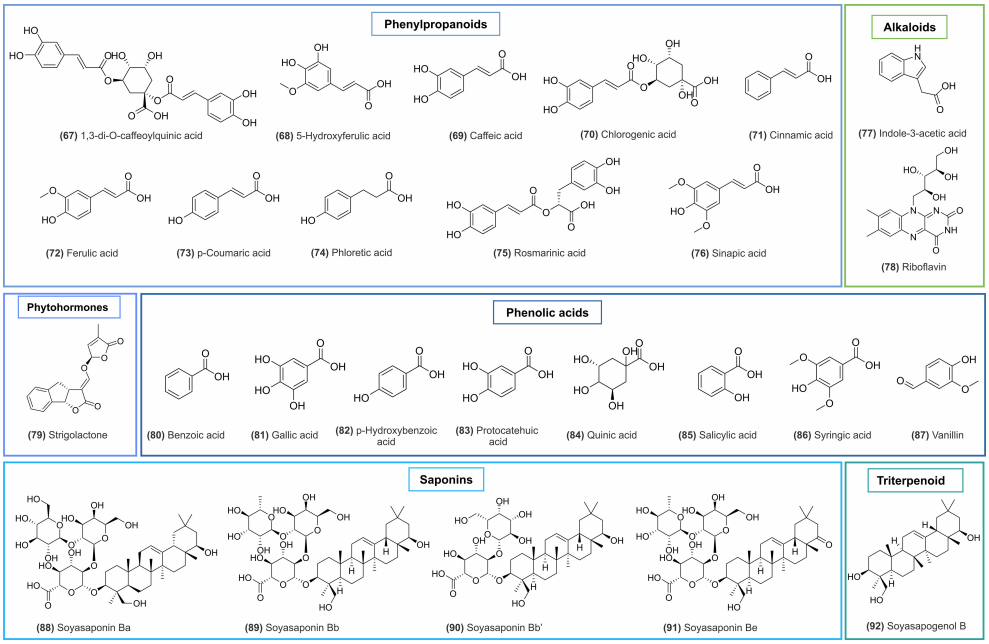


FIGURE 5
Chemical structures of the main classes of secondary metabolites described in legume root exudates, including phenylpropanoids, alkaloids, phytohormones, phenolic acids, saponins, and triterpenoids.

methods (Salomonsen et al., 2024). Root exudates are commonly collected in hydroponic systems, mainly due to the accessibility of the roots. However, these systems alter the physiology and metabolic activity of roots, compared to those growing in soils. By contrast, metabolite analyses under soil-grown conditions reflect more accurately the root exudation complexity (Ma et al., 2022). Therefore, the analysis of root exudates under more realistic, soil-based conditions is necessary to better capture ecologically relevant metabolite fingerprint, but requires root washing, which may stress plants and further bias metabolite profiles (Williams et al., 2021). These methodological biases complicate direct comparisons using different collection systems and underscore the need for careful technical selection when interpreting secondary metabolite profiling in root exudate analyses (Ma et al., 2022). Recent methodological advances have yielded optimized and cost-effective protocols that enhance the reliability and adaptability of sampling from potted or field-grown plants, thereby significantly improving reproducibility under realistic conditions (Döll et al., 2024).

Table 2 summarizes the collection methods and sample preparation procedures for legume root exudates. When considering nutrient solutions, the Hoagland solution is generally regarded as the standard choice for hydroponic systems. It supplies a balanced composition of the essential macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Fe, B, Mn, Zn, Cu, and Mo), which support optimal plant development. This solution has been successfully applied to different species, including *M. sativa*, *A. hypogaea*, and *D. uncinatum* (Pino et al., 2016; Cesari et al., 2019; Jiang et al., 2022; Were et al., 2022). The diluted Long Ashton solution, which is rich in phosphorus and micronutrients, has been widely employed in symbiosis studies involving *D. uncinatum*, *P. sativum*, and *L. japonicus* (Hooper et al., 2010; Ueda and Sugimoto, 2010; Foo and Davies, 2011; Salomonsen et al., 2024). The Rigaud-Puppo solution, on the other hand, was specifically formulated for legumes. Its composition is nutrient-rich with adjusted nitrogen supply, which is essential for evaluating nitrogen fixation and other legume-specific physiological processes (Liu et al., 2021). The nitrogen-free solutions stimulate the release of exudates and the production of flavonoids as a response to environmental stress, as reported in *R. pseudoacacia* under different nitrogen sources (Li et al., 2024) and in chickpea with nitrogen deficiency (Fujimatsu et al., 2024).

In soil-grown systems, labile compounds are subject to rapid microbial uptake and enzymatic reactions, leading to the transformation of metabolites within the rhizosphere. Such biotransformation and degradation by enzymatic activities are the major challenges for accurately characterizing root exudate composition (Martins et al., 2026). In hydroponic systems, the environmental variations as microbial activities are reduced, but labile metabolites still be lost or chemically altered during the collection step (Ritter et al., 2025), due to dilution, oxidation, or prolonged exposure in the nutrient solution.

After collection, exudates are typically concentrated by lyophilization to stabilize compounds and enrich secondary metabolites before being resuspended in organic solvents for downstream analyses. Other procedures include sample clean-up using solid-phase extraction (SPE) cartridges (Foo and Davies, 2011; Mori et al., 2020; Fujimatsu et al., 2024), as well as purification techniques such as solvent partitioning (with ethyl acetate or chloroform), and chromatographic separation using TLC (Steele et al., 1999), silica gel (Ueda and Sugimoto, 2010), or Sephadex (Makarova et al., 2012). Differences in extraction solvents, solid-phase extraction strategies, and concentration steps enrich or exclude specific classes of secondary metabolites, leading to

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TABLE 2 Plant growth conditions, collection methods, and sample preparation procedures employed in legume root exudates.

| Reference | Plant species | Growth conditions | Exudation solution | Recovery time/ Exudation time | Extraction/Sample preparation |
|---|---|---|---|---|---|
| Steele et al., 1999 | *L. pedunculatus* | Hdp | Nitrogen-free solution | 2 days/4 days | Lyophilization; resuspension in organic solvent; TLC separation |
| Hooper et al., 2010 | *D. uncinatum* | Hdp | Long Ashton solution | ND/2 days | Lyophilization and resuspension in organic solvent |
| Pini et al., 2017 | *P. sativum* | GM | Fahraeus medium | ND/ND | Lyophilization and resuspension in water |
| Kneer et al., 1999 | *L. luteus* | Hdp | Hydro-Sol/$Ca(NO_3)_2$ | ND/24h | Lyophilization and resuspension in organic solvent |
| Tsanuo et al., 2003 | *D. uncinatum* | Hdp | B5 media in perspex tray | ND/ND | Cleaning up through SPE – C18 cartridge, resuspension in organic solvent |
| Evidente et al., 2007 | *T. foenum-graecum* | Hyb | Water | ND/2 days | Lyophilization and resuspension in organic solvent, purification by silica gel column chromatography |
| Foo and Davies, 2011 | *P. sativum* | Hdp | Long Ashton solution | ND/24h | Extraction with EtOAc, cleaning up through SPE – C18 cartridge, resuspension in organic solvent |
| Liu et al., 2013 | *T. pratense* | ND | ND | ND/ND | Lyophilization and resuspension in organic solvent |
| Michalet et al., 2013 | *E. falcata* | BSf | ND | ND/72h ($CaCl_2$ solution) | Lyophilization and resuspension in organic solvent |
| Pino et al., 2016 | *M. sativa* | Hdp | Hoagland solution | ND/ND | Lyophilization and resuspension in organic solvent |
| Cesari et al., 2019 | *A. hypogaea* | Hdp | Hoagland solution | ND/overnight | Lyophilization and resuspension in water |
| Ueda and Sugimoto, 2010 | *L. japonicus* | Hdp | Long Ashton solution | 14 days/ND | Lyophilization, resuspension in $CHCl_3$, purification by silica gel chromatography and elution with *n*-hexanes and EtOAc |
| Fujimatsu et al., 2020 | *G. max* | BS | ND | ND/ND | Lyophilization and resuspension in organic solvent |
| Seitz et al., 2024 | *V. villosa* | GM | MS media | ND/24h | Lyophilization and resuspension in water |
| Salomonsen et al., 2024 | *L. japonicus* | Hdp; Hyb | Water and Long Ashton solution | ND/3 days,3h | Lyophilization and resuspension in organic solvent |
| Qiu et al., 2024 | *G. max* | BS | Water | ND/24h | Lyophilization and resuspension in organic solvent |
| Li et al., 2024 | *R. pseudoacacia* | Hdp | (1) Nitrogen free nutrient solution; (2) Nitrogen-free nutrient solution containing *M. amorphae*; (3) Hoagland solution | ND/6h ($H_2O$) | Lyophilization and resuspension in organic solvent |
| Fujimatsu et al., 2024 | *C. arietinum* | Hdp | Nitrogen-deficient hydroponic solution (diluted Broughton and Dilworth medium) | ND/24h | Cleaning up through SPE – C18 cartridge, resuspension in organic solvent |
| Ben Gaied et al., 2024b | *P. sativum* | Sterile sand: vermiculite 1:2 *v/v* mixture | Water and nitrogen-free solution | ND/7days | Lyophilization and resuspension in organic solvent |
| Ben Gaied et al., 2024a | *C. arietinum* | Sterile sand: vermiculite 1:2 *v/v* mixture | Water and nitrogen-free solution | ND/7days | Lyophilization and resuspension in organic solvent |
| Seitz et al., 2023 | *P. sativum, T. incarnatum, T. pratense, V. villosa, V. unguiculata* | GM | MS media | 14 days/24h $H_2O$ | Lyophilization and resuspension in water |
| Ding et al., 2024 | *M. sativa* | BS | ND | ND7ND | Refluxing with 70% EtOH, at 80 °C for 3 h |

*(Continued)*

TABLE 2 Continued

| Reference | Plant species | Growth conditions | Exudation solution | Recovery time/ Exudation time | Extraction/Sample preparation |
|---|---|---|---|---|---|
| Paniagua-López et al., 2023 | *P. vulgaris* | Hdp | Nitrogen-free solution | 5 days/ND | Lyophilization and resuspension in organic solvent |
| Heuermann et al., 2023 | *M. alexandrinum* | Hdp; BSf | Nutrient solution ($NH_4NO_3$, $Ca(NO_3)_2$, $CuSO_4$, $MgSO_4$, $KH_2PO_4$, $K_2SO_4$, Fe-EDTA, $CaCl_2$, $H_3BO_3$, $MnSO_4$, $ZnSO_4$, $NaMoO_4$) pH 5.8 | ND/4h Hdp, 2h BSf, aerated $H_2O$ | Lyophilization and resuspension in organic solvent |
| Were et al., 2022 | *D. uncinatum*, *M. pruriens* | Hdp | Hoagland solution | ND/6h (0.2 mM $CaCl_2$, pH 6.2 | Lyophilization and resuspension in organic solvent |
| Shimamura et al., 2022 | *L. japonicus* | GM | B&D liquid medium | ND/ND | Lyophilization and resuspension in organic solvent |
| Zhang et al., 2022 | *A. hypogaea* | BS | ND | ND/ND | Lyophilization and resuspension in organic solvent |
| Makarova et al., 2012 | *G. max*, *P. sativum*, *V. faba* | ND | ND | ND/ND | Partition with acidified EtOAc; resuspension in EtOH. The compounds were isolated sequentially by paper chromatography, Sephadex LH20 chromatography column, and TLC |
| Leoni et al., 2021 | *M. polymorpha*, *M. sativa*, *T. incarnatum*, *T. subterraneum* | Hdp | Hoagland solution | ND/ND | Lyophilization and resuspension in organic solvent |
| Mori et al., 2020 | *L. japonicus* | Hdp | M medium containing $KH_2PO_4$ | 9 weeks/ND | Cleaning up through SPE-C18, resuspension in organic solvent |
| Gupta et al., 2020 | *A. hypogaea* | Gnotobiotic conditions | PNS solution (KCl, $MgSO_4$, $KH_2PO_4$, $KNO_3$, $CaNO_3$, micronutrients) | ND/2 days, $H_2O$ | Lyophilization and resuspension in water |
| Fagorzi et al., 2021 | *M. sativa* | Perlite: vermiculite 1:1 | Fahraeus N-free solution | ND/14 days | NA |
| Jiang et al., 2022 | *A. hypogaea* | Hdp | Hoagland solution | ND/ND | Resuspension in acetone and derivatization (silylation) |
| Liu et al., 2021 | *G. max* | Hdp | Rigaud-Puppo solution supplemented inorganic sitrogen | ND/7 days | Lyophilization and resuspension in organic solvent |

Hdp, growth in hydroponic conditions; BS, growth in bulk soil in pot; BSf, growth in bulk soil from field; GM, growth media; Hyb, growth in substrate and hydroponic; ND, not described.

divergent metabolite profiles even when similar plant systems are studied across different studies (Salem et al., 2022). This variability reflects the wide range of methodological combinations employed in metabolomics studies.

## 6.2 Analytical techniques for the detection, annotation, and identification of root exudates

The strategic selection of an analytical technique for the analysis of secondary metabolites is primarily guided by the study's objective, which is to achieve a comprehensive profiling of the metabolome or to conduct a targeted analysis of a specific metabolite or class. To this end spectroscopic and spectrometric methods offer distinct advantages for different analytical needs. Furthermore, the coupling of analytical techniques, particularly the hyphenation of separation methods with detection systems, enhances the detection, annotation and identification of secondary metabolites (Seger et al., 2013).

Hyphenated techniques are particularly valuable for analyzing complex matrices, including natural product extracts, as they streamline the dereplication process by providing a detailed chemical fingerprint. Consequently, it often reduces the need for extensive fractionation and isolation procedures. The most prominent hyphenated methods in natural products research comprehend the coupling of liquid or gas chromatography (LC or GC, respectively) with mass spectrometry (MS) or with Nuclear Magnetic Resonance (NMR), as well as capillary electrophoresis coupled to mass spectrometry (CE-MS) (Ernst et al., 2014).

In fact, the combination of chromatography with mass spectrometry is the most effectively used technique in the research in natural products and metabolomics, as it enables both separation and annotation of organic compounds with molecular masses below 2000 Da. In these workflows, the chromatographic separation resolves metabolites based on their physicochemical properties, while MS detection provides structural information by measuring the precise mass-to-charge ratio ($m/z$) of analytes. This combination enabled the identification of individual metabolites within the chemically diverse pool of legume root exudates (Dettmer et al., 2007; Alseekh et al., 2021).

Given the exudation mechanisms and the belowground chemistry, LC-MS (using single-stage or tandem mass

spectrometry) is the best choice technique for the detection and analysis of secondary metabolites, offering a broad analytical coverage, as shown in the Table 1. Other methods include the GC-MS - for volatile and non-polar compounds - and NMR for structural elucidation of purified compounds. In the subsequent sections, we discuss analytical techniques used to detect the secondary metabolites from legume root exudate, as reported in the literature, and highlight their utility in metabolomics studies.

### 6.2.1 Liquid chromatography-mass spectrometry

After the collection of root exudates (mostly performed in hydroponic conditions followed by lyophilization, and subsequently resuspension in an organic solvent (e.g., methanol or ethyl acetate) LC-MS is the most suitable and employed technique for characterizing and quantifying secondary metabolites in legume root exudate, as summarized in Table 1. Either in target or untargeted metabolomics, the prior liquid chromatographic step enables compounds separation usually in reverse mode, using a stationary phase such as a C18 column, and a mobile phase, typically composed by mixtures of methanol or acetonitrile and water. The separation step is aimed to achieve a good peak resolution, minimizing ion suppression in mass spectrometry analysis. In the next subsequent step, the analytes are then ionized to be detected in the MS. With this regard, the most used technique for the compounds from root exudates is the electrospray ionization (ESI) operating in positive or negative ionization modes, which directly influences the sensitivity and reliability of metabolite detection (Fenn et al., 1989; Crotti et al., 2006). In metabolomics using LC-MS, two scanning modes are commonly employed: data-dependent acquisition (DDA) and data-independent acquisition (DIA). Both methods generate spectra containing precursor and fragment ions, which are crucial for elucidating chemical structures. In DDA, typically in liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), the most intense precursor ions detected in a survey scan are selectively isolated and fragmented to produce MS/MS spectra. In contrast, DIA operates by systematically isolating and fragmenting all ions within predefined, wide *m/z* windows, regardless of their intensity, thereby generating comprehensive fragmentation data. Although DIA is versatile and can be coupled with either LC-MS (single-stage) or GC-MS, the specific fragmentation processes result in distinct spectral patterns compared to LC-MS/MS (Guo and Huan, 2020). Choosing the most suitable data acquisition strategy is a challenge in metabolomics studies and a central step for the subsequent processing and analysis of large datasets using bioinformatic tools.

If target quantification is intended, high selectivity and sensitivity are essential, particularly when measuring specific metabolites within a highly complex matrix, such as root exudates. The most common technique for this purpose utilizes a triple quadrupole or quadrupole-Orbitrap mass analyzers in Multiple Reaction Monitoring (MRM) mode, which is able to monitor a specific transition from a precursor ion to a characteristic product ion. MRM is highly effective for quantifying specific compounds, such as phytohormones (Foo and Davies, 2011) and secondary metabolites such as flavonoids (Li et al., 2024) and triterpenes (Fujimatsu et al., 2020; Seitz et al., 2023), whose levels can signal symbiotic interactions between legumes and rhizobia. Although MRM does not provide the untargeted chemical fingerprint, it offers complementary data that enhances the reliability of the target metabolomics approach.

Indeed, for comprehensive chemical profiling and qualitative analysis, the combination of chromatography with tandem mass spectrometry has become the most widely used approach. The sensitivity and versatility make it ideal for evaluating complex mixtures, such as root exudates, using hybrid analyzers like quadrupole/Orbitrap (Qiu et al., 2024) or quadrupole/time-of-flight (Fujimatsu et al., 2024; Salomonsen et al., 2024; Seitz et al., 2024) for MS/MS fragmentation and high-resolution mass spectrometric data (HRMS). Besides the high-resolution spectrometric data, the coupling of these mass analyzers allows the fragmentation of analytes by Collision-Induced Dissociation (CID) for recognition of characteristic fragmentation patterns, which are essential for compound annotation and/or identification. Advances in data processing tools have been enabling the development of diverse workflows to effectively interpret the complex data generated by these techniques (Guo and Huan, 2020).

It is worth emphasizing that analytical limitations in metabolite detection and characterization still exist; however, a range of methodological strategies should be considered to improve metabolome coverage and accuracy. For example, the choice of ionization method is critical, as it varies in suitability across compound classes, polarity ranges, and molecular weights. Likewise, the dissociation energies applied during fragmentation strongly influence the resulting mass spectra and, consequently, metabolite identification. These analytical variations are further compounded by differences in mass spectrometry platforms, acquisition settings, and data-processing workflows, which limit comparability across studies. In addition, metabolite annotation in untargeted metabolomics largely depends on spectral databases that are continually expanded through community contributions. Although these resources are dynamic, they still do not comprehensively cover the full diversity of metabolites. Therefore, continued advances in bioinformatic tools and metabolomics methodologies are essential to achieve more comprehensive and reliable coverage of the metabolome (Ritter et al., 2025).

In conclusion, untargeted metabolomics based on LC-MS/MS has emerged as a powerful approach for characterizing the complex mixture of secondary metabolites in root exudates (Escolà Casas and Matamoros, 2021). The high sensitivity and selectivity of this technique are particularly suited for analyzing these dilute and chemically diverse samples. Subsequently, tandem mass spectrometry provides fragmentation patterns for annotating metabolites by matching against spectral libraries, which is a backbone in untargeted metabolomics. This approach has successfully uncovered how legume root exudate profiles are modulated under different conditions (Döll et al., 2024; Salomonsen et al., 2024). The applications include the identification of compounds related to the defense mechanism (Qiu et al., 2024), the role of specific flavonoids in initiating plant-*Rhizobium* symbiosis (Fagorzi et al., 2021; Tao et al., 2024),

the exudation of organic acids in response to phosphorus or nitrogen deficiency (Fujimatsu et al., 2024; Li et al., 2024), and the comparison of metabolic profiles among crops (Seitz et al., 2023, 2024). Summarized in Table 1, these studies highlight the use of LC-MS/MS in deciphering the chemical fingerprint of legume root exudates.

### 6.2.2 Capillary electrophoresis-mass spectrometry

Capillary electrophoresis–mass spectrometry (CE-MS) offers exceptional separation efficiency for polar metabolites, such as organic acids and amino acids, with minimal sample volumes, yet remains underutilized in exudate studies. The root exudates of common bean (*P. vulgaris*) under phosphorus deficiency have been successfully profiled using CE-TOF-MS, revealing the elevated secretion of organic acids and amino acids in response to P stress (Tawaraya et al., 2014). Stable isotope labeling using $^{13}C$, $^{15}N$, or $^{33}P$, combined with MS or NMR, provides dynamic insights into metabolic fluxes from plants into the soil. For example, a recent dual-labeling study with $^{13}C$, $^{15}N$, and $^{33}P$ in *Canavalia brasiliensis* Mart. ex Benth. (Brazilian jackbean) tracked element-specific rhizodeposition over time, revealing that younger root segments and carbon moved more rapidly into soil than nitrogen or phosphorus (Stevenel et al., 2025). Together, these approaches enrich our toolkit for dissecting complex exudation dynamics and plant–microbe–soil interactions.

### 6.2.3 Gas chromatography-mass spectrometry

Plant volatile as well as semi-volatile compounds, whether collected using adsorbing matrices or isolated using solvent extraction, are commonly analyzed using gas chromatography (GC). This standard technique utilizes different types of columns, including non-polar dimethyl polysiloxanes (e.g., DB-1) or more polar phases, such as polyethylene glycol-based columns (e.g., DB-Wax). After separation on a GC column, metabolites are typically analyzed using mass spectrometry (MS) detectors, resulting in the mainly used GC-MS hyphenated instrument. The compounds eluting from the GC column are ionized via electron impact (EI), producing charged molecular ions and fragments.

These ions are then separated based on their mass-to-charge (*m/z*) ratio after entering a quadrupole ion trap. The analysis yields total ion chromatograms, providing both the retention time and a characteristic mass spectrum for each compound, defined by its unique fragmentation pattern. Compounds separated by GC-MS can be identified by comparing their mass spectra to reference spectra in established mass spectral libraries, such as the NIST MS databases, complemented with Kovat's retention indices (Tholl et al., 2006).

Research on volatile compounds in legume root exudates remains scarce, with only a handful of studies (mentioned below) addressing this area. To investigate the volatile emissions from rhizobacteria associated with Bambara groundnut, researchers employed GC-MS analysis after extracting compounds using seven solvents: benzene, butanol, petroleum ether, chloroform, ethyl acetate, hexane, and methanol. A total of 68 compounds were identified based on their retention times, structural characteristics, and peak area percentages. The compound dimethylfuvene extracted with ethyl acetate from *B. amyloliquefaciens* and *B. thuringiensis* showed the highest abundance (89.11%), while formic acid 2-methylpropyl ester from hexane extraction had the lowest (6.25%). Other important compounds identified included tridecane, acetic acid butyl ester, paraldehyde, S-(+)-1,2 propanediol, as well as p-xylene (Ajilogba and Babalola, 2019).

In another study conducted by Makarova et al. (2012), several aromatic compounds were identified in the root exudates of three legume species, namely *Pisum sativum, Vicia faba, and Glycine max*. These compounds included N-phenyl-2-napthyl amine, dibutyl and dioctyl esters of ortho-phthalic acid. These compounds act as negative allelopathic substances, regulating legume–rhizobia symbiosis, particularly under conditions unfavorable for symbiotic interactions following rhizobial inoculation.

Additionally, non-volatile root exudate metabolites can be identified by derivatizing them with silylating reagents such as *N, O*-bis (trimethylsilyl)trifluoroacetamide (BSTFA) with trimethylchlorosilane (TMCS), and subsequently analyzing them by GC-MS. In a study examining the impact of maize-peanut intercropping on root exudation profiles, exudates were collected from both monoculture and co-culture hydroponic systems. GC-MS analysis of derivatized extracts revealed the presence of sixteen isoflavonoids, including isoflavone, genistein, formononetin, taxifolin, rotenone, biochanin A, daidzein, and quercetin. Additionally, fatty acid methyl esters such as methyl hexadecanoate (13.33-fold), methyl linoleate (2.9-fold), and methyl linolenate (6.83-fold) were found to be significantly increased in the intercropped peanut/maize system (Jiang et al., 2022).

In another related study by Ankati and Podile (2019), the metabolite profiling of groundnut root exudates was performed to compare bacterized (inoculated with plant growth-promoting rhizobacteria, PGPR) and non-bacterized treatments. Root exudates were derivatized using methoxyamine hydrochloride and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) and analyzed via GC-MS/MS. A total of 75 metabolites were identified, including amino acids, sugars, organic acids, and phenolic compounds. Notably, threonine and glyoxylic oxime acid were detected in *Pseudomonas aeruginosa*-bacterized root exudate. In contrast, serine, pentanoic acid, glucopyranoside, tartaric acid, and 2-pyrrolidinone were detected in root exudates of seedlings bacterized with *Bacillus sonorensis* and *Pseudomonas aeruginosa*. Kidd et al. (2018) analyzed root exudate profiles from various pasture and grain legume species to identify citramalates. Root exudates were derivatized with methoxyamine in pyridine, followed by silylation with MSTFA, and subsequently analyzed via GC-MS. Among the metabolites detected, citrate and malate were present in the rhizosheath across all species studied.

Collectively, these studies indicate that reports on the identification of volatile compounds in legumes' root exudates using GC-MS are still limited. A significant knowledge gap remains regarding the characterization of volatile metabolites in root exudates emitted by leguminous plants. However, several studies have successfully identified root exudate-related primary and non-volatile secondary metabolites through derivatization with silylating agents, followed by GC-MS analysis.

### 6.2.4 Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectroscopy is among the most powerful techniques for the structural elucidation of organic molecules, primarily applied to previously isolated and purified compounds, but also applicable to complex mixtures. It is considered a "gold standard" in natural products research for new molecule discovery. An advantage of NMR is its capacity for direct chemical structure and conformation elucidation. Furthermore, its multinuclear capability allows for the analysis of both complex mixtures and purified molecules. NMR has lower sensitivity compared to MS, but it provides a distinct advantage in metabolomics by detecting analytes regardless of their ionization efficiency. This capability yields more comprehensive and unbiased metabolome coverage (Nagana Gowda and Raftery, 2015; Wishart et al., 2022).

Its principle relies on the fact that only nuclei with a non-zero spin quantum number ($I \neq 0$) can absorb or emit electromagnetic radiation. Even–even nuclei, such as $^{12}C$, $^{16}O$, and $^{32}S$, exhibit $I = 0$ and are thus inactive in NMR. In contrast, nuclei with $I = n/2$, where n is an odd integer (e.g., $^{1}H$, $^{13}C$, $^{19}F$, $^{15}N$, $^{31}P$), interact with the applied magnetic field, generating detectable resonance signals. Analysis of resonance frequencies and relaxation times enables characterization of these nuclei. The chemical shift (δ), expressed in parts per million (ppm), represents the difference between a nucleus's resonance frequency and that of a reference standard (Ross et al., 2007).

In natural product research, $^{1}H$ and $^{13}C$ nuclei are most frequently analyzed. Their one-dimensional spectra provide information on the number and types of nuclei, chemical shifts, multiplicities, and coupling constants. Spectroscopic investigation typically begins with $^{1}H$-NMR, which offers insights into molecular framework complexity, sample purity, minor components, solvent effects, and acquisition parameters. This is followed by $^{13}C$-NMR and complemented by two-dimensional techniques - such as COSY, TOCSY, NOESY, ROESY, HSQC, and HMBC - for comprehensive structural elucidation (Lubbe et al., 2013).

Although less sensitive than MS-based methods, NMR spectroscopy offers unique advantages for root exudate studies. It is highly reproducible, inherently quantitative, and requires minimal sample preparation (Gowda et al., 2025). Importantly, NMR can resolve structural isomers that are difficult to distinguish using MS alone. In the study of legume root exudates, Evidente et al. (2007) reported the isolation and the chemical structure characterization of trigoxazonane, a new monosubstituted trioxazonane, isolated from root exudates of *Trigonella foenum-graecum* using NMR, FT–IR and UV spectrometry. This compound showed inhibitory activity against the *Orobanche crenata* seed germination, which is a significant threat to grain legume production.

In another study, in which greater quantities of the purified compounds was required, three isoflavanones, 5,7,2′,4′-tetrahydroxy-6-(3-methylbut-2-enyl)isoflavanone, 4″,5″-dihydro-5,2′,4′-trihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone and 4″,5″-dihydro-2′-methoxy-5,4′-dihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone, and a previously known isoflavone 5,7,4′-trihydroxyisoflavone (genistein) were isolated and characterized spectroscopically from the root exudate of *D. uncinatum* (Jacq.) DC (Tsanuo et al., 2003). For compound isolation, the authors trapped the active root exudate material directly from a hydroponic system using C18 adsorbent. The procedure consisted of *D. uncinatum* seedlings growing on trays using distilled water for 3 days, followed by nutrient B5 medium for another 3 days, and then returning to distilled water. Over 4 weeks, the trapping of root exudate material was carried out when the *D. uncinatum* plants were growing in distilled water. The trapped material was then desorbed with methanol, yielding 120 mg of bulk crude root exudate, which was further isolated using reverse-phase liquid chromatography. The isolated compound was characterized using NMR and assayed for *S. hermonthica* germination stimulant activity.

Other applications have demonstrated that NMR can detect shifts in exudate composition under abiotic stress, thereby linking metabolic fingerprints - such as phenolic acids, sugars, and amino acids - to plant stress responses and microbial recruitment. Recent advances, particularly high-resolution magic angle spinning (HRMAS) NMR, further extend its utility by enabling the *in-situ* analysis of semi-solid samples, such as intact root tissues and rhizosphere material (Mazzei and Piccolo, 2017). Aiming at a non-targeted metabolomics approach, a tailored NMR-based protocol was used to analyze exudates from hydroponically grown pea and faba bean plants, showing that osmotic stress affects exudate quantity without altering chemical diversity, highlighting both methodological refinements and biological relevance (Fortier et al., 2023).

### 6.2.5 Data analysis, annotation, visualization, integration, and interpretation

The study of root exudates has evolved in tandem with advancements in metabolomics and in bioinformatics, enabling scientists to better comprehend the chemical diversity and ecological significance of these compounds. For a holistic view of root exudate chemical patterns and rhizosphere interactions, we highlight the integrative analysis using multi-omics approaches, data collection at scale, plus well-established computational tools.

One of the bottlenecks in metabolomics analysis is managing the large volume of data required to assess the metabolome, obtained using techniques such as GC-MS, LC-MS, or NMR. Pre-processing raw data from analytical platforms is the first step in root exudate analysis, employing softwares such as MZmine, XCMS, and MS-DIAL, to extract good features, which assures accuracy and reproducibility in later studies (Gowda et al., 2014; Tsugawa et al., 2015; Schmid et al., 2023; Heuckeroth et al., 2024). For instance, efficient data pre-processing of root exudates from *L. japonicus* is a starting point for correlating the impact of the nitrogen regimen on the chemical profile (Tao et al., 2024).

In mass spectrometry analysis, the determination of metabolites is based on comparing the consensus spectra with those deposited in spectral databases. Currently, several open-source platforms assist in compound annotation and/or class predictions. SIRIUS combined with the CANOPUS module (Dührkop et al., 2019, 2021), for example classifies metabolites into superclasses and

subclasses (Salomonsen et al., 2024) according to NPClassifier criteria (Kim et al., 2021). The GNPS (Global Natural Products Social Molecular Networking) platform is widely used to connect chemical structures with biological and ecological roles and visualize the chemical fingerprint through molecular network (Wang et al., 2016). Flavonoids and isoflavonoids in legume root exudates have recently been profiled with GNPS molecular networking, a powerful platform that connects the chemical diversity of these compounds though structural similarity (Heuermann et al., 2023).

Supervised and unsupervised multivariate analysis examines the entire metabolome simultaneously. Unsupervised methods explore inherent data structures without predefined sample groups, making them ideal for initial pattern recognition and data exploration. In contrast, supervised methods utilize known group classifications to identify metabolic features that best distinguish between them. For instance, the Partial Least Square-Discriminant Analysis (PLS-DA) indicates the relevant features in metabolome correlating with plant functional trait (Seitz et al., 2023; 2024).

Large datasets from root exudate studies are complex, and visualization is critical for interpreting all results and validating the workflow. Without adequate visualization, several chemical, biological, and ecological answers remain hidden. In the statistical analysis, the Heatmaps, PCA plots, and sunburst plots highlight the distributions of metabolites across treatments (Döll et al., 2024). In short, a good analysis makes the data visible, clear enough to reveal patterns, yet detailed enough to guide interpretation.

Multi-omics approaches enable the integration of metabolite profiles with microbial community structures, connecting metabolite patterns to gene expression while assessing broader ecological effects. This integration reshapes our understanding of how plants and microbes communicate below ground. The process is facilitated by computational tools such as DRAM (for functional genome annotation), GTDB-tk (for taxonomic classification), and QIIME 2 (for microbiome analysis). Together, these tools provide a clearer picture of how rhizosphere microbiomes interact with root exudates (Seitz et al., 2022). Tao et al. (2024) demonstrated in their research article that nitrogen availability alters the composition of root exudates in legumes, resulting in shifts in microbial community formation.

Although many studies investigating root exudate-microbiome interactions in legumes rely primarily on multi-omics integration, the combination with metabarcoding-based community analyses allows the scientific community to perform correlation-based inferences (Dutta et al., 2020). However, root exudation is a highly dynamic process regulated by intrinsic developmental programs and environmental cues; consequently, single time-point measurements may fail to capture transient pulses of signaling molecules involved in rhizobial recruitment (Bais et al., 2006). Furthermore, many exuded metabolites are rapidly metabolized or transformed by surrounding microorganisms, complicating the distinction between actively secreted plant compounds and secondary microbial products (Oburger and Jones, 2018; Martins et al., 2026). Without spatially resolved sampling, time-series analyses, or experimental validation, bulk metabolomic approaches may therefore overestimate direct metabolite-driven effects and obscure fine-scale ecological processes in legume rhizospheres.

Conversely, innovative trends such as AI and machine learning breakthroughs encourage the application of these approaches to help in the understanding such interactions. For example, neural network algorithms and support vector machines are increasingly used to categorize root exudate patterns based on treatments, environmental conditions, and microbial interactions (Zhao et al., 2023). Machine learning models differentiate exudate profiles under varying nitrogen levels, linking them to metabolite diversity (Tao et al., 2024). AI platforms, such as Omics Integrator (Tuncbag et al., 2016) and Multi-Omics Factor Analysis (MOFA), integrate metabolomics with other omics data to provide a comprehensive understanding (Dutta et al., 2020). AI tools such as t-SNE and UMAP aid in visualizing complex interactions within metabolomics datasets (McInnes et al., 2018). Predictive models forecast changes in exudate composition in response to environmental stresses, aiding in the development of sustainable agricultural practices (Na and Na, 2024).

By utilizing LC-MS/MS, machine learning and AI tools such as MS2DeepScore and DreaMS, it is possible to precisely predict metabolite structures (Huber et al., 2021; Bushuiev et al., 2025). DeepLC can accurately predict retention times (Bouwmeester et al., 2021), thereby facilitating the identification of novel metabolites. AlphaFold is being adapted to predict protein-metabolite interactions, revealing how exudates affect microbial communities (Abramson et al., 2024).

Emerging applications in spatial metabolomics use machine learning to analyze mass spectrometric data, mapping exudate distribution and interactions with soil bacteria (Deng et al., 2021). Tools such as MALDI-MSI (Escolà Casas and Matamoros, 2021) and AI-based image analysis (Zhang et al., 2024) facilitate *in situ* visualization of exudate hotspots. Generative AI models, including Large Language Models, can assist in hypothesis generation and experimental design under human supervision (Ding and Li, 2025), accelerating discovery by analyzing literature and identifying knowledge gaps.

## 7 Conclusions and future research perspectives

Legume root exudates, particularly their secondary metabolites, are essential in mediating ecological interactions, as reported over the last 25 years in pulses and leguminous oilseed crops. These compounds regulate symbiotic relationships, modulate rhizosphere microbial communities, and enhance plant defense mechanisms under abiotic and biotic stress. Beyond their contribution to nitrogen fixation and nutrient acquisition, root exudates are involved in allelopathy, pathogen suppression, and pollutant remediation, emphasizing their ecological and agronomic significance.

Undoubtedly, the rapid progress of metabolomics and high-resolution analytical techniques has greatly expanded our understanding of exudate chemical diversity. Among the diverse classes of metabolites, phenolic compounds, particularly flavonoids

and isoflavones, are the most frequently identified compounds in legume root exudates. They function as central regulators of symbiosis, influence microbial community composition, and strengthen plant resilience to environmental challenges. However, other compounds classes such as volatile organic compounds, which participate in a wide range of biotic and abiotic interactions (both below- and above-ground) remain understudied. Therefore, comprehensive metabolomics studies, integrating other non-volatile compound classes (*e.g.*, common chemical markers for legumes such as saponins and other glucosides) and volatile metabolites, would provide invaluable information on the root exudation dynamics.

In this context, emerging technologies such as spatial metabolomics and advanced root imaging will enable *in situ* visualization and precise localization of exudation within root zones. The integration of these datasets with computational tools, including machine learning and artificial intelligence, will further enhance metabolite identification, pathway reconstruction, and the interpretation of plant responses under variable environmental conditions.

It is important to point out that despite growing insight into the chemistry and functions of root exudates, this knowledge remains fragmented across few legume taxa, with most research concentrated on a limited number of model and crop species. Wild and underutilized members of the Fabaceae family, particularly those native of biodiversity-rich regions, represent a largely unexplored reservoir of metabolic diversity. Therefore, besides selecting and including wild species in future studies, prioritizing legume species that represent a broader phylogenetic diversity and exhibit contrasting ecological or functional traits, such as root architecture, nitrogen-fixation strategy, or habitat adaptation, would greatly contribute to better understand how exudate chemistry shapes rhizosphere microbial interactions. Ultimately, expanding research beyond cultivated species, while combining metabolomics with genomics, ecology, and data-driven modeling, could uncover new mechanisms governing multitrophic interactions.

This knowledge, consequently, will aid the design of microbiome-based cropping systems that minimize agrochemical dependence, enhance climate resilience, and promote soil and ecosystem restoration. Collectively, these advances provide a foundation for translating root exudate research into practical strategies for sustainable agriculture and ecological stewardship.

## Author contributions

NK: Methodology, Data curation, Visualization, Writing – review & editing, Conceptualization, Writing – original draft. AG: Methodology, Writing – review & editing, Writing – original draft, Data curation, Visualization, Conceptualization. PZ: Writing – review & editing, Methodology, Writing – original draft, Data curation. KV: Writing – review & editing, Writing – original draft, Data curation, Methodology. MB: Data curation, Methodology, Writing – original draft, Writing – review & editing. ER: Methodology, Data curation, Writing – review & editing, Writing – original draft. RG: Supervision, Funding acquisition, Conceptualization, Writing – review & editing. NL: Writing – review & editing, Supervision, Resources, Funding acquisition. ND: Conceptualization, Supervision, Funding acquisition, Resources, Writing – review & editing. PB: Project administration, Writing – original draft, Visualization, Conceptualization, Writing – review & editing, Supervision, Methodology.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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