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Review Article

Linking Untargeted Metabolomics to Functional Bioactivity in Nutmeg (*Myristica fragrans* Houtt.): Current Advances, Bioactivity Integration, and Analytical Challenges

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Abstract

Myristica fragrans Houtt. has been widely explored due to its high chemical composition and many functional features, nevertheless, the analytical framework applied to analyze this species has progressed unevenly. This study critically discusses the shift from conventional GC–MS-based phytochemical profiling to more integrated metabolomics techniques incorporating LC-HRMS, chemometrics, and bioactivity analysis in nutmeg research. A literature study was undertaken in five scientific databases using PRISMA guidance. Studies were categorized as MAIN and SUPPORT to distinguish between metabolomics-driven studies and studies providing complementary evidence of phytochemical and bioactivity data. The synthesis illustrates that most of the published work still depends on GC–MS analysis of volatile fractions and essential oils, but fully untargeted metabolomics investigations are rare. Recent developments have increased metabolite coverage, sample discrimination and helped identification of potential markers related to bioactivity interpretation, species authentication and quality assessment. Most studies still tend to report relationships between metabolites and bioactivity mostly as compositional or statistical associations, biomarker verification remains patchy across the literature and metabolite annotation is still often limited to MSI Level 2 or 3, although examples of higher-confidence identification are emerging. In sum, contemporary M. fragrans research can best be characterized as a shift from extended phytochemical profiling to more biologically interpretable metabolomics. Future advances will depend on increased standardization, more confident metabolite annotation, stricter chemometric validation, and wider integration of chemical signatures with functional biological evidence to enable more robust and translatable applications of nutmeg metabolomics.

Keywords: bioactivity, biomarker discovery, chemometrics, nutmeg, untargeted metabolomics

1. INTRODUCTION

The nutmeg plant (*Myristica fragrans* Houtt.) contains various metabolites that have been associated with antioxidant, antimicrobial, anti-inflammatory, and neuroprotective activities [1–3]. The chemical diversity and biological functions make nutmeg a relevant system for examining the relationship between metabolite complexity and functional bioactivity. So far, phytochemical studies and metabolite profiles of nutmeg have been dominated by the analysis of volatile fractions of essential oil based on GC–MS, which has greatly contributed to identifying dominant components such as sabinene, myristicin, elemicin, terpinen-4-ol, and eugenol, as well as supporting studies on aroma, chemotype, authentication, and safety [4]. Nevertheless, the strength of GC–MS in mapping volatile profiles has not always been followed by equivalent biological integration, so the relationship between chemical composition and biological activity in many studies is still primarily explained through compositional association or statistical analysis [5]. Although some recent studies have begun to apply more targeted metabolite feature selection, stronger analytical validation, and mechanistic approaches such as molecular docking, network pharmacology, and experimental verification, the relationship between the chemical profile of nutmeg and its functional bioactivity generally remains indicative [3,6,7].

Metabolomic research on natural products has evolved from mere descriptive profiling to a more integrative analytical framework [8,9]. This approach combines high-resolution mass spectrometry with multivariate analysis, feature prioritization, and computationally assisted interpretation [10,11]. In that paradigm, metabolomics no longer stops at the inventory of compounds. The focus is directed toward identifying discriminative features, enhancing

confidence in annotations, and supporting more meaningful biological interpretations. However, the extent to which this transition is truly realized in nutmeg research has rarely been evaluated critically and systematically.

Although the term metabolomics is increasingly used in nutmeg studies, the actual application of untargeted metabolomics is relatively limited because most of the literature still relies on profiling volatile components based on GC–MS without applying the complete metabolomics workflow, such as systematic feature extraction, alignment, confidence-based annotation, multivariate discrimination, and biomarker validation. Even in studies that have used LC-MS or LC-HRMS, metabolite identification is often still putative, and its correlation with bioactivity has generally not been well established. As a result, the term "metabolomics" in many cases still more reflects the expansion of phytochemical profiles rather than modern metabolomics that are truly biologically interpretative. Furthermore, the integration between metabolomics and bioactivity in nutmeg is still dominated by correlational relationships, although several recent studies have begun to incorporate molecular docking, network pharmacology, and experimental verification [3]. However, the biological validation of the proposed features or biomarker candidates remains uneven, and the inferences drawn are often limited by potential overfitting, lack of external validation, limited permutation testing, and low standardization of the analytical workflow. Thus, current nutmeg research remains dominated by metabolite-associated bioactivity, although the field is beginning to shift toward more biologically validated metabolomics.

This review critically evaluates the application of metabolite profiling and untargeted metabolomics to nutmeg, with particular emphasis on their integration with functional bioactivity. This article not only summarizes the compounds and activities reported but also reviews the gradual developments in the field. These developments include the dominance of GC–MS for volatile fractions, the shift toward LC-HRMS for broader metabolite discrimination, the use of chemometrics and biomarker discovery, and the still-limited efforts to link chemical signatures to validated biological activitie.

2. MATERIALS AND METHODS

2.1. Study Design

This review is prepared following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency, methodological consistency, and traceability of the literature selection process [12]. The review protocol was established before the screening stage and includes search strategies, selection criteria, data extraction, article classification, and data synthesis approaches. This review is a critical narrative synthesis that focuses on the use of metabolomic approaches in nutmeg research, particularly with respect to functional bioactivity. Therefore, this review not only summarizes the compounds and biological activities reported but also evaluates the quality of the analytical approaches used, including extraction, solvent systems, instrumentation platforms, chemometric strategies, metabolite annotation levels, and the strength of the integration between metabolite profiles and bioactivity.

2.2 Data Sources and Strategy for Literature Search

A systematic literature search was performed in five international scientific databases: Scopus, ScienceDirect, PubMed, Wiley Online Library, and Taylor & Francis Online. All searches were made on April 20, 2026, and the included articles were published between 2010 to 2026. The search technique was constructed around four primary domains; plant species, analytical methods, functional bioactivity and safety and authentication.

Table 1. Database and literature search strategy.

Database	Search String	Records Retrieved
Scopus	TITLE-ABS-KEY(("Myristica fragrans" OR nutmeg) AND (metabolomics OR "metabolic profiling" OR "GC-MS" OR "LC-HRMS") AND (bioactivity OR antioxidant OR antibacterial OR authentication OR adulteration OR adulteration))	78
ScienceDirect	("Myristica fragrans" OR nutmeg) AND (metabolomics OR "LC-MS" OR "GC-MS") AND (bioactivity OR antioxidant OR antibacterial OR authentication)	420
PubMed	("Myristica fragrans"[Title/Abstract] OR nutmeg[Title/Abstract]) AND (metabolomics OR "LC-MS" OR "GC-MS") AND (bioactivity OR antioxidant OR antibacterial OR authentication OR adulteration)	40
Wiley Online Library	("Myristica fragrans" OR nutmeg) AND (metabolomics OR "LC-MS" OR "GC-MS") AND (bioactivity OR antioxidant OR antibacterial OR authentication OR adulteration)	71
Taylor & Francis Online	("Myristica fragrans" OR nutmeg) AND (metabolomics OR "LC-MS" OR "GC-MS") AND (bioactivity OR antioxidant OR antibacterial OR authentication OR adulteration)	83

In the species realm, the terms are like “Myristica fragrans” and “nutmeg”. The analytical domain comprises terms relating to metabolite profiling and mass spectrometry based metabolomics such as “metabolomics”, “metabolic profiling”, “GC-MS”, “LC-MS”, “LC-HRMS”, “UPLC-QTOF/MS” and similar terms. The bioactivity domain includes terms such as "bioactivity," "antioxidant," "antibacterial," "anti-inflammatory," "anti-aging," and "enzyme inhibition," while the safety and authentication domain includes terms such as "toxicity," "toxicology," "metabolic activation," "food safety," "authentication," "adulteration," and "quality control." The addition of the final domain was carried out to ensure that the literature collected not only represents the positive bioactivity dimension but also includes issues of material authentication, biosafety, and the potential toxicity of nutmeg components, which are increasingly relevant in the development of cutting-edge metabolomic research [13,14]. Details of the search strategy and the number of articles obtained from each database are presented in Table 1.

2.3 Eligibility Criteria and Study Selection

The inclusion criteria in this review encompass original research articles in English that focus on nutmeg or its derivatives and involve metabolite analysis, phytochemical profiling, or mass spectrometry-based metabolomic approaches, including, but not limited to, GC-MS, LC-MS, LC-HRMS, UPLC-QTOF/MS, Orbitrap-MS, and similar platforms. The included studies must also report at least one form of functional bioactivity or have clear relevance to the authentication, quality, safety, or chemical discrimination of nutmeg. Only articles with the full text available are included in the selection process.

Exclusion criteria include review articles, conference proceedings, editorials, and book chapters. Studies that focus solely on agronomy, food processing, or simple phytochemical identification, without relevance to the integration of metabolites with bioactivity, authentication, quality, or safety, are also excluded. Duplicate articles, articles with insufficient methodological information, and articles with inaccessible full texts were excluded during the screening and eligibility assessment stages.

All articles obtained from the five databases were first compiled into a single reference list to identify and remove duplicates. After the deduplication stage, the remaining articles are screened by title and abstract to assess their relevance to the review's focus. Articles that pass the initial screening are then evaluated through full-text review to ensure substantive and methodological alignment with the review's objectives. Exclusion reasons at the full-text assessment stage are recorded to maintain transparency in the selection process.

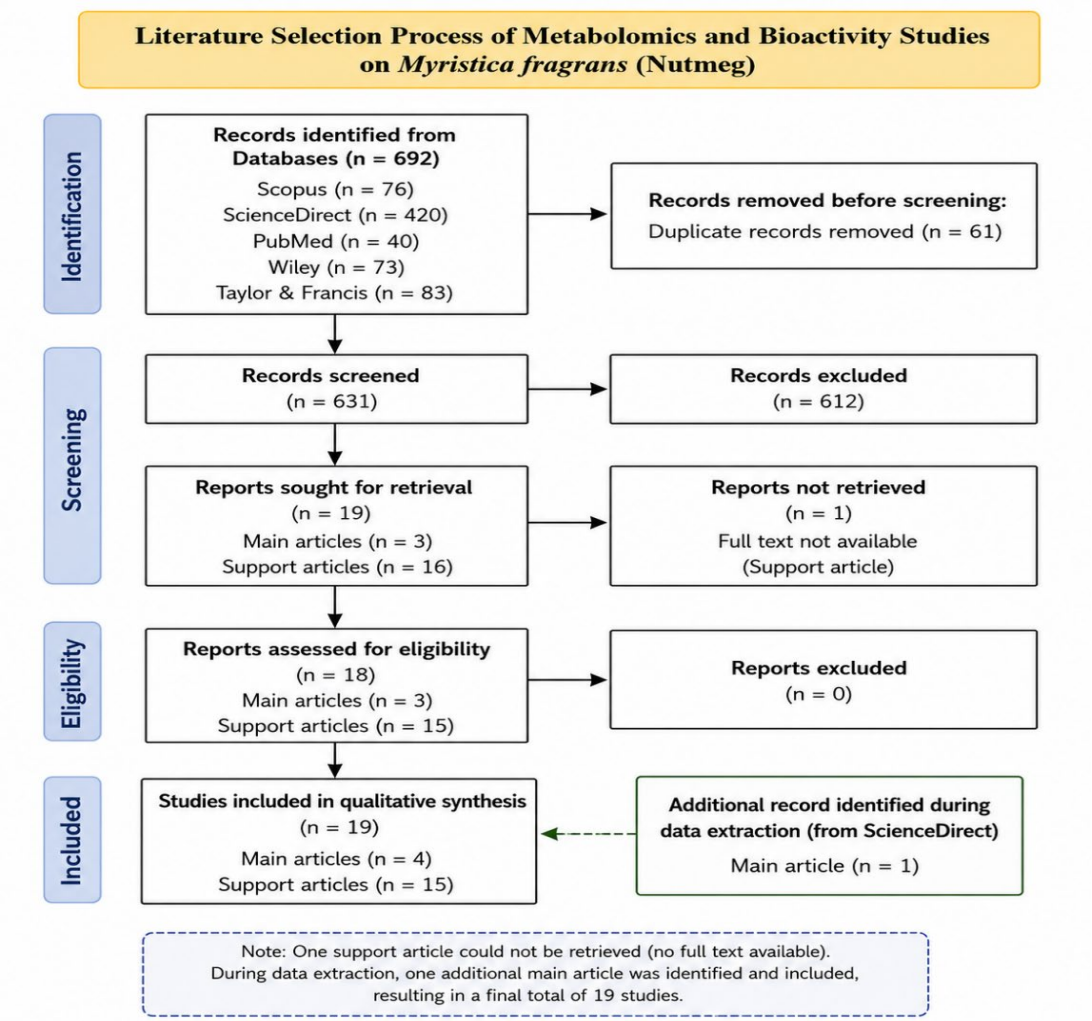

Figure 1. PRISMA 2020 flow diagram illustrating the literature selection process for studies on nutmeg metabolomics and bioactivity. The screening process included identification, duplicate removal, title and abstract screening, full-text eligibility assessment, and the final inclusion of relevant studies for this review.

In addition to the PRISMA-based selection process, the included studies were appraised qualitatively prior to synthesis. Given the heterogeneity of the literature, this appraisal did not rely on a formal clinical risk-of-bias tool but instead considered criteria more relevant to metabolomics research, including sample description, extraction strategy, analytical platform suitability, metabolite annotation confidence, chemometric rigor, and the extent of biological validation.

2.4 Article Classification

The articles that passed the selection process were then classified as MAIN or SUPPORT studies to allow a more targeted and analytical balance in the narrative synthesis. This classification was based on thematic relevance to the review objectives, as well as on the relative degree of analytical depth, interpretative strength, and integration of metabolite profiling with biological or applied significance. The approach was necessary because not all studies contributed equally to the review's central aims. The included literature was heterogeneous in terms of study design, platform selection, analytical scope, and intended application.

Studies were classified as MAIN studies if they met at least two of the following criteria: (i) application of untargeted or integrated mass spectrometry-based metabolomics platforms, e.g., LC-HRMS, UHPLC-QTOF-MS, or coupled HRMS/GC-MS workflows; (ii) use of chemometric tools for sample discrimination, feature prioritization or marker selection; (iii) explicit connection between metabolite features and bioactivity, authentication, safety or processing effects; and (iv) analytical

interpretation that advanced beyond descriptive compound listing to biologically or functionally meaningful inference. SUPPORT studies, conversely, were defined as those that did not reach this analytical depth but provided relevant contextual evidence, particularly regarding phytochemistry, metabolite composition, extraction strategies, authentication, and constituents related to safety, chemical quality, or functional bioactivity.

This classification was used so that MAIN studies served as the primary basis for constructing the review's critical arguments, especially regarding advances in metabolomics, chemometric discrimination, biomarker discovery, and metabolite–function interpretation. SUPPORT studies were retained to provide phytochemical context, broaden comparative interpretation, and capture relevant evidence that was not wholly metabolomics-oriented yet still important for understanding the wider analytical and functional landscape of *Myristica fragrans* research.

3.5 Data Extraction and Narrative Synthesis

Data are extracted methodically from each article in a pre-specified format. The data collected includes study identity, plant part, type of extract or fraction, extraction method, solvent system, analytical platform, profiling or metabolomic approach, chemometric strategy, type of bioactivity, main reported metabolites, and suggested association between chemical profile and biological activity. Where available, the level of annotation of metabolites, the software used for data processing, statistical validation and the methodological restrictions are also reported. This technique is consistent with the scope of the review, which not only inventories the results but also analyzes the quality of the integration of the chemical data with the biological interpretation in the selected literature.

The substantial heterogeneity among the included studies in terms of research designs, extraction methodologies, instrument platforms, metabolite annotation strategies, and bioactivity characteristics made quantitative meta-analysis difficult and the data were synthesized using a narrative synthesis strategy. The synthesis aims to assess the development of analytical platforms, the evolution of metabolomic workflows, the use of chemometrics, the reliability of metabolite annotation, and the extent to which the relationship between metabolite profiles and bioactivity is supported by adequate biological and methodological inferences. In the process, the review critically discusses many recurrent problems in the literature such as extraction bias, ion suppression, annotation ambiguity, over-reliance on spectral libraries and poor validation of discriminant biomarkers.

3. RESULTS AND DISCUSSIONS

3.1. General Characteristics of Included Studies

In total, 19 studies that met the inclusion criteria were included in a qualitative synthesis of 4 MAIN and 15 SUPPORT studies. This composition reveals that nutmeg research has still been dominated mostly by traditional phytochemical and bioactivity studies, rather than by the application of a current metabolomic framework. While the majority of papers still use GC–MS for profiling volatile chemicals and characterizing essential oils, only a small proportion of primary studies employ untargeted metabolomics techniques based on high-resolution mass spectrometry (HRMS). This trend implies that the metabolomic investigation of nutmeg is still in the process of a slow evolution and the entire metabolomics procedure has not been uniformly implemented over the existing literature.

The distribution of the included research also reflects a methodological change in recent years. In early research, the identification of volatile components and aroma using GC–MS was generally the focus, whereas more recent studies have started to involve platforms such as UHPLC-QTOF-MS, LC-HRMS/MS and UPLC-ESI-QTOF/MS(E) to analyze both volatile and

non-volatile metabolites more comprehensively [15]. However, this transition has not been uniform. The dominance of GC–MS remains strong, especially in SUPPORT studies, while HRMS-based studies, accompanied by chemometrics, metabolite discrimination, and deeper biological interpretation, remain limited. Thus, the development of this field is more accurately viewed as a transitional phase from extended phytochemical profiling to integrative metabolomics, with more advanced approaches beginning to emerge but not yet constituting the majority of published studies.

From a biological materials perspective, seeds are the most frequently used plant part, indicating that they remain the primary source of bioactive

Table 2. General characteristics of included studies on metabolomics and bioactivity of nutmeg.

Ref	Plant Part	Analytical Platform	Metabolomics Type	Bioactivity
[4]*	Seed, aril, powder	GC-MS	Fingerprinting (semi-metabolomics)	No direct bioassay (only safety discussion)
[20]*	Seed	UHPLC-QTOF-MS + GC-MS	Untargeted metabolomics (HRMS) + integrated platform	Not directly tested (processing-based study)
[21]*	Seed (oil, total extract, spent material, residual water)	GC-MS + LC-HRMS/MS	Untargeted metabolite profiling	Antioxidant (DPPH, ABTS, FRAP, CUPRAC), Enzyme inhibition, Antimicrobial
[22]*	Seed	UPLC-ESI-QTOF/MS(E), HS-GC-MS/MS, E-nose	Untargeted metabolomics	Antioxidant (TPC, TFC, DPPH, ABTS, FRAP, CAA)
[23]**	Seed	GC-MS	Targeted / compositional profiling	No experimental bioactivity (only literature context)
[24]**	Seed	GC-MS	Targeted compositional analysis	Antifungal
[25]**	Seed (essential oil)	GC-MS (reported from previous study)	Non-metabolomics / targeted EO profiling	Antifungal, antiaflatoxigenic, antioxidant (lipid peroxidation)
[26]**	Bud	GC-MS, HPLC, LC-HRMS Orbitrap	Untargeted	None
[27]**	Mace (fuli)	GC-MS, FTIR	Non-metabolomics	Antibacterial, antioxidant
[28]**	Seed	GC-MS, SEM, RSM (BBD)	Non-metabolomics (targeted volatile profiling)	Not experimentally tested (only cited literature)
[19]**	Seed	GC-MS, UV-Vis (TPC, DPPH, β-carotene, reducing power), microbiology assays	Non-metabolomics (targeted GC-MS profiling)	Antioxidant (DPPH, β-carotene, reducing power, chelation), Antimicrobial (disk diffusion, MIC)
[29]**	Seed, mace, leaf, pericarp	GC-MS (SIM), GC-QTOF-MS	Non-metabolomics (volatile profiling)	None
[30]**	Seed	GC-MS, GC-FID	Targeted/Profiling (non-metabolomics)	Not experimentally tested (literature-based only)
[31]**	Seed essential oil	GC-MS, FTIR, DSC, TGA, XRD, 1H NMR	Non-metabolomics (volatile profiling and encapsulation study)	Antioxidant (DPPH, OH scavenging, reducing power), nitrite scavenging activity
[32]**	Seed essential oil	GC/MS and GC/FID	Non-metabolomics (volatile profiling)	Antioxidant (DPPH) and antimicrobial activity
[33]**	Fruit	GC-MS	Non-metabolomics (volatile and oleoresin profiling)	Antioxidant activity (DPPH, reducing power, chelating activity, peroxide value, TBA, p-anisidine)
[34]**	Seed extract	GC-MS, AAS, µPADs, UV-Vis	Non-metabolomics phytochemical profiling	Antioxidant (DPPH, total phenolic), antimicrobial, anti-inflammatory (COX-2 inhibition)
[35]**	Fruit flesh, seed, and arillus (mace) essential oils	GC-MS, FT-IR, UV-Vis	Non-metabolomics phytochemical profiling	Antioxidant (DPPH) and antibacterial activity
[36]**	Mace essential oil	GC-MS, FT-IR	Non-metabolomics phytochemical profiling	Antioxidant (DPPH, ABTS), antibacterial, antiaging

Note: *) Main acrticle; **) Support article

metabolites in nutmeg [16,17]. The most commonly reported biological activities are antioxidant and antimicrobial, primarily assessed using DPPH, ABTS, and FRAP assays, as well as basic antibacterial testing [18,19]. However, in most studies, the relationship between metabolite profiles and bioactivity remains dominated by correlational approaches, although recent research has begun to integrate chemometrics [9], molecular docking, network pharmacology [7], and more in-depth biological validation. Overall, the characteristics of the analyzed studies indicate that nutmeg research is beginning to move toward modern metabolomics, but it remains limited by low adoption of HRMS, inconsistent use of multivariate analysis, and weak integration of chemical profiles with functional bioactivity.

3.2 Advances in Untargeted Metabolomics of Nutmeg

The development of metabolite analysis in nutmeg reflects a gradual shift from GC–MS-based volatile profiling to more integrative strategies that capture both volatile and non-volatile metabolites. This shift, however, has not occurred evenly across the literature. Most studies still focus on essential oils, oleoresins, or other volatile fractions, whereas those that more closely align with the logic of untargeted metabolomics remain limited to a relatively small number of key reports.

In earlier work, the main analytical contribution lay in defining the volatile fingerprint of nutmeg and repeatedly identifying major constituents such as sabinene, α-pinene, β-pinene, terpinen-4-ol, myristicin, elemicin, and safrole [4,29,36–38]. That foundation remains important because it supports authentication, quality evaluation, and the early recognition of metabolites relevant to both safety and bioactivity. At the same time, its analytical reach is still narrow, since it is largely confined to volatile compounds. In that sense, most studies from this phase are better regarded as expanded volatile profiling rather than untargeted metabolomics in the broader sense, because they establish a useful phytochemical base without yet capturing the wider metabolome of semi-polar and non-volatile constituents [29,32,39].

A more noticeable methodological step emerged once volatile analysis was no longer treated simply as a compositional description, but was combined with chemometrics for sample discrimination. This changed the role of GC–MS from a tool for listing dominant compounds into a platform that could also support species authentication, adulterant detection, and the prioritization of candidate chemical markers relevant to quality and safety. Even so, the analytical foundation at this stage still relies heavily on peak area percentages and is not consistently supported by peak deconvolution. As a result, risks of co-elution, misidentification, and bias in selecting discriminant features remain difficult to ignore. For that reason, this phase is more convincingly interpreted as a maturation of chemical fingerprinting than as a full transition to robust untargeted metabolomics [4].

More substantive progress becomes visible when HRMS-based platforms are integrated with GC–MS or used within a multiplatform design. Here, the emphasis begins to shift from merely reporting dominant constituents to prioritizing differential features, interpreting sample-to-sample variation more systematically, and identifying marker candidates that may be relevant not only to bioactivity but also to authentication and processing effects. Compared with GC–MS-dominated studies, these approaches provide broader metabolite coverage and a more informative basis for discrimination, particularly when volatile and non-volatile layers are interpreted together. This is evident in studies combining UPLC-ESI-QTOF/MS(E), HS-GC–MS/MS, electronic nose, or UHPLC-QTOF-MS with GC–MS to evaluate species-level differences and processing-related changes in a more integrated manner [20–22].

Even so, the field remains in transition rather than maturity. The number of studies that apply an untargeted metabolomics workflow in a

Table 3. Core untargeted metabolomics studies and bioactivity integration in nutmeg.

Ref	Key Metabolites	Analytical Relevance	Integration Strategy	Analytical Significance
[4]	Myristicin, sabinene, elemicin, safrole, α-pinene, β-pinene	Volatile markers relevant to species discrimination, authentication, and safety-oriented quality control	GC–MS fingerprinting combined with PCA, PLS-DA, HCA, and VIP analysis	Enabled discrimination between *M. fragrans* and *M. argentea* and suggested volatile markers relevant to authentication, adulterant detection, and safety monitoring, although interpretation remained restricted to the volatile fraction.
[20]	Malabaricone derivatives, flavonoids, myristicin, and neolignan-related compounds	Metabolites associated with antioxidant and antibacterial activity	Integrated LC-HRMS profiling, GC-MS, molecular docking, and bioactivity-guided interpretation	Identified phenolic and neolignan-related candidates associated with antioxidant and antibacterial activities and provided preliminary mechanistic support through docking-based analysis, while full biomarker validation remained limited.
[21]	5-Hydroxymaltol, maltol, dipterine, adipic acid, fragransin B2, lignans, volatile terpenoids	Processing-responsive candidate markers and bioactivity-related metabolites	Multiplatform metabolomics using UHPLC-QTOF-MS and GC-MS with PCA, PLS-DA, and machine-learning-based external validation	Revealed processing-induced metabolite shifts and proposed candidate markers for differentiating raw and bran-roasted nutmeg, supported by chemometric discrimination and external model validation.
[22]	Malabaricone C, alkyl-DHAP, (R)-Shinanolone, volatile discriminatory compounds	Discriminant metabolites associated with antioxidant capacity and variety differentiation	Untargeted metabolomics integrated with UPLC-ESI-QTOF/MS(E), HS-GC-MS/MS, electronic nose, OPLS-DA, HCA, and Pearson correlation analysis	Expanded volatile and non-volatile metabolite coverage and identified discriminant features associated with antioxidant performance and species-level differentiation, although the biological interpretation remained predominantly correlation-based.

genuinely comprehensive way is still limited, and many investigations still stop at descriptive links among dominant compounds, sample discrimination, and putative functional relevance. In other words, the main difference across the literature is no longer simply one of instrumentation, but of analytical depth: GC–MS-based studies tend to be stronger in volatile characterization and authentication-oriented fingerprinting, whereas HRMS and multiplatform studies offer broader metabolite coverage and more informative feature prioritization, though not always with equally strong annotation confidence or biological validation. Nutmeg metabolomics is therefore better understood as an emerging field moving toward broader integration among metabolite coverage, authentication, chemical discrimination, and biological validation, rather than one that has already reached a fully established untargeted metabolomics framework [20–22].

3.3 Linking Metabolomics to Functional Bioactivity

The studies summarized in Table 4 indicate that the antioxidant activity of nutmeg is most often attributed to phenolic compounds, flavonoids, lignans, phenylpropanoids, and certain volatile components. At the level of support studies, this pattern is clearly evident in nutmeg seed extracts, where the acetone extract shows a total phenolic content of 93.12 ± 1.48 mg GAE/100 g, DPPH radical scavenging activity of 63.04 ± 1.56%, metal chelation activity of 64.11 ± 2.21%, and β-carotene bleaching inhibition of 74.36 ± 1.94%. In the same extract, GC–MS identified sabinene, β-pinene, α-pinene, myristicin, isoeugenol, p-cymene, carvacrol, eugenol, and β-caryophyllene, which are then proposed as candidate contributors to antioxidant and antimicrobial activities. However, the relationships established in such studies still primarily remain at the level of compositional associations, as the contribution of each metabolite has not yet been determined through

biomarker validation or systematic mechanistic testing [19].

The connection between metabolites and antioxidant activity is enhanced when bioassays are performed simultaneously and combined with non-volatile fractions richer in phenolic compounds. It was found that the spent material extract after distillation of nutmeg by-products had the maximum phenolic and flavonoid content, which were 63.31 ± 0.72 mg GAE/g and 8.31 ± 0.06 mg RE/g, respectively. It also has considerable radical scavenging activity, metal reduction capacity, butyrylcholinesterase inhibition and antibacterial activity. These findings are crucial in that they move the focus away from the only essential oils and emphasize post-distillation fractions as additional sources of bio-active diarylnonanoids, phenolics, flavonoids and lignans. At the same time, the biological meaning of these connections requires careful interpretation. The extracts do show clear functional significance but data is still lacking to identify which individual metabolites are driving the observed reactions and which are just co-occurring in an active chemical matrix [21].

A more systematic metabolomics-based interpretation begins to emerge when volatile, non-volatile, and bioactivity-related data are considered together. Comparative studies using UPLC-ESI-QTOF/MS(E), HS-GC–MS/MS, and electronic nose analysis showed that M. fragrans had higher antioxidant capacity than Alpinia galanga, with positive relationships across TPC, TFC, DPPH, ABTS, FRAP, and cellular antioxidant activity. Within this framework, 195 non-volatile metabolites were putatively identified, with annotation confidence broadly corresponding to MSI Level 2 or 3, and Malabaricone C, alkyl-DHAP, and (R)-Shinanolone emerged as key discriminant features. This marks an important methodological shift. Interpretation is no longer based only on the most abundant compounds, but begins to draw on wider metabolite patterns that co-vary with biological function. Still, a discriminant feature is not automatically a biologically validated marker. In most cases, the evidence remains strongest at the level of correlation, while independent confirmation, targeted quantification, and causal validation remain limited [22].

A similar pattern appears in studies of antibacterial activity. Nutmeg seed acetone extract has been reported to show the strongest antibacterial and antifungal activity, particularly against *Staphylococcus aureus* and *Aspergillus niger*, while α-pinene, β-pinene, p-cymene, β-caryophyllene, carvacrol, and eugenol were proposed as major contributors based on GC–MS data. Related observations have also been reported for nutmeg essential oil obtained by microwave water hydrodistillation, which is rich in methyl eugenol, eugenol, safrole, terpinen-4-ol, and myristicin and shows strong antibacterial activity against *S. aureus*, moderate activity against *Escherichia coli*, and moderate antioxidant capacity. These findings are useful, but they still come from chemically mixed systems. As a result, the proposed metabolite–antibacterial relationships more often reflect phytochemical association than direct proof that a given feature is the primary determinant of the biological effect. The presence of myristicin and safrole also calls for a more careful reading, since recent work has begun to emphasize the toxicological and safety dimensions of these compounds. This means that functional interpretation in nutmeg cannot be separated entirely from questions of safety and biological risk [19,40].

From a biomarker perspective, the literature is beginning to move beyond simple co-occurrence. GC–MS fingerprinting combined with PCA, PLS-DA, and VIP has shown that sabinene, α-pinene, β-pinene, safrole, and myristicin can distinguish *M. fragrans* from *M. argentea*, supporting the use of volatile metabolites as markers for authentication and material differentiation [4]. More recent studies have gone further by integrating molecular docking, network pharmacology, and experimental validation to strengthen the interpretation of

specific discriminant metabolites [41]. These advances matter because they begin to separate metabolites that are merely statistically informative from those that may also be biologically meaningful. Even here, however, the field has not fully resolved that distinction. The strongest evidence still tends to cluster around a relatively small number of studies, while the broader literature remains more uneven in analytical depth and biological follow-up.

Nevertheless, the literature no longer supports the view that nutmeg research is confined to simple compositional association alone. A more mechanistically informed direction is clearly emerging. What remains unresolved is not whether such integration exists, but how widely and how rigorously it has been implemented across the field. Most studies still stop short of isolating active compounds, performing targeted quantification, validating biomarkers across platforms, confirming dose–response relationships, or linking prioritized metabolites to biological targets in a reproducible way. Future work therefore needs to do more than expand the number of reported metabolites. It needs to clarify which features are simply discriminant in statistical space and which can withstand the stronger demands of biological validation. Only then can nutmeg metabolomics move from metabolite-associated bioactivity toward metabolite-validated bioactivity.

3.4 Chemometric Strategies for Metabolite Discrimination

The application of metabolomics to nutmeg increasingly relies on chemometric strategies to extract biological patterns from complex and high-dimensional data [43]. In the analyzed literature, chemometrics is used not only to separate samples by species, processing, or extraction systems, but also to identify the most relevant metabolite candidates for chemical differences, material authentication, and

Table 4. Representative metabolites of nutmeg associated with functional bioactivities and chemometric relevance.

Metabolite	Reported Bioactivity	Chemometric / Functional Role	Validation Level	Source
Myristicin	Antioxidant, antimicrobial, neuroactive	Major discriminant metabolite associated with geographical and volatile profile differentiation	Partially validated	[21,29]
Malabaricone A	Antioxidant, antibacterial	Diarylnonanoid biomarker associated with antimicrobial activity	Experimentally validated	[21]
Malabaricone B	Antioxidant, antibacterial	Associated with bioactive differentiation among extracts	Partially validated	[21]
Malabaricone C	Strong antioxidant, antibacterial	Frequently linked with radical scavenging and antibacterial activity	Experimentally validated	[21]
Fragransin C1/C2	Antioxidant	Lignan metabolite associated with functional clustering	Association only	[21]
Giganteone A	Antioxidant potential	Diarylnonanoid dimer associated with metabolite diversity	Association only	[21]
Eugenol	Antioxidant, antifungal	Contributed to antimicrobial efficacy and aroma differentiation	Experimentally validated	[24]
Methyl eugenol	Antimicrobial, antioxidant	Important volatile marker in MAHD and GC–MS studies	Partially validated	[27]
Safrole	Antimicrobial phenylpropanoid	Monitored due to toxicological relevance and extraction variation	Experimentally validated	[42]
Terpinen-4-ol	Antioxidant, antimicrobial	Associated with oxygenated monoterpene fraction	Experimentally validated	[27]
α-Pinene	Antioxidant, antibacterial	Major monoterpene contributing to volatile fingerprint discrimination	Partially validated	[29]
β-Pinene	Antioxidant, antibacterial	Dominant volatile constituent associated with chemotaxonomic differentiation	Partially validated	[29]
Sabinene	Antioxidant, antimicrobial	Most abundant volatile contributing to GC–MS clustering patterns	Partially validated	[21]
Elemicin	Neuroactive, antioxidant	Important aromatic ether contributing to metabolomic variability	Association only	[29]
Catechin	Strong antioxidant	Associated with radical scavenging activity	Experimentally validated	[21]
Apigenin	Antioxidant, anti-inflammatory	Flavonoid contributing to antioxidant capacity	Partially validated	[21]

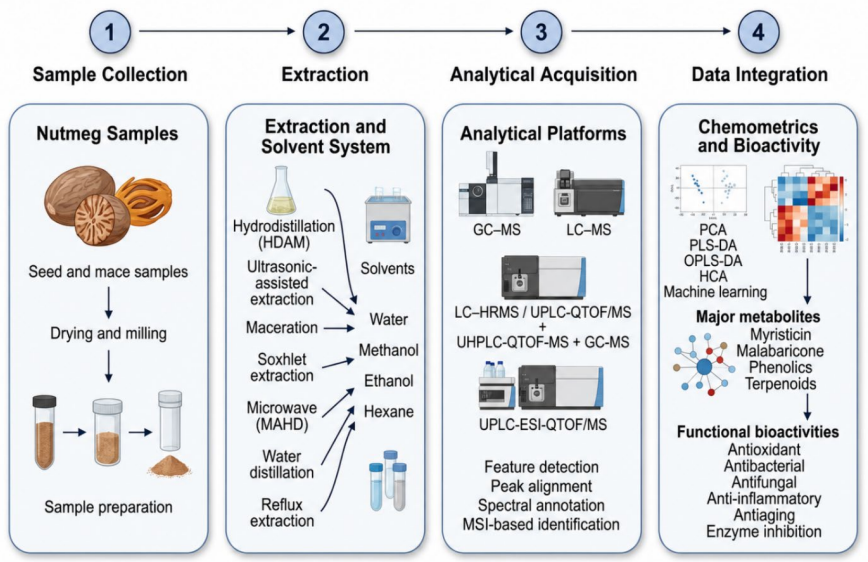

Figure 2. Workflow for untargeted metabolomics and functional bioactivity analysis of Nutmeg.

bioactivity interpretation. Thus, the main value of this approach lies not merely in the visualization of sample separation, but in its ability to transform metabolite profile data into more directed and interpretative discriminative [4,20,43].

Figure 2 summarizes the general workflow linking untargeted metabolomics to functional bioactivity analysis in nutmeg, beginning with sample preparation, LC-HRMS or GC–MS acquisition, preprocessing, dimensionality reduction, multivariate modeling, and feature prioritization, followed by biological interpretation. In practice, PCA remains the most commonly applied unsupervised approach for exploring initial clustering patterns and detecting natural variation among samples [44]. In nutmeg studies, PCA has been particularly useful for showing separation according to species, processing, or volatile profile variation, although its interpretive strength remains largely exploratory and is generally insufficient on its own for establishing reliable marker metabolites [4,20]. Interpretation becomes more discriminative when studies move to supervised methods such as PLS-DA and OPLS-DA, which enable the identification of metabolite features contributing most strongly to class separation. These models have highlighted compounds such as sabinene, α-pinene, β-pinene, safrole, myristicin, elemicin, malabaricone C, and several processing-related markers as influential variables in distinguishing nutmeg samples according to authenticity, thermal treatment, or bioactivity-related characteristics. In this setting, VIP scores are useful for prioritizing candidate discriminant features, but a high VIP value should not be treated as direct evidence of biological relevance. Without further validation, features that emerge as statistical markers may still reflect dataset structure more than reproducible biological meaning [45].

Chemometric applications in nutmeg are also becoming increasingly relevant for authentication and safety-oriented assessment. GC–MS fingerprinting, when combined with multivariate analysis, can distinguish M. fragrans from M. argentea through discriminant volatile markers, thereby supporting adulteration detection and quality control in a food safety context [4]. This is particularly important because compounds such as safrole and myristicin are not only discriminant variables within classification models, but also compounds with toxicological relevance. For this reason, chemometric analysis in nutmeg should not be confined to bioactivity-oriented pattern recognition, but should also contribute to the monitoring of safety-relevant metabolites and to the interpretation of chemical variation in relation to biological risk [4,46].

Additional tools such as HCA and heatmap clustering further clarify similarity relationships

among samples and co-occurrence patterns among metabolites, especially when interpreted alongside PCA or PLS-DA. Still, the strength of chemometric interpretation in nutmeg remains highly dependent on the analytical quality of the preceding workflow. Models based on limited sample numbers, metabolite annotations that still predominantly fall within MSI Level 2 or 3, and the absence of peak deconvolution, permutation testing, or independent validation datasets can make statistical separation appear persuasive even when biological reliability remains uncertain. At the same time, recent progress should not be overlooked. Several studies have achieved MSI Level 1 annotation for selected key metabolites and have begun to integrate molecular docking, network pharmacology, and experimental verification to strengthen the mechanistic interpretation of discriminant features [2,21,47]. Ultimately, progress in this area is not defined by the sheer number of multivariate models applied, but by the extent to which those models yield discriminant features that are consistent, reproducible, and biologically meaningful. Accordingly, the use of PCA, PLS-DA, OPLS-DA, HCA, and VIP in nutmeg research should increasingly move beyond statistical separation toward a biomarker selection framework validated across platforms and datasets, while remaining sensitive to questions of authentication, safety, and functional relevance [3,21,47].

3.5 Current Issues and Limitations

The research on nutmeg metabolomics has evolved tremendously yet the current studies suffer from some analytical and methodological limitations, which hamper biological interpretation. One of the most persistent challenges is the continued dominance of GC–MS-based profiling, which remains highly effective for volatile compounds and essential oils and has contributed substantially to authentication, species discrimination, and the identification of major constituents such as myristicin, sabinene, safrole, terpinen-4-ol, and methyl eugenol [4,29,35,40]. At the same time, this analytical emphasis still biases chemical representation toward dominant volatile constituents, while semi-polar and non-volatile metabolites such as lignans, neolignans, diarylnonanoids, flavonoids, and other polar phenolics remain less consistently explored across nutmeg matrices [21,48].

This limitation becomes more apparent because the adoption of LC-HRMS, UHPLC-QTOF-MS/MS, Orbitrap-MS, and genuinely integrated multi-platform workflows remains relatively limited [21,48]. HRMS-based platforms are far better suited to detecting minor, semi-polar, and non-volatile metabolites with higher sensitivity and mass resolution, yet only a small number of studies apply comprehensive untargeted workflows that include systematic feature extraction, alignment, annotation, and multivariate prioritization [21,48]. As a result, metabolite candidates that may be more informative for bioactivity, authentication, or safety assessment are likely to remain undetected when analysis is restricted to dominant volatile fractions.

Another major constraint concerns the confidence of metabolite annotation. Annotation confidence in nutmeg metabolomics is also shaped by the availability and effective use of curated spectral libraries, molecular networking platforms, and publicly accessible metabolomics databases. Wider use of these resources, together with machine-learning-assisted annotation tools, would improve compound assignment, reduce annotation ambiguity, and strengthen cross-study comparability and reproducibility. Many reported metabolites are still assigned primarily through spectral matching against databases, leaving a substantial proportion of features at MSI Level 2 or 3 [10,49]. Although some recent studies have reported MSI Level 1 confirmation for selected metabolites, this remains the exception rather than the standard across the nutmeg literature [21]. Such uncertainty weakens downstream interpretation, because the biological relevance

of a prioritized feature depends heavily on the reliability of its structural assignment.

The use of chemometrics also remains uneven in rigor. PCA, HCA, PLS-DA, and OPLS-DA are increasingly used to distinguish species, processing effects, and chemical variation, but validation through permutation testing, external datasets, or cross-platform confirmation is still inconsistently applied [4,50,51]. Under these conditions, statistically discriminant features may not always remain stable, reproducible, or biologically meaningful. This issue is particularly important in high-dimensional metabolomics datasets supported by relatively small sample sizes, where the risk of overfitting can easily inflate confidence in apparently discriminant markers [50,51].

From a biological interpretation perspective, the main challenge is no longer the absence of links between metabolite profiles and functional properties, but the uneven depth of validation supporting those links. Most studies still infer functional relevance from compositional patterns, correlation analysis, or chemometric separation, while mechanistic confirmation is available only in a smaller subset of the literature [19]. Although some recent studies have begun to integrate molecular docking, network pharmacology, and experimental verification, these approaches have not yet become standard practice in nutmeg metabolomics research [47]. Consequently, it often remains difficult to distinguish statistically discriminant metabolites from biologically validated markers.

These analytical restrictions also reach beyond bioactivity. In nutmeg research volatile indicators such as safrole and myristicin are important for chemical identification, authenticity, adulteration monitoring and safety assessment [4,52]. This implies that the bottlenecks in annotation, quantification and validation affect not only the interpretation of bioactivity, but also the assessment of product quality and safety [52]. Moreover, changes in solvent systems, extraction methods and sample types may affect the recovery of metabolites and change the representation of volatiles vs. non-volatiles, making comparison between studies more difficult [4,21,35].

Overall, the biggest constraints in the current research on nutmeg metabolomics are not the lack of data, but the still unequal quality of metabolite coverage, confidence in annotation, chemometric validation and biological verification. Clinical relevance, pharmacokinetic support, and broader systems-level integration also remain limited, even though selected studies have begun to move in that direction [2,46,47]. The major analytical challenges, their implications, and recommended directions are summarized in Table 5.

Overall, the main challenge in nutmeg metabolomics research does not lie solely in the lack of data, but in the immaturity of workflow standardization, annotation quality, model validation, and the integration of chemical features with biological evidence. Thus, the advancement of this field will be largely determined by the ability to shift from merely expanding metabolite coverage to enhancing inference quality, so that the resulting biomarkers are not only statistically discriminative but also reproducible, biologically interpretable, and relevant to authentication, safety, and functional applications.

3.6 Future Perspectives

Future progress in nutmeg metabolomics will depend less on simply expanding metabolite coverage and more on improving how those data are translated into biologically meaningful and practically usable knowledge. The next phase should therefore move beyond broad chemical discrimination and focus on identifying markers that remain informative across more complex biological systems, processing conditions, and real-use contexts. In this respect, clinical metabolomics offers a useful direction, not because nutmeg research is already operating at that level, but because it provides a clearer framework for asking whether candidate markers

are still relevant once they are removed from simplified analytical settings.

Another important priority is the strengthening of systems-level interpretation. Selected studies have already begun to combine metabolomics with network pharmacology, molecular docking, and experimental verification, but these efforts remain uneven and still depend heavily on annotation quality and the depth of biological validation. Their future value will lie not in adding another interpretive layer to descriptive profiling, but in clarifying how

Table 5. Current Analytical Challenges, Methodological Limitations, and Recommended Directions in Nutmeg Metabolomics Research.

Challenge Area	Current Limitations in Nutmeg Research	Impact on metabolomics–bioactivity integration	Recommended Directions
Overreliance on GC-MS profiling	Most studies still emphasize volatile fractions and essential oils, while semi-polar and non-volatile metabolites remain less explored across nutmeg matrices [4,29,35].	Narrows metabolome coverage and constrains interpretation of bioactivity, authentication, and safety-relevant chemistry.	Integrate LC-HRMS, UHPLC-QTOF-MS/MS, and multi-platform workflows alongside GC-MS.
Limited application of true untargeted metabolomics	Only a small number of studies apply comprehensive, untargeted workflows that include feature extraction, alignment, annotation, and multivariate prioritization [21,22,48].	Restricts biomarker discovery and reduces the ability to detect discriminant metabolites beyond dominant compounds.	Expand untargeted metabolomics studies using high-resolution MS and data-independent acquisition strategies such as MS^e and SWATH.
Uneven metabolite annotation confidence	Many annotations still rely on library matching without authentic standards or orthogonal confirmation, although some recent studies have achieved MSI Level 1 for selected metabolites [10,21,49,53].	Weakens biological interpretation and increases the risk of over-annotation or ambiguous compound assignment.	Improve annotation confidence through authentic standards, MS/MS matching, molecular networking, and complementary platforms such as LC-NMR.
Lack of standardized workflows	Considerable variation persists in sampling, extraction, ionization mode, preprocessing, normalization, and reporting across available studies [50,51,54].	Reduces reproducibility and complicates comparison across datasets and laboratories.	Develop harmonized workflows for extraction, acquisition, preprocessing, normalization, and reporting.
Limited chemometric rigor	PCA, HCA, PLS-DA, and OPLS-DA are increasingly used, but permutation testing, external validation, and cross-platform confirmation remain inconsistently applied [50,51,55,56].	Increases the risk of overfitting and limits confidence in discriminant features and predictive interpretation.	Strengthen model validation through external validation, larger-fold cross-validation, permutation testing, and reporting of stability metrics such as Q^2.
Predominantly correlative bioactivity interpretation	Most studies still infer functional relevance from compositional patterns, correlation analysis, or chemometric separation, while mechanistic confirmation is available only in a smaller subset of the literature [19,54,57].	Makes it difficult to distinguish statistically discriminant metabolites from biologically validated markers.	Extend metabolomics findings through targeted quantification, dose–response testing, pathway analysis, network pharmacology, molecular docking, and experimental validation.
Incomplete coverage of bioactive diversity	Research still focuses heavily on major volatile markers such as myristicin and sabinene, while low-abundance or matrix-specific metabolites remain undercharacterized [13,58].	Potentially important compounds relevant to antioxidant activity, antimicrobial effects, authentication, or safety may be overlooked.	Prioritize low-abundance metabolites using high-sensitivity HRMS, enrichment strategies, and integrated volatile/non-volatile profiling.
Matrix effects and extraction bias	Differences in solvents, sample matrices, and cleanup strategies influence metabolite recovery and ion suppression [59,60].	Produces inconsistent metabolite profiles, affecting quantitative interpretation.	Optimize extraction protocols and incorporate matrix-cleaning approaches, such as SPE and QuEChERS, as well as standardized QC samples.
Still limited translational and multiomics integration	Clinical, pharmacokinetic, and multiomics validation remains scarce, and systems-level interpretation is still emerging [54,61].	Constrains the transition from analytical discrimination to translationally relevant metabolomics.	Integrate metabolomics with clinical validation, bioavailability studies, and multiomics approaches to support precision herbal medicine and nutraceutical development.

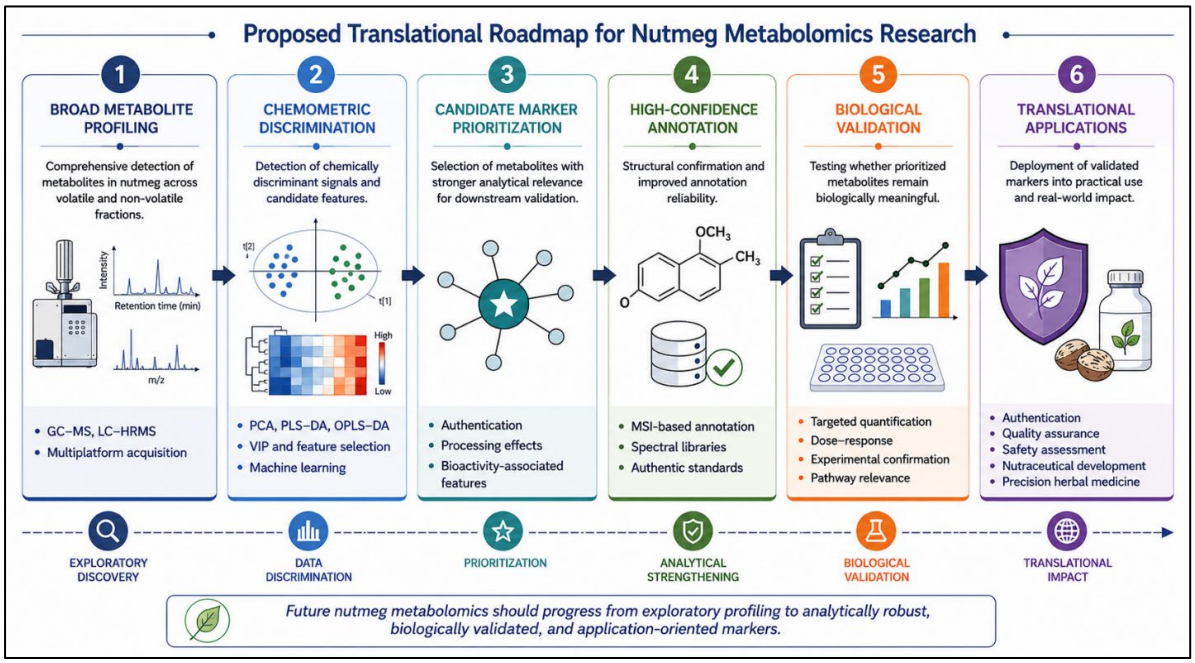

Figure 3. Proposed translational roadmap for nutmeg metabolomics research.

discriminant metabolites connect to molecular targets, biological pathways, and measurable functional outcomes. A more explicit translational framework is therefore needed to organize this progression more coherently, as illustrated in Figure 3.

Within that framework, future work should progress from broad metabolite profiling to feature prioritization, higher-confidence annotation, biological validation, and ultimately to application-oriented outcomes such as authentication, quality assurance, safety assessment, and evidence-based nutraceutical development. Framing nutmeg metabolomics in this way would help shift the field from descriptive metabolite reporting toward biologically interpretable and practically relevant use.

At the analytical level, artificial intelligence is likely to play an increasingly important role in feature prioritization, biomarker prediction, and cross-platform classification. Its contribution, however, will only be meaningful if predictive performance is matched by interpretability, reproducibility, and rigorous validation. More broadly, these advances could support stronger quality differentiation, more reliable authentication strategies, better formulation design, and, eventually, more evidence-based applications in precision herbal medicine and nutraceutical development. Future progress will also depend on broader integration with open-access repositories, curated MS/MS libraries, molecular networking resources, and machine-learning-assisted annotation tools, as these infrastructures are increasingly central to reproducible metabolite identification and interoperable metabolomics datasets.

All of these directions ultimately converge on one requirement: standardization. Without more harmonized workflows for extraction, data acquisition, annotation, chemometric analysis, and biological validation, methodological progress will remain fragmented and difficult to reproduce. A more standardized and cross-platform framework is therefore essential if nutmeg metabolomics is to move from analytical differentiation toward genuinely translational use.

4. CONCLUSIONS

This review shows that nutmeg research has shifted from GC–MS-based phytochemical profiling to a more integrative metabolomic approach using LC-HRMS, chemometrics, and bioactivity analysis. However, most studies remain dominated by statistical or compositional

associations, although recent research has begun to integrate molecular docking, network pharmacology, and experimental verification to strengthen mechanistic interpretations. Thus, current nutmeg research has not yet fully achieved biologically validated metabolomics uniformly across the literature, but is moving from expanded phytochemical profiling toward more functionally interpretative metabolomics. Future progress will be largely determined by greater confidence in metabolite annotation, stricter biomarker validation, and stronger integration of chemical profiles with biological functions, enabling nutmeg metabolomics to evolve toward more robust and translational applications. A translational progression from profiling to validation and application will be essential if nutmeg metabolomics is to move beyond descriptive analytical advancement and deliver practical value for authentication, nutraceutical development, and biologically informed product standardization.

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Conflict of Interest

The authors declare no conflict of interest.

DECLARATION OF GENERATIVE AI

Not applicable.

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