Review Article

Research Progress on using Omics Technology to Examine the Antimicrobial Mechanisms of Natural Active Substances

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Abstract
Pathogenic microbial metabolism during food storage can lead to food spoilage, which can cause food poisoning and foodborne infections, posing a significant risk to human health and safety. Additionally, food spoilage generates greenhouse gases, which could contribute to global warming and have significant impacts. These challenges prompt us to explore effective solutions to reduce food spoilage, aiming to mitigate its adverse impacts. Many secondary plant metabolites have been used in the food and pharmaceutical industries due to their natural antimicrobial activity and low drug resistance. However, the reported targets of antibacterial action are complex, and with the continuous development of research methods, it has become possible to deeply analyze the antibacterial mechanisms using omics technologies. This article discussed the trends and application of transcriptomics, metabolomics, and proteomics in investigating the antimicrobial and antifungal properties of essential oils (EOs) and their active ingredients, aiming to provide a theoretical basis for the use of plant EOs and their active ingredients in addressing health risks and environmental challenges posed by food spoilage.

Keywords: Active ingredients, Antimicrobial mechanism, Multi-omics techniques, Omics technology, Plant essential oils

1 Introduction

Food is susceptible to bacterial, fungal, and yeast contamination during both production and supply chains., causing spoilage, unpleasant odors, and even food poisoning and foodborne illnesses after accidental ingestion, presenting a serious risk to human health and safety. Despite improvements in modern food production and preservation techniques, food safety remains a public health concern. According to the World Health Organization (WHO), foodborne diarrheal infections resulting from food

spoilage cause around six million annual fatalities worldwide, with a significant proportion being young children. The severity of this issue is most pronounced in developing nations [1]. *Vibrio* spp., *non-typhoidal Salmonella* spp., *Listeria monocytogenes*, and various types of pathogenic *Escherichia coli* represent the most common foodborne pathogens [2]. Additionally, food spoilage generates greenhouse gases such as methane, leading to significant carbon emissions that exacerbate global warming and climate change. Many studies in recent years have shown notable progress in exploring natural antimicrobial compounds. Compared with other synthetic preservatives, essential oils (EOs) are consistent with the trend of "green," "safe," and "healthy" food additives. Plant EOs consist of diverse aromatic compounds with bioactive capabilities, including flavor enhancement, antibacterial and anticancer effects, antioxidant activity, insecticidal properties, and soothing effects [3], [4]. Extensive research has investigated the utilization of EOs and their active ingredients for food preservation, yielding promising results. For example, using cinnamaldehyde and allspice in packaging can extend the shelf-life of baked foods [5]. Thymol-containing edible coating significantly extends the longevity of post-harvest cherry tomatoes [6]. Biodegradable biofilm containing *Ferulago angulata* EO extends the shelf-life of rainbow trout fillets in refrigerated conditions [7]. Nanofibers containing tea tree EO can increase the shelf-life of beef in refrigerated conditions [8]. Thymol, carvacrol, eugenol, carvacryl ketones, and cinnamaldehyde inhibit microbial deterioration of cheese, prolonging its shelf life [9].

Considering the diversity of EO components, each may interact with the same or different targets, resulting in varying action mechanisms. Research has confirmed the germicidal impact of plant EOs and their components on subcellular microbial structures. However, the interaction between these mechanisms at the subcellular level and their relevance regarding the antimicrobial efficacy of plant EOs and their constituents remain unclear. Therefore, this review examined the impact of essential oils and their active ingredients on microbial subcellular structures, as well as how this impact varies due to microbial differences. These findings lay the groundwork for the application and promotion of plant-derived natural antimicrobial agents. Additionally, we discussed how transcriptomics, metabolomics, and proteomics aid in understanding the antimicrobial mechanisms of essential oils against microbial interactions, which holds significance in developing biomimetic plant-derived antimicrobial agents. Lastly, we explored how multi-omics integration technologies further reveal potential targets of action and the antimicrobial potential of essential oils and their active ingredients to address health risks and environmental challenges posed by food spoilage.

2 Antimicrobial Substances in EOs and Their Active Ingredients

Essential oils are complex mixtures containing a wide range of volatile constituents. It mainly includes alkaloids, flavonoids, terpenes, and aromatic compounds. The chemical composition of essential oil samples extracted from the same plant species can vary significantly depending on plant components, growth time, geographic location, harvesting time, extraction method, and storage conditions. More than 10% of the 3,000 known essential oils have been successfully used in the food and pharmaceutical industries [10]. Figure 1 illustrates the structural formulas of some common active components of essential oils.

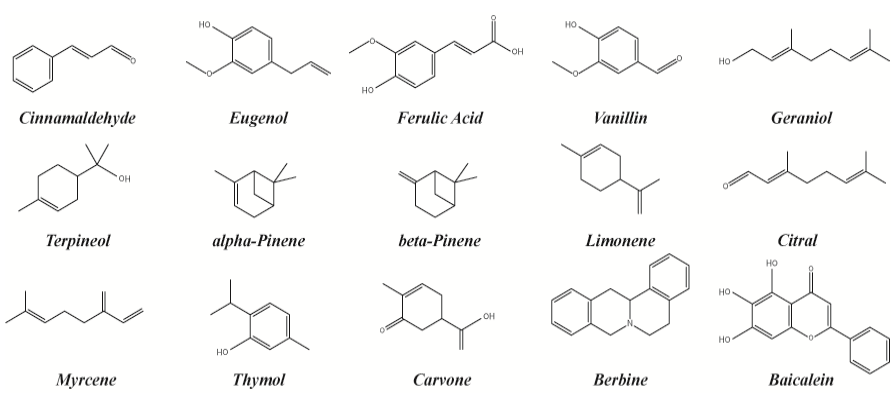

Figure 1: Structural formulas of some common active components of essential oils.

2.1 *Alkaloids*

Alkaloids are complex natural antimicrobial compounds containing nitrogen. Many known alkaloids, such as berberine, hydrochloride, indirubin, and tetrandrine, are often found in traditional herbal medicine [11]. Berberine hydrochloride extracted from Chinese herbal plants can inhibit *Actinobacillus pleuropneumoniae* growth and the synthesis of genetic material and protein at a minimum inhibitory concentration (MIC) of 0.3125 mg/mL [12]. Ponnusamy *et al*. [13] identified the natural active ingredient, indirubin, from South Indian ethnomedicine, showing inhibitory activity against *Trichophyton rubrum, Epidermophyton floccus, and Aspergillus niger,* with MICs between 0.75 μg/mL and 25 μg/mL. Carbazole alkaloid represents the primary antibacterial compound in curry leaves (*Murraya koenigii*), significantly inhibiting *Aspergillus niger* at concentrations between 11 μg/mL and 20 μg/mL [14].

2.2 *Flavanoids*

Flavonoids, secondary plant metabolites mainly present as glycosides, may contain various flavonoid glycosides due to the different types, quantities, positions and connecting sugars. Flavone glycosides consist of monosaccharides, disaccharides, tri-sugars and acylated sugars [15]. Echeverria *et al.* [16] assessed the antibacterial activity of five flavonoids against different Gram-positive and Gram-negative bacteria. The results showed higher efficacy against Gram-positive bacteria. Wu *et al*. [17] found that several common flavonoids, such as baicalein, quercetin, tangerine, daidzein, puerarin, genistein, and onion, displayed strong antibacterial activity against *Escherichia coli* by inhibiting its expression in genetic material. Various solvent extracts derived from periwinkle leaves displayed significant antifungal activity. UV spectrum analysis identified the active component as 5-hydroxyflavone [18]. Bravo *et al*. [19] found that AgNPs synthesized using quercetin enhanced the bacteriostatic activity of *Escherichia coli*.

2.3 *Terpenes*

Terpenes can be divided into monoterpene, sesquiterpene, and diterpene derivatives according to their structures [20], representing a main EO component subgroup. Some monoterpenes are classified generally recognized as safe (GRAS) by the FDA [21], and provide candidate materials for developing novel antibiotic alternatives [22]. Studies have shown that α-terpinol, linalool, eucalyptol and α-pinene can inhibit *S. aureus, E. coli O157:H7*, and *S. aureus* [23]. Furthermore, carvyl alcohol, citronellol, and geraniol display bactericidal activity against Gram-negative bacteria [24], possibly due to peptidoglycan compositional differences between the cell walls of Gram-positive and Gram-negative bacteria. Yu *et al*. [25] found that limonene showed strong inhibitory activity against the food-spoilage yeast *C. tropicalis* at a MIC of 20 μg/mL. D-limonene may damage the integrity of the cell wall and membrane of *C. tropicalis*, increasing protein misfolding and disrupting metabolic coordination. Linalool displayed a MIC of 1.5 mL/L against *Pseudomonas aeruginosa*, destroying its cell structure, which caused content leakage and affected its metabolic activities [26].

2.4 *Aromatic compounds*

Alkaloids are important antimicrobial substances, second only to terpenes in plant-derived EOs. They can be divided into terpene and phenylpropane derivatives, such as common phenolic compounds and aldehydes, according to their structure [27]. A 0.4% eugenol concentration effectively prevented *Vibrio parahaemolyticus* biofilm formation, significantly reducing extracellular polysaccharide (EPS) secretion and metabolic activity [28]. Doyle *et al*. [29] summarized the antimicrobial activity of trans-cinnamaldehyde as an FDA-approved additive against both Gram-positive and Gram-negative bacteria. Other studies combined various aromatic substances (eugenol, cinnamaldehyde, thymol, and carvall) to synergically inhibit *Escherichia coli*. The effect was superior to using a single bioactive compound, with a significant decline in the MIC value. Antibacterial activity is primarily attributed to aromatic nuclei with polar functional groups. These nuclei establish hydrogen bonds with the active site of the target enzyme, leading to its inactivation, which induces cell membrane malfunction or rupture [24], [30].

3 The Microbial Subcellular Interference of Plant EOs and Their Active Components

As natural active compounds, plant essential oils and their components can interfere with various subcellular functions in microorganisms, inducing

their antibacterial ability. The disruption of subcellular processes by EOs and their components may vary depending on their structural composition and specific subcellular targets (Figure 2). Here is an overview of how plant essential oils and their components interfere with subcellular functions in microorganisms (Table 1).

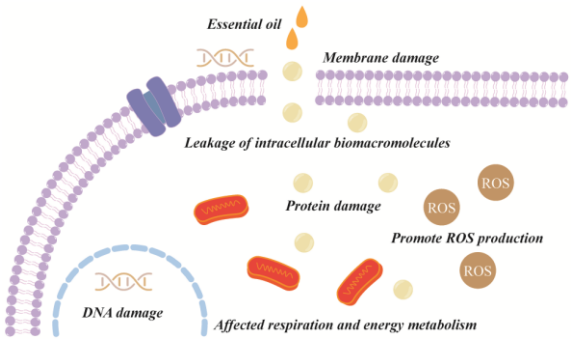

Figure 2: Schematic diagram of the possible action mechanism of EOs.

3.1 *Cell wall formation inhibition*

Function changes in the cell wall barrier are associated with the sensitivity of microorganisms to antimicrobial substances [31]. Due to the lipophilic nature of plant EOs and their active components, they can disrupt the lipid bilayer structure of cell walls [32], interfere with the main cell wall structure components [33], inhibit the enzymes associated with cell wall synthesis [34], and exert microbial cell toxicity. Bacterial cell wall susceptibility to damage varies due to peptidoglycan and lipopolysaccharide content differences in Gram-positive and Gram-negative bacteria, respectively [35]. For example, Mehmet *et al*. [36] utilized the agar diffusion method to investigate the antibacterial activity of resin essential oils against 25 microorganisms, yielding inhibition zones of 13.70 ± 0.10 mm for the Gram-negative bacterium *Salmonella typhimurium* and 9.30 ± 0.10 mm for the Gram-positive bacterium *Staphylococcus aureus*. Certain terpenes, such as transglycosylase and transpeptidase, can inhibit the enzymes involved in peptidoglycan biosynthesis [37], disrupting the normal peptidoglycan assembly and cross-linking, weakening the cell wall and impairing bacterial growth. Ergosterol and β-1,3-glucan are unique fungal cell wall components and are considered as targets for plant EOs and their active components [38]. Plant EOs and their active components also target cell wall-related enzymes [39]. Studies have shown that eugenol can inhibit all candida species at 0.08–0.64 μL/mL concentration, and reduce ergosterol synthesis, resulting in cell wall damage [40]. Additionally, the natural plant product poacic acid exhibited antifungal activity against brewer's yeast at a concentration of 110 μg/mL. In vivo and *in vitro* experiments indicated that phosphoric acid can inhibit the synthesis of β-1,3-glucan [41]. García *et al*. [42] investigated Poacic acid binding to β-1,3-glucan synthase active sites via modeling and molecular docking.

3.2 *Cell membrane barrier disruption*

Plant EOs and their constituent components can penetrate exterior and cytoplasmic membranes of microbial cells and are believed to have an irreversible impact on bacterial and fungal cell membrane structures [43]. The cell membrane isolates the external environment, prevents intracellular substance leakage, and facilitates substance transport. Plant EOs and their constituents may affect the fatty acids in the cell membranes, altering the saturation, acyl chain length, branch position, cis-trans isomerization, and cyclic fatty acid proportion. After the MICs of synergistic combination treatment of 0.06 mg/mL eugenol and 0.17 mg/mL citral; the cell membrane of *Penicillium roqueforti* irreversible damage, altering the structural proportion of fatty acids [44]. Additionally, many active plant EO components, such as terpenes, can reduce the pH gradient on the cytoplasmic membrane, leading to proton collapse and ATP depletion, ultimately causing cell death [45].

3.3 *Intracellular genetic material damage*

DNA and RNA represent genetic material in all organisms, playing a pivotal role in fundamental biological processes such as growth, development, reproduction, and mutation [46]. It controls protein synthesis and metabolism and ensures the continuity of genetic inheritance between generations. Therefore, plant EOs and their active components interact with microbial nucleic acids, disrupting essential cellular processes including replication, transcription, and translation. Research has shown that *Artemisia* essential oil attacks microbial DNA, causing strand breakage or fragmentation [47]. Carvacrol, the primary component in oregano essential oil, can intercalate with DNA to inhibit gene expression, after

treatment at 0.2 mg/mL oregano essential oil concentration for 8 h, the amount of Meticillin-resistant *Staphylococcus aureus* was reduced by 98.98% [48].

3.4 *Mitochondrial dysfunction induction*

Mitochondria represent the primary sites for aerobic respiration and ATP energy production in eukaryotes, playing a crucial role in maintaining normal energy metabolism and function in microorganisms [49]. Some EOs and their constituent components affect mitochondrial function by inhibiting the mitochondrial dehydrogenases involved in ATP biosynthesis, such as lactate dehydrogenase, citrate synthase, and succinate dehydrogenase. For example, at a minimum inhibitory concentration of 20 mL/L, limonene can inhibit the TCA cycle pathway and its key enzymes in *Staphylococcus aureus*, disrupting microbial energy metabolism [50]. Additionally, EOs and their constituents induce oxidative stress in microbial cells, generating reactive oxygen species (ROS) that damage mitochondrial function by impairing respiratory chain complexes, leading to mitochondrial dysfunction [51].

Table 1: The action mechanisms of natural active substances in different microorganisms.

Categories	Plant-derived Products	Species	Main Target	MIC	Ref.
Bacteria	Thymol	*Staphylococcus aureus*	Loss of cell membrane integrity	0.12 mg/mL	[24]
	Cinnamic aldehyde	*Escherichia coli*	Cell membrane permeability changes	125 μg/mL	[51]
		Aeromonas hydrophila	Cell membrane damage and nucleic acid leakage	128 μg/mL	[52]
	Linalool	*Pseudomonas aeruginosa*	Tricarboxylic (TCA) cycle induced oxidative stress inhibition	1.5 mL/L	[26]
	Ferulic acid	*Listeria monocytogenes*	Irreversible cell membrane properties changes	1250 μg/mL	[53]
	Eugenol	*Shigella sonnei*	Cell membrane permeability changes and cell membrane dysfunction	0.5 mg/mL	[54]
	Citronellal	*Penicillium digitatum*	Cell membrane damage	1.6 μL/mL	[55]
Fungi	Carvacrol	*Cryptococcus neoformans*	Exogenous ergosterol and cholesterol bonding	25 g/mL	[56]
		Botrytis cinerea	Structure cell membrane damage	120 μL/L	[57]
		Candida albicans	Change sterol content	256 μg/mL	[58]
	Cinnamic aldehyde Citronellal	*Green mold*	Cell wall and cell membrane damage	0.4 mL/L	[59]
	Citral Limonene Eugenol	*Zygosaccharomyces rouxii*	Cell membrane damage and cellular protein destruction	0.188 μL/mL 0.75 μL/mL 0.4 μL/mL	[60]
	D-Limonene	*Candida tropicalis*	Cell membrane potential, permeability and integrity disruption	20 μL/mL	[25]
	Citronellol Geraniol	*Trichophyton rubrum*	Ergosterol synthesis inhibition	8 μg/mL 16 μg/mL	[61]

4 Antimicrobial Mechanism Determination using Single-omics Technologies

Studies have verified the germicidal impact of plant EOs and their components on subcellular microbial structures. However, the interaction between these mechanisms at the subcellular level and their relevance regarding the antimicrobial efficacy of plant EOs and their constituents remain unclear. Advancements in transcriptomics, metabolomics, and proteomics technology (Figure 3) have enabled the analysis of organisms at the genetic, metabolic, and protein levels. Transcriptomics is widely applied to assess the germicidal mechanisms of various antimicrobial agents, including natural plant compounds and antimicrobial drugs [62], [63]. This section provides a detailed overview of omics technology application in antimicrobial mechanism studies (Table 2).

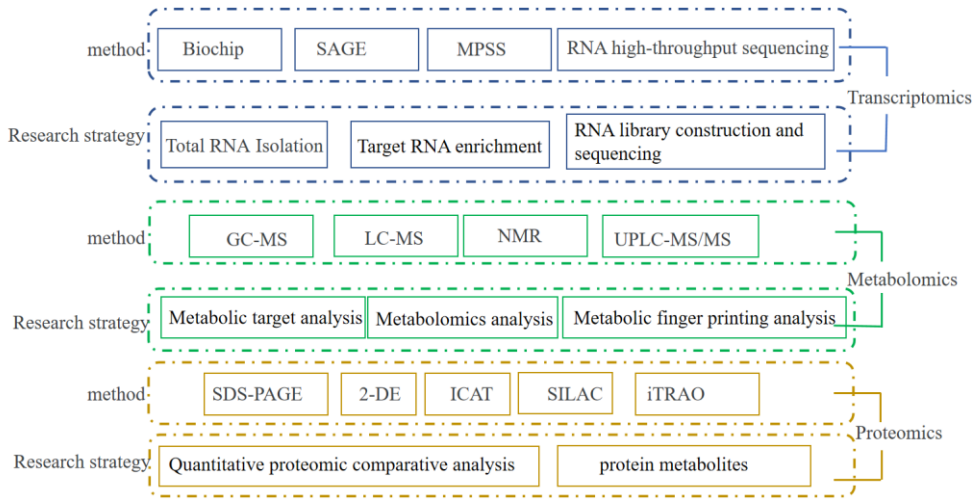

Figure 3: Omics research technology method and strategy.

4.1 *Transcriptomics*

Transcriptomics emerged in the 1990s with the development of the first oligonucleotide gene chip by Affymetrix. However, the chip could not simultaneously analyze a large number of genome expressions in cells. Consequently, serial analysis of gene expression (SAGE) and massively parallel signature sequencing (MPSS) technologies have emerged, becoming indispensable tools in biological research. The popularity of high-throughput transcriptome sequencing (mRNA) during transcriptomic analysis has increased due to advancements in next-generation sequencing technologies. This method enables the precise, quantitative analysis of RNA samples, facilitating research on biological development, variation, and environmental adaptation [64]. Li *et al*. [65] used transcriptomic sequencing analysis to show that the expression of cell cycle protein genes *CLB1*, *CLB2*, and *CLN2* is downregulated. They found that un-ionized calcium propionate aggregates on the yeast cell membrane or enters the cell, this restricted the transition from the G1 to the S phase and significantly inhibited DNA replication, which impeded microbial growth. Another study on the inhibitory mechanism of D-limonene on *Candida tropicalis* SH1 revealed that at the transcriptomic level, D-limonene disrupted C. *tropicalis* SH1 cell wall integrity by downregulating the genes encoding β-(1,3)-glucan (*fks-2* and *fks-3*), mitogen-activated protein kinase (MAPK) signaling cascade genes (*mck1* and *mkk1*). In the cell membrane, it downregulated the transcription levels of ergosterol biosynthesis pathway genes *(erg11*, *erg2*, *erg5*), glycerophospholipid synthesis-related genes, and those involved in sphingolipid synthesis. This caused metabolic disruption and physicochemical changes in the membrane structure, resulting in structural damage and morphological transformation [66]. Similarly, the MAPK pathway controls stress responses, cell proliferation, and cell differentiation in eukaryotes [67]. Transcriptomics has also revealed that the mechanisms employed by prokaryotic microorganisms to tolerate plant EOs and their components differ from those of eukaryotes. Sun *et al*. [68] conducted a Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to examine the effect of quercetin nanoparticles on *Escherichia coli*, revealing notable differentially expressed gene (DEGs) enrichment in three pathways compared to the control group. The results indicated that quercetin nanoparticles affected the bacterial metabolic pathways, which inhibited *Escherichia coli* growth. Furthermore, flavonoid compounds disrupted 721 DEGs during *Bacillus cereus* transcription, most of which were involved in lipopolysaccharide biosynthesis, glycerophospholipid metabolism, amino acid biosynthesis, purine metabolism, ABC transport, and stimuli response. The results indicated that flavonoid compounds intervened in *Bacillus cereus* cell wall and membrane destruction at the transcriptional level, disrupting metabolic processes, oxidative balance, and nucleic acid content,

ultimately leading to cell death [62]. A study involving the antibacterial activity of tea tree oil against *Staphylococcus aureus* showed that the tea tree oil-treated group contained 304 DEGs compared to the control group. In the presence of tea tree oil, significant changes occurred at the gene level, which was related to glycine, serine, threonine, purine, and pyrimidine metabolic pathways and amino acid biosynthesis [69]. Therefore, transcriptomic methods can accurately demonstrate the inhibitory mechanism of antimicrobial agents on microorganisms at the molecular level, providing a solid theoretical foundation for further application.

4.2 *Metabolomics*

Metabolomics was first reported in a review in 1994 using two-dimensional thin-layer chromatography to analyze the overall metabolic spectrum changes of slow-growing *Escherichia coli* [70]. It is a relatively new omics technology after genomics and transcriptomics. Common metabolomics tools include gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), LC-MS, and UPLC-MS/MS. Metabolomics reflects cellular vitality at the functional level, primarily by examining changes in small-molecule metabolites in response to external perturbations [71], [72]. By utilizing various data analysis methods, metabolomics can elucidate potential metabolic mechanisms in organisms. Metabolomics involves collecting all low-molecular-weight metabolites (<1000) in an organism or cell during a specific physiological period, including signaling molecules, metabolic intermediates, and secondary metabolites. This is fundamental in the interaction between cellular changes and phenotypes, directly reflecting the physiological state of cells [73]. Therefore, metabolomics is widely applied in various biological and biomedicine fields to examine the pathogenic mechanisms and antibiotic resistance in microorganisms, as well as to discover new bacterial species, functional genes, and natural products. Elmroth *et al*. [74] used GC-MS in metabolomics to analyze the fatty acids, amino acids, and sugars in *Candida albicans* during its growth process to evaluate the level of contamination. Hao *et al*. [75] employed ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF MS) system to analyze metabolites. Data were subjected to orthogonal partial least squares discriminant analysis and screened using Metaboanalyst 4.0 to identify differential metabolites. The result revealed significant metabolic changes in specific metabolites, including organic acids, lipids, nucleosides, and organic oxidation compounds in the liver, through metabolomics analysis in response to the polysaccharides produced by *Lactobacillus paracasei*. Villas-Bôas and Khoomrung [76], [77] conducted several studies on microbial metabolomics using GC-MS. They obtained amino acid profiles from various filamentous fungi using chloroformate derivatization and analyzed the fatty acids in yeast samples using microwave derivatization. Two-dimensional GC-MS technology significantly improves the separation and sensitivity of complex samples, making it effective for microbial metabolomics. In the context of antimicrobial resistance mechanisms. Kurwadkar *et al*. [78] revealed the *Staphylococcus aureus* resistance to methicillin using targeted metabolomics with liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology. They differentiated sensitive strains from resistant strains of *Staphylococcus aureus* strains using targeted metabolic profiles, metabolic intermediates, and metabolic pathways. Furthermore, 1H NMR was used to analyze the metabolic impact of oregano EO on *Staphylococcus aureus* and *Escherichia coli* in different media, confirming that the amino acid and TCA cycle metabolic pathways were most affected [79]. Although metabolomics data can reveal changes in small molecules or secondary metabolites acting as signaling molecules or inhibitory effectors, minimal studies are available regarding the role of metabolomics in antifungal mechanisms.

4.3 *Proteomics*

The concept of proteomics, which was proposed at the 1994 Italian Science Meeting, is defined as “the set of proteins expressed by a genome” [80]. Protein expression depends on host regulation. Several protein analysis techniques have been developed for protein assessment, including conventional methods such as ion-exchange chromatography (IEC), enzyme-linked immunosorbent assay (ELISA), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and two-dimensional gel electrophoresis (2-DE). Additionally, a series of novel protein quantification methods were created, such as isotope-coded affinity tags (ICAT), stable isotope labeling by amino acids in cell culture

(SILAC), and isobaric tags for relative and absolute quantification (iTRAQ), which have enabled protein function examination [81]. Proteomics provides a comprehensive understanding of cell tissues and reflects the activities and essence of phenomena at the cellular and biological levels [82]. Rapid development has been evident in genome sequencing technology, mass spectrometry technology, computational capabilities, bioinformatics, and proteomics. Proteomics research has emerged as a prominent subject and area of interest in drug development, fermentation engineering, and food science [83]. Various studies have used reversed-phase LC-MS/MS (RP-LC-MS/MS) to analyze induced bacterial classes in pea and lentil root nodules. Comparative analysis revealed significant host-specific differences that affected bacterial proteins, including stress-related proteins, transcriptional regulatory proteins, and those involved in carbon and nitrogen metabolism [84]. Proteomics has shown that the D-limonene tolerance of brewer's yeast primarily relies on cellular stress responses, sugar metabolism, respiration, amino acids, and lipids, among other small molecule metabolisms [85]. Studies used proteomics to reveal the adaptation mechanisms of *Vibrio vulnificus* biofilms to non-biological surfaces, where functional proteins were involved in metal ion stress, nutrition, osmotic stress, and sugar utilization, among other biological processes [86]. A quantitative proteomic approach was used to investigate the differentially expressed proteins in response to berberine treatment of *Streptococcus pyogenes*. KEGG pathway analysis demonstrates that berberine exerts its antibacterial effect by disrupting carbohydrate metabolism [87]. The application of proteomics has clarified changes in strain proteomes, providing a theoretical foundation for further in-depth research and practical applications.

Table 2: The application of single-omics technologies in the antibacterial mechanisms of EOs.

Omics Technologies	Research Content	Results	Ref.
Transcriptomics	D-limonene inhibits *Candida tropicalis* by disrupting metabolism	Disrupted biosynthesis of cell wall and membrane, the transcription of genes encoding key enzymes in glycolysis pathway, tricarboxylic acid cycle and oxidative phosphorylation pathway, and the proper folding of nascent protein in endoplasmic reticulum.	[66]
	Perilla frutescens essential oil against *Aspergillus flavus* by transcriptomic analysis	*Perilla frutescens* essential oil treatment could inhibit spore development, induce oxidative stress, and increase membrane permeability.	[88]
	Transcriptomic analysis of *Staphylococcus aureus* under the stress condition caused by *Litsea cubeba* L. essential oil	After *Litsea cubeba* L. essential oil treatment breaks the cell membrane, cell wall, and the biosynthesis of carotenoid organisms interferes nitrogen metabolism, and pyruvate metabolism.	[89]
Metabolomics	Metabolomics reveals the antimicrobial action of oregano essential oil against *Escherichia coli* and *Staphylococcus aureus* in broth, milk, and beef	The amino acid metabolism was identified as the route most affected by oregano essential oil treatment	[79]
	Antibacterial mechanism of rose essential oil against *Pseudomonas putida*	Rose oil disrupts the TCA cycle, amino acid metabolism, nucleotide metabolism, carbohydrate metabolism, lipid metabolism, and affects the biosynthesis of pantothenic acid and coenzyme A, as well as the biosynthesis of aminoacyl tRNAs.	[90]
	Antibacterial mechanism of canthin-6-one against *Staphylococcus aureus*	Fifty-three biochemical pathways were altered in S. aureus after treated by canthin-6-one mainly involved in lipid metabolism, amino acid metabolism, carbohydrate metabolism, metabolism of cofactors and vitamins, and nucleotide metabolism.	[91]
Proteomics	Mechanism is involved in the Antifungal Activity of Citral against *Penicillium digitatum*	Citral affects oxidative phosphorylation complex, TCA cycle, and RNA transport-related protein synthesis in *Penicillium digitatum*.	[92]
	Antibacterial effect of *Blumea balsamifera* (L.) DC. essential oil against *Staphylococcus aureus*	*Blumea balsamifera* (L.) DC. essential oil affects disorder of amino acid metabolism and affects the activity of various enzymes and the transport of substances.	[93]
	Proteomic investigation into the action mechanism of berberine against *Streptococcus pyogenes*	berberine effect proteins were mainly involved in carbohydrate metabolism, fatty acid biosynthesis, pyrimidine metabolism, RNA degradation, ribosome, purine metabolism, DNA replication and repair and oxidative phosphorylation pathways.	[87]

5 The Trends in the Application of Multi-omics Approaches to Examine Antibacterial Mechanisms

The importance of multi-omics technologies in the study of essential oil antibacterial mechanisms is becoming increasingly prominent. With the growing application and demand for natural plant extracts, understanding the deeper and more comprehensive levels of essential oil antibacterial mechanisms has become particularly crucial. The introduction of multi-omics technologies provides us with a fresh perspective, aiding in the unraveling of the complexity of interactions between essential oils and microorganisms, thereby enhancing our understanding of their antibacterial effects (Figure 4).

5.1 *The limitations of traditional methods*

Although the utilization of omics technology has witnessed significant growth in various fields, such as food science, medicine, agriculture, and microbiology, in recent years, these approaches have also shown certain limitations. For example, single transcriptomics may not fully capture biological features [94], It has certain limitations in capturing expression differences at the single-cell level, low-abundance transcription signals, real-time information on dynamic changes, and cell heterogeneity. Metabolomics faces challenges related to sample collection, extraction, platform variability, and variations in results. It is limited to identifying differences between samples and often relies on literature references for the functional interpretation of key differential metabolites, consequently elucidating the mechanisms underlying metabolite changes [95]. Proteomics faces challenges in comprehensively covering the proteome, especially when high-abundance proteins may overshadow the detection of low-abundance proteins.

5.2 *Application of multi-omics technologies*

While the limitation of a single analytical method hinders direct comparisons between data, researchers have overcome some of these challenges via omics method integration and targeted experimental validation, addressing issues such as the ambiguity of antibacterial targets, variations in antibacterial effects, and microbial resistance, and providing a more comprehensive understanding of antibacterial mechanisms. For instance, transcriptomics and proteomics have been integrated to provide a top-down research strategy, transitioning from the gene level to examining protein function [96]. Gene expression changes in transcriptomics data can be validated by confirming the corresponding protein abundance variation via proteomics. Combined with metabolomics analysis, connections can be established between upstream genotypes and downstream phenotypes, providing a bottom-up approach. Linking differentially expressed proteins and DEGs to specific metabolic pathways or biological processes can provide deeper insight into the functional consequences of inhibition and identify the critical pathways involved. A recent study combined transcriptomics and metabolomics analysis to compare the inhibitory mechanisms of gallic acid and quinic acid against *Staphylococcus aureus*. The results showed that both organic acids disturbed the oxidative phosphorylation pathways, altered membrane fluidity, and affected ribosomal function and aminoacyl-tRNA synthesis, consequently disrupting protein synthesis. Additionally, it was suggested that these two organic acids exert their effect via specific metabolic pathways [97]. The intrinsic mechanisms behind metabolite changes at multiple levels, including proteins, enzymes, and genes, were examined in-depth by combining in vitro and in vivo experiments and molecular biology techniques. Gao *et al*. [98] evaluated the impact of epigallocatechin gallate on *Streptococcus suis* using metabolomics and proteomics. The analysis revealed differential expression of the proteins involved in DNA replication, cell wall and membrane synthesis, and virulence, which affected the ABC transporters, glycolysis/gluconeogenesis, and aminoacyl-tRNA biosynthesis pathways. Tang *et al*. [99] reported that *Pseudomonas fragi* exhibited differential expression of 519 proteins, 136 positive ion mode metabolites, and 100 negative ion mode metabolites after 3-Carene treatment. These differences were mainly observed in amino acid metabolism, fatty acid degradation, and physiological TCA cycle activity. Additionally, the response of *Salmonella typhimurium* to thyme and cinnamon EOs was evaluated using metabolomics and transcriptomics. The results showed that transcriptomics identified 161-324 DEGs, while metabolomics analysis revealed changes in 47 important metabolites and pathways. Studies have also shown that the aminoacyl-tRNA biosynthesis pathway is involved in the stress response to EOs [100]. The application of these omics technologies demonstrates the significant role of EOs and their active components in antibacterial processes.

However, most of the mentioned omics applications focus on foodborne pathogenic bacteria, with limited reports on their utilization for fungi. Analyzing the suitability of multi-omics technologies for examining the antifungal mechanisms of EOs and their active components offers a comprehensive perspective on the molecular interactions and regulatory networks underlying antifungal mechanisms. Consequently, this area of research can provide a comprehensive view of the molecular interactions and regulatory networks behind antifungal mechanisms.

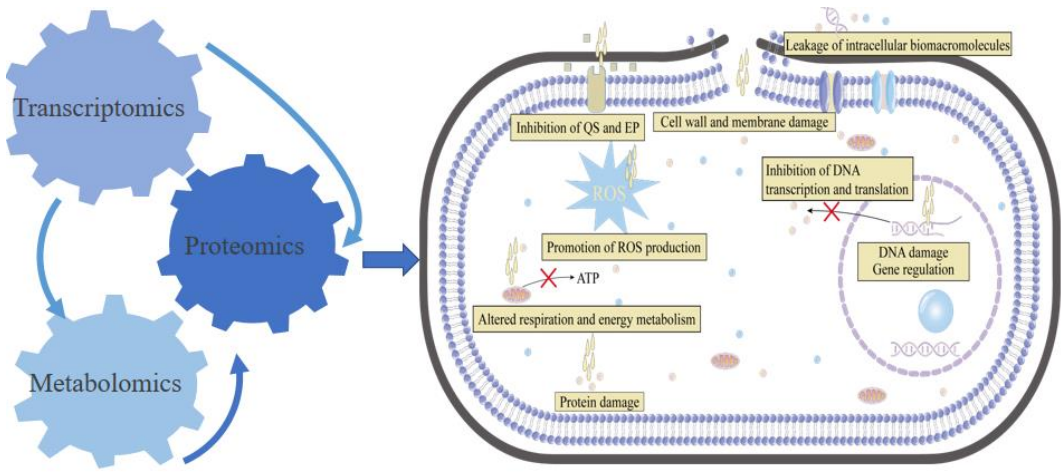

Figure 4: Multi-omics techniques in the antimicrobial mechanism.

6 Conclusions

In the face of serious issues such as food safety and carbon emissions caused by food spoilage, finding effective antibacterial strategies is paramount. Essential oils, as natural plant extracts, possess potential antibacterial and antifungal activities. However, their antibacterial mechanisms are complex, with unclear targets and variability efficacy inhibition, different plant EOs and their components on microbial subcellular structures, as well as the research progress of transcriptomics, proteomics, and metabolomics in the antibacterial mechanisms of EOs. Although single omics technologies have made certain progress in analyzing the antibacterial mechanisms of EOs, they still fail to comprehensively elucidate the antibacterial mechanisms of EOs and their components. The integrated application of multi-omics technologies addresses the limitations of traditional methods. By combining transcriptomics, metabolomics, and proteomics, we can comprehensively study the effects of EOs on microorganisms, from gene expression levels to changes in metabolites and protein expression levels, thereby forming a more comprehensive explanation of antibacterial mechanisms. In the future, with the development of multi-omics technologies, the integration and complementarity among different techniques will be realized, providing comprehensive insights into the antibacterial effects of EOs and their components. This not only contributes to a better understanding of the complex interactions between EOs and microorganisms but also offers a more reliable theoretical basis for the development and application of EO antibacterial agents.

Author Contributions

C.Z.: conceptualization, investigation, methodology, writing an original draft; research design, data analysis; P.Y., A.T, S.K., T.L., W.K., T.P., and P.V.: conceptualization, data curation, writing—reviewing and editing; J.T.: funding acquisition, project administration. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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