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Applications of FTIR, GC-MS, and LC-MS in the Identification of Antimicrobial Phytochemicals

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Abstract

Medicinal plants are rich sources of structurally diverse phytochemicals with potent antimicrobial properties, offering alternatives in the fight against antimicrobial resistance (AMR). Accurate identification and characterization of these bioactive compounds are essential for validating traditional uses, standardizing herbal formulations, and advancing drug discovery. This review highlights the applications of Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography–Mass Spectrometry (GC–MS), and Liquid Chromatography–Mass Spectrometry (LC–MS) in profiling antimicrobial phytochemicals. FTIR provides rapid functional group analysis, GC–MS is suited for volatile and semi-volatile metabolites such as terpenoids and essential oils, while LC–MS effectively profiles polar, non-volatile, and thermolabile compounds, including flavonoids, alkaloids, and phenolic acids. This review explains that integrating these techniques with bioassays and chemometric tools enables correlation of chemical profiles with antimicrobial activity, identification of lead compounds, detection of synergistic interactions, and quality control of herbal products. The review also emphasizes the importance of standardized protocols, metabolomics integration, and in silico approaches to accelerate phytochemical-based antimicrobial discovery.

Keywords: Antimicrobial Phytochemicals, FTIR Spectroscopy, GC–MS Analysis, LC–MS Profiling

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1. Introduction

Medicinal plants have long served as a primary source of therapeutic agents, particularly in traditional healthcare systems (Angelini, 2024). They produce a wide variety of secondary metabolites that protect them against microbial infections, many of which exhibit potent antimicrobial properties in humans (Khare

et al., 2021; Kabir *et al.*, 2025). In recent decades, the rapid emergence of antimicrobial resistance (AMR) has renewed scientific interest in plant-derived compounds as alternative or complementary antimicrobial agents (Kabir *et al.*, 2023; Kadda *et al.*, 2026a&b). Unlike many synthetic antibiotics, medicinal plants offer chemically diverse and structurally complex molecules that can act on multiple microbial targets. Their wide availability, structural novelty, and historical safety profiles make medicinal plants valuable reservoirs for the discovery of new antimicrobial drugs (Anand *et al.*, 2019; Danjuma, 2024)

Phytochemicals such as alkaloids, flavonoids, phenolic acids, terpenoids, tannins, and saponins play a crucial role in combating antimicrobial resistance (Diass *et al.*, 2021; Aziz *et al.*, 2022; Ouahabi *et al.*, 2023; Kabir and Lawan, 2025). These compounds exert antimicrobial effects through diverse mechanisms, including disruption of microbial cell membranes, inhibition of nucleic acid and protein synthesis, enzyme inhibition, efflux pump suppression, and interference with quorum sensing and biofilm formation (Khare *et al.*, 2021; Danjuma, 2025). Importantly, many phytochemicals act as resistance-modifying agents by restoring the activity of conventional antibiotics against multidrug-resistant (MDR) pathogens. Their multi-target modes of action reduce the likelihood of resistance development, making phytochemicals promising tools in the global fight against antimicrobial resistance (Nsofor *et al.*, 2023; Kabir and Lawan, 2025)

The complex chemical composition of medicinal plants necessitates accurate identification and characterization of bioactive compounds to ensure reproducibility, efficacy, and safety (Ugboko *et al.*, 2020). Crude plant extracts often contain hundreds of metabolites, and antimicrobial activity is frequently attributed to specific compounds or synergistic interactions among them (Ella *et al.*, 2024). Without proper characterization, inconsistencies in plant species, extraction methods, or phytochemical profiles may lead to variable biological outcomes (Mouloudi *et al.*, 2023). Accurate identification is therefore essential for understanding structure–activity relationships, standardizing herbal formulations, validating traditional claims, and advancing plant-derived compounds toward drug development and clinical application (Kumar *et al.*, 2024; Danjuma, 2026).

Spectroscopic and chromatographic techniques play a central role in the identification and characterization of antimicrobial phytochemicals. Fourier Transform Infrared Spectroscopy (FTIR) is widely used for rapid functional group analysis and preliminary compound characterization (Teoh *et al.*, 2023). Gas Chromatography–Mass Spectrometry (GC-MS) enables the separation and identification of volatile and semi-volatile phytochemicals, particularly essential oil components with antimicrobial activity. Liquid Chromatography–Mass Spectrometry (LC-MS) is highly effective for profiling non-volatile, polar, and thermolabile compounds such as flavonoids, alkaloids, and phenolic acids. The complementary use of these techniques provides comprehensive phytochemical profiling, linking chemical composition to antimicrobial activity and supporting natural product-based drug discovery (Sharma *et al.*, 2021; Ouahabi *et al.*, 2024 and 2025).

2. Methodology

This review systematically examined the use of FTIR, GC-MS, and LC-MS in identifying antimicrobial phytochemicals. Relevant literature was retrieved from PubMed, Scopus, Web of Science, and Google Scholar using targeted keywords, focusing on peer-reviewed articles published between 2015 and 2025. Studies included reported the application of these analytical techniques for plant-derived antimicrobial compounds, while conference abstracts, editorials, and unrelated studies were excluded. Key data—such as plant species, analytical methods, identified phytochemicals, and antimicrobial activity—were extracted using a standardized sheet, and study quality was assessed based on methodological accuracy and assay reliability. The collected information was synthesized qualitatively to compare the strengths and limitations of each technique, highlight commonly identified bioactive compounds, and provide insights for future research in plant-based antimicrobial discovery (Danjuma, 2024).

3. Classification of Phytochemicals

Phytochemicals are naturally occurring bioactive compounds synthesized by plants as part of their secondary metabolism (Khameneh *et al.*, 2019). Unlike primary metabolites, which are essential for plant growth and development, phytochemicals primarily function in plant defense against pathogens, herbivores, and environmental stress. These compounds are responsible for many of the therapeutic properties of medicinal plants, including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. Phytochemicals are broadly classified based on their chemical structure and biosynthetic origin into groups such as alkaloids, phenolics, terpenoids, and glycosides, each encompassing diverse subgroups with distinct biological activities (Moo *et al*., 2020; Aourabi *et al*., 2021; Mrani *et al*., 2024)

3.1 Alkaloids

Alkaloids are nitrogen-containing heterocyclic compounds commonly derived from amino acids (**Figure 1**). They exhibit strong antimicrobial activity by interfering with DNA replication, protein synthesis, and enzymatic processes in microbial cells. Many alkaloids disrupt cell membrane integrity or inhibit key metabolic pathways, leading to microbial cell death. Their structural complexity and presence of basic nitrogen atoms enable strong interactions with microbial targets, making them effective against a wide range of bacteria and fungi (Danjuma, 2025)

3.2 Flavonoids

Flavonoids are polyphenolic compounds characterized by a C6–C3–C6 carbon framework (**Figure 2**) (Górniak *et al*., 2019; Merimi *et al*., 2025). They are widely distributed in plants and exhibit antimicrobial activity through mechanisms such as membrane disruption, inhibition of nucleic acid synthesis, energy metabolism interference, and suppression of virulence factors (Mahady, 2016). The hydroxylation pattern

and degree of conjugation in flavonoid structures significantly influence their antimicrobial potency, with increased hydroxyl groups often enhancing activity (Moo *et al.*, 2020).

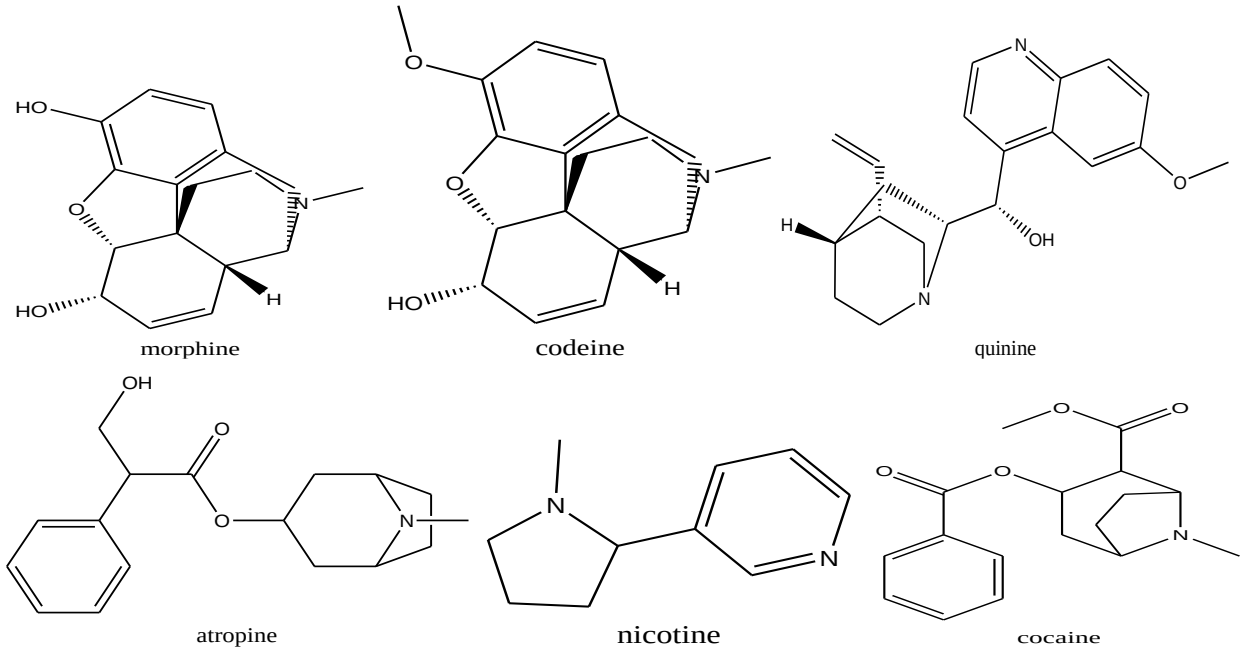

Figure 1: Examples of alkaloids

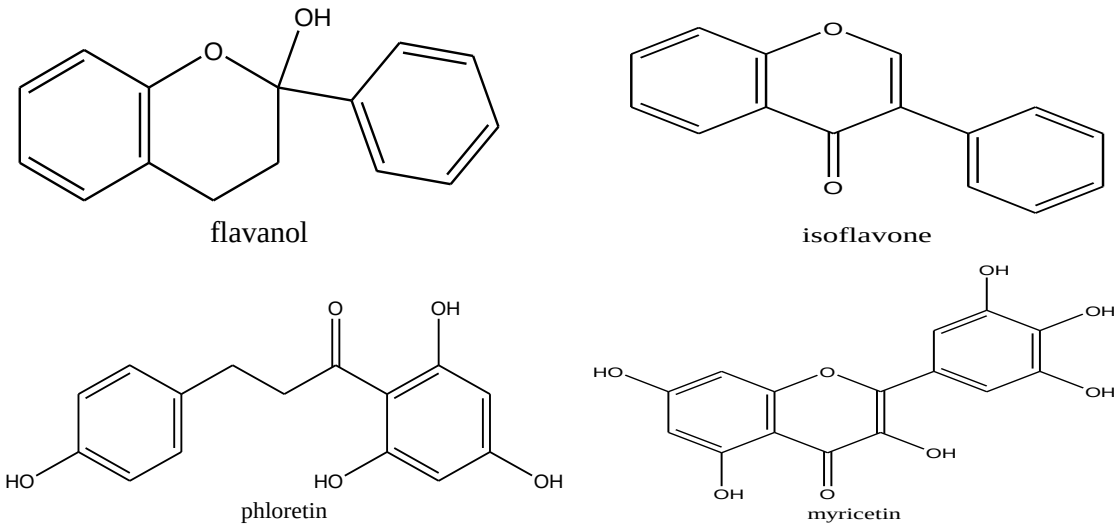

Figure 2: Examples of flavonoids

3.3 Phenolic Compounds

Phenolic compounds include simple phenols, phenolic acids, and complex polyphenols (Daglia, 2017; El Amri *et al.*, 2025). Their antimicrobial effects are primarily attributed to their ability to denature proteins, disrupt microbial cell walls, and interfere with enzyme function (**Figure 3, Table 1**). The presence of hydroxyl groups enables phenolics to form hydrogen bonds with microbial proteins, leading to enzyme inactivation. Their redox properties also contribute to oxidative stress in microbial cells (Cowan, 2019).

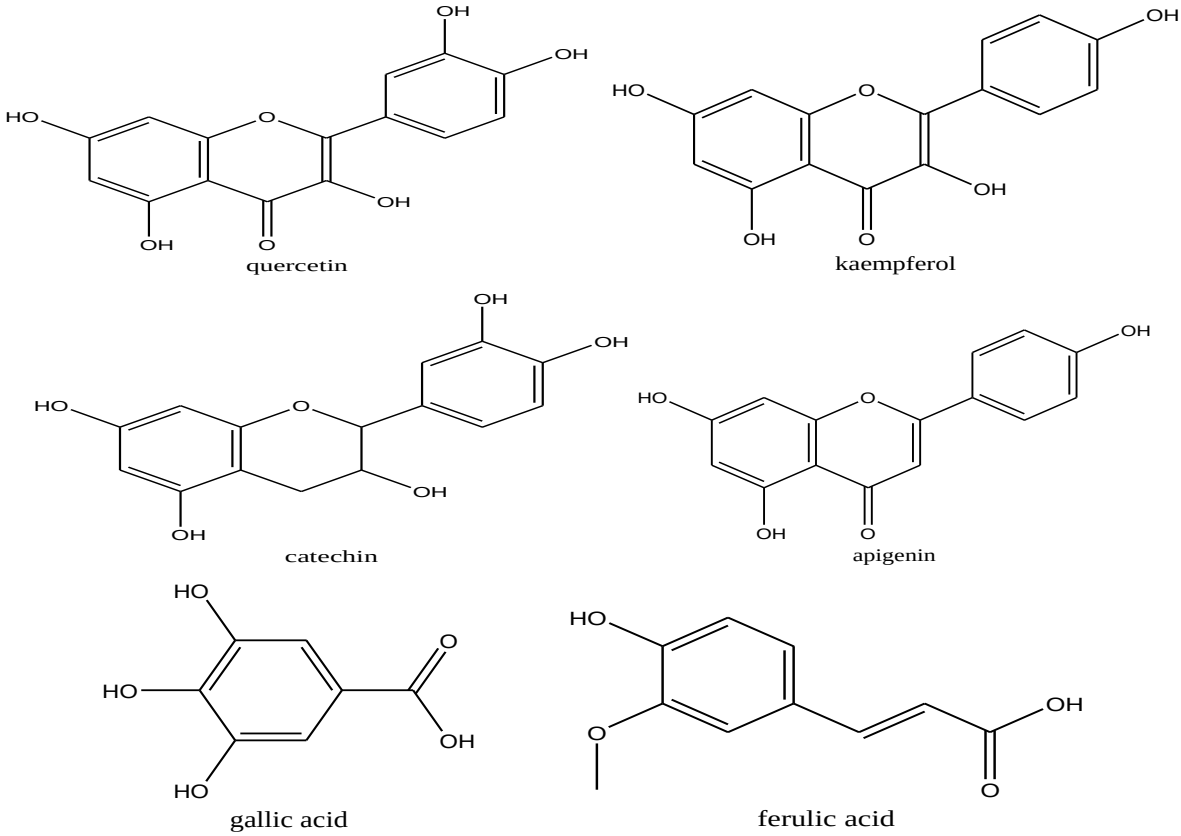

Figure 3: Examples of phenolic compounds

Table 1: Major phytochemical classes, representative compounds and their mechanisms of antimicrobial action

Phytochemical Class	Example	Mechanism of Antimicrobial Action	Microbial Targets
Alkaloids	Berberine, Quinine, Sanguinarine	Interfere with DNA/RNA synthesis, inhibit enzymes, disrupt cell membrane integrity	Bacteria (Gram-positive & Gram-negative), Fungi
Flavonoids	Quercetin, Kaempferol, Catechin	Disrupt microbial membranes, inhibit nucleic acid synthesis, suppress virulence factors	Bacteria, Fungi, Viruses
Phenolics Compounds	Gallic acid, Caffeic acid, Tannic acid	Denature microbial proteins, disrupt cell walls, induce oxidative stress	Bacteria, Fungi
Terpenoids & Essential Oils	Thymol, Carvacrol, Limonene	Integrate into lipid membranes, increase permeability, cause leakage of cellular contents	Bacteria, Fungi
Tannins	Ellagitannin, Proanthocyanidins	Precipitate proteins, inhibit microbial enzymes, complex with cell wall proteins	Bacteria, Fungi
Saponins	Aescin, Diosgenin	Interact with membrane sterols, form pores, cause cell lysis	Bacteria, Fungi

3.4 Terpenoids and Essential Oils

Terpenoids are derived from isoprene units and are major constituents of essential oils (**Figure 4**). These compounds are predominantly lipophilic, allowing them to integrate into microbial cell membranes and disrupt membrane structure and function (Mahady, 2016). This leads to increased permeability, leakage of cellular contents, and eventual cell death. Monoterpenes and sesquiterpenes are particularly effective antimicrobial agents due to their small molecular size and high volatility (Nazzaro *et al.*, 2017).

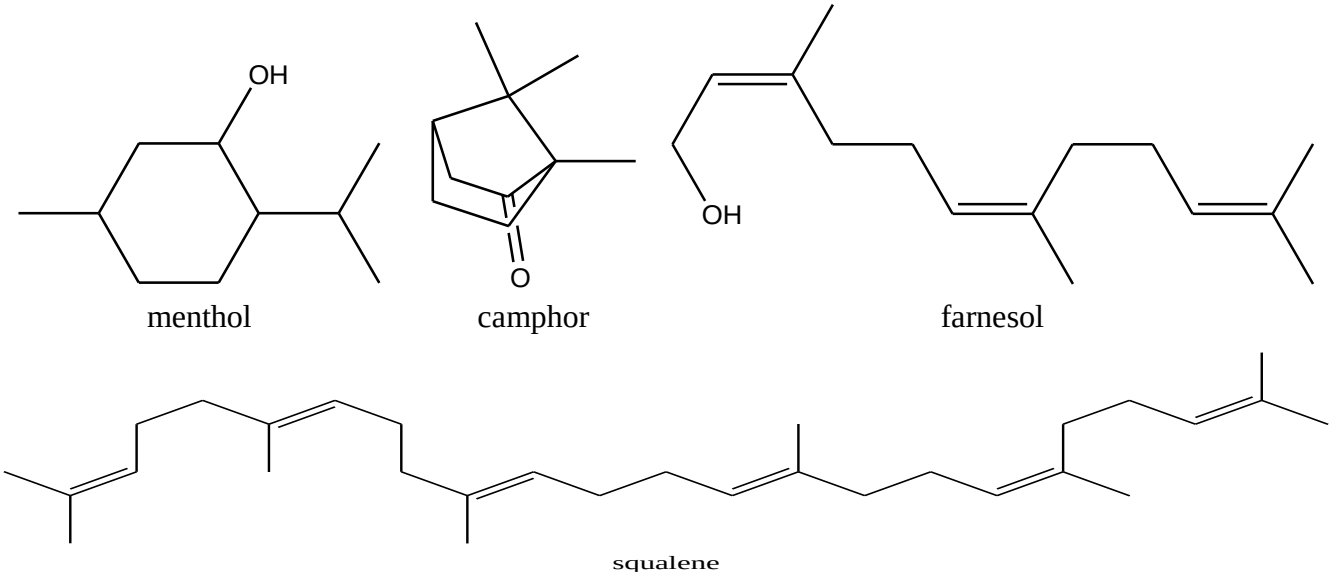

Figure 4: Examples of terpenoids

3.5 Tannins and Saponins

Tannins are high-molecular-weight polyphenolic compounds (**Figure 5**) capable of precipitating proteins and inhibiting microbial enzymes (Othman *et al.*, 2019). They exert antimicrobial effects by forming complexes with cell wall proteins and membrane-bound enzymes. Saponins are glycosides characterized by their soap-like properties, which allow them to interact with membrane sterols, leading to pore formation and cell lysis. Both classes contribute significantly to the antimicrobial activity of many medicinal plants (Silva *et al.*, 2017).

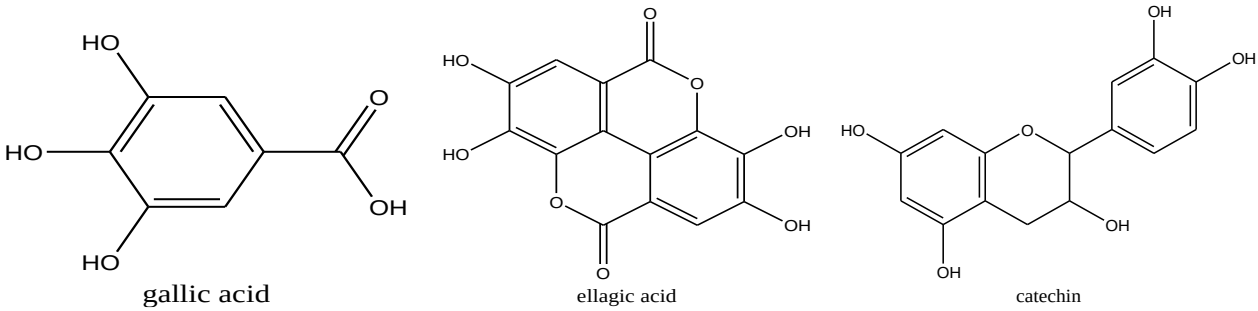

Figure 5: Examples of tannins

4. Relationship Between Chemical Structure and Antimicrobial Activity

The antimicrobial activity of phytochemicals is strongly influenced by their chemical structure. Functional groups such as hydroxyl, carbonyl, and amino groups determine the ability of compounds to interact with microbial targets. Molecular size, polarity, lipophilicity, and degree of unsaturation also affect membrane permeability and target binding (Savoia, 2018). Structural features that enable multi-target interactions reduce the likelihood of resistance development. Understanding structure–activity relationships (SAR) is essential for identifying potent antimicrobial phytochemicals, optimizing their efficacy through chemical modification, and advancing them as lead compounds in antimicrobial drug development (Tiwari *et al.*, 2018; Louafi *et al.*, 2025).

5. Sample Preparation and Extraction of Phytochemicals

5.1 Plant Selection and Authentication

Medicinal plants are selected based on ethnobotanical relevance, reported biological activity, or traditional use (Azmir *et al*., 2015). Correct botanical identification is essential to ensure reproducibility and validity of phytochemical studies. Authentication is typically carried out by a qualified taxonomist, and voucher specimens are deposited in a recognized herbarium with an accession number for future reference (Fitri *et al.*, 2025)

5.2 Drying, Grinding, and Storage

Fresh plant materials are thoroughly cleaned to remove soil and debris, then dried to reduce moisture content and prevent microbial growth (Herrero *et al.*, 2015). Drying may be done under shade, in an oven at controlled temperatures (40–50 °C), or using freeze-drying to preserve thermolabile compounds. The dried materials are ground into fine powder using a mechanical grinder to increase surface area and extraction efficiency. Powders are stored in airtight containers, protected from light, heat, and humidity until extraction (Dai and Mumper, 2015; Pandey and Tripherti, 2018).

5.3 Extraction Methods

Extraction is a crucial step aimed at separating bioactive phytochemicals from plant matrices using suitable solvents and techniques (Kadda *et al*., 2022).

5.3.1 Maceration

Maceration involves soaking powdered plant material in a solvent at room temperature for a defined period with occasional agitation (Bitwell, 2023; El Guerrouj *et al*., 2023). It is a simple and cost-effective method, suitable for heat-sensitive compounds, although it requires longer extraction times and may yield lower efficiency compared to advanced methods (Hlatshwayo, 2025).

5.3.2 Soxhlet Extraction

Soxhlet extraction is a continuous hot extraction technique in which solvent repeatedly passes through the plant material until exhaustive extraction is achieved. It is efficient for non-volatile and thermostable compounds but may lead to degradation of heat-sensitive phytochemicals and requires large solvent volumes (Bitwell *et al.*, 2023).

5.3.3 Ultrasound- and Microwave-Assisted Extraction

Ultrasound-assisted extraction (UAE) enhances solvent penetration through acoustic cavitation, improving extraction yield while reducing time and solvent consumption (Chemat *et al.*, 2017). Microwave-assisted extraction (MAE) uses microwave energy to rapidly heat solvents and plant cells, leading to efficient cell wall rupture and rapid phytochemical release. Both methods are considered green and efficient alternatives to conventional techniques (Suárez, 2023; Gallo *et al.*, 2020; Kaufmann *et al.*, 2017; Husaini et al., 2023).

5.4 Solvent Selection Based on Polarity

Solvent choice plays a critical role in determining the types of phytochemicals extracted (Do *et al.*, 2018). Polar solvents such as water, methanol, and ethanol are effective for extracting phenolics, flavonoids, and glycosides, while moderately polar solvents (ethyl acetate, acetone) extract alkaloids and some terpenoids. Non-polar solvents like hexane are suitable for lipids, steroids, and essential oils. Sequential extraction using solvents of increasing polarity is often employed to maximize phytochemical recovery (Altıok and Gallo, 2021)

5.5 Fractionation and Purification Strategies

Crude extracts are often subjected to fractionation to separate complex mixtures into simpler fractions (Tiwari *et al.*, 2016). Liquid–liquid partitioning using immiscible solvents is commonly employed. Further purification may be achieved through chromatographic techniques such as column chromatography, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or preparative chromatography. These strategies enhance compound isolation, improve bioactivity assessment, and facilitate structural characterization (Ignat *et al.*, 2016; Sasidharan *et al.*, 2016).

6. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a rapid and non-destructive analytical technique widely used for the identification and characterization of chemical compounds based on their infrared absorption patterns (**Figure 6**) (Kamnev and Tugarova, 2023). The technique relies on the interaction between infrared radiation and molecular bonds, where absorption at specific wavenumbers corresponds to vibrational modes of functional groups such as O–H, N–H, C=O, and C–H (Trisnawativ *et al.*, 2022). Using a Michelson

interferometer and Fourier transformation, FTIR generates a characteristic spectrum that serves as a molecular fingerprint, enabling qualitative and semi-quantitative analysis of complex biological and chemical samples (Pakbin *et al.*, 2022).

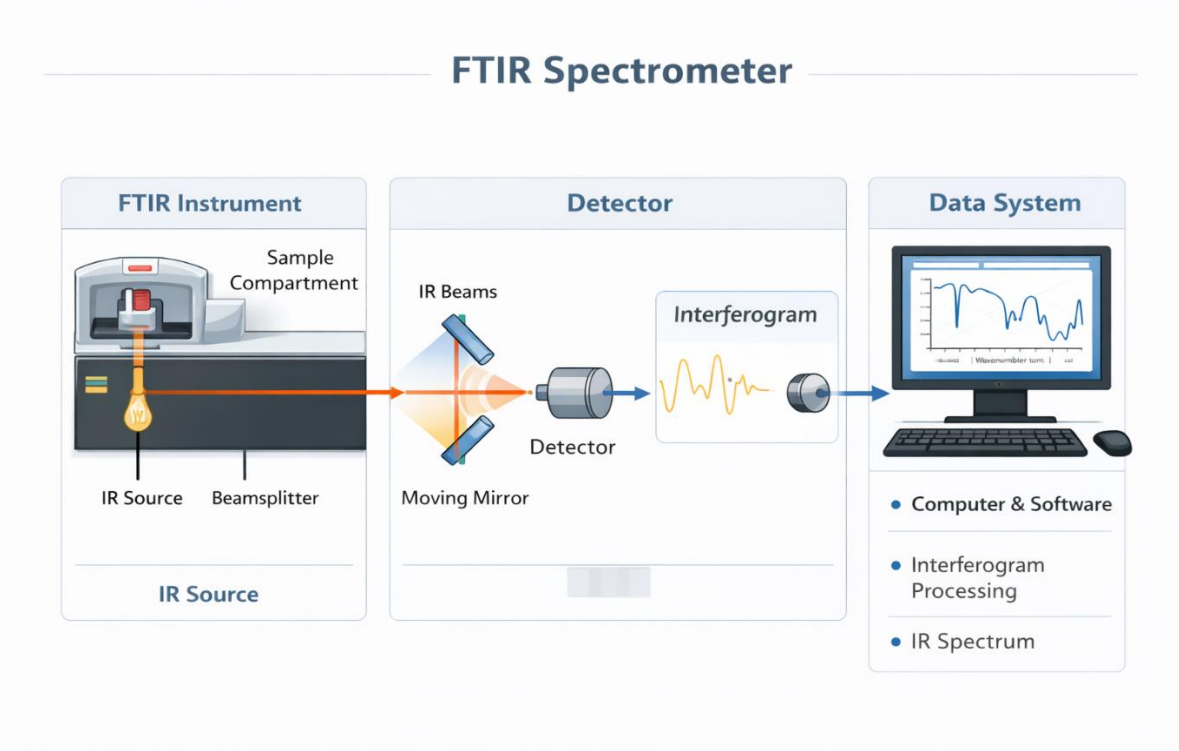

Figure 6: Principles of Fourier Transform Infrared Spectroscopy (FTIR)

In antimicrobial-resistance (AMR) research, FTIR has gained importance due to its ability to detect biochemical and structural alterations associated with resistant microorganisms and antimicrobial compounds (Yang *et al.*, 2019; risnawati *et al.*, 2022). Resistance mechanisms often involve modifications in bacterial cell walls, membranes, proteins, and polysaccharides, which produce measurable changes in FTIR spectral regions corresponding to lipids, proteins (amide I and II bands), and carbohydrates (Winning and Scherer, 2017). By comparing spectra from resistant and susceptible strains, FTIR can reveal molecular-level differences linked to antimicrobial exposure, degradation of antibiotic compounds, and adaptive cellular responses (Lasch and Neumann, 2018).

Furthermore, when combined with chemometric techniques such as principal component analysis and cluster analysis, FTIR enables effective discrimination and classification of resistant and non-resistant microbial strains (Helm *et al.*, 2018). The technique has also been applied to monitor antibiotic–bacteria interactions and biofilm-related resistance by detecting extracellular polymeric substances and metabolic changes. Although FTIR does not directly identify resistance genes, its speed, low cost, and minimal sample preparation make it a valuable complementary tool for routine screening and surveillance of antimicrobial resistance in clinical, pharmaceutical, and environmental studies (Kirschner *et al.*, 2019; Sandt *et al.*, 2020; Lyngby *et al.*, 2026).

7. Gas Chromatography–Mass Spectrometry (GC–MS)

7.1 Principle of GC–MS

Gas Chromatography–Mass Spectrometry (GC–MS) is a powerful analytical technique widely used for the separation, identification, and quantification of volatile and semi-volatile compounds in complex mixtures (**Figure 7**) (Nielsen, 2017). The technique combines the separation capability of gas chromatography (GC) with the identification capability of mass spectrometry (MS), making it one of the most reliable tools for chemical characterization in biological, pharmaceutical, and environmental research (Kataoka, 2018).

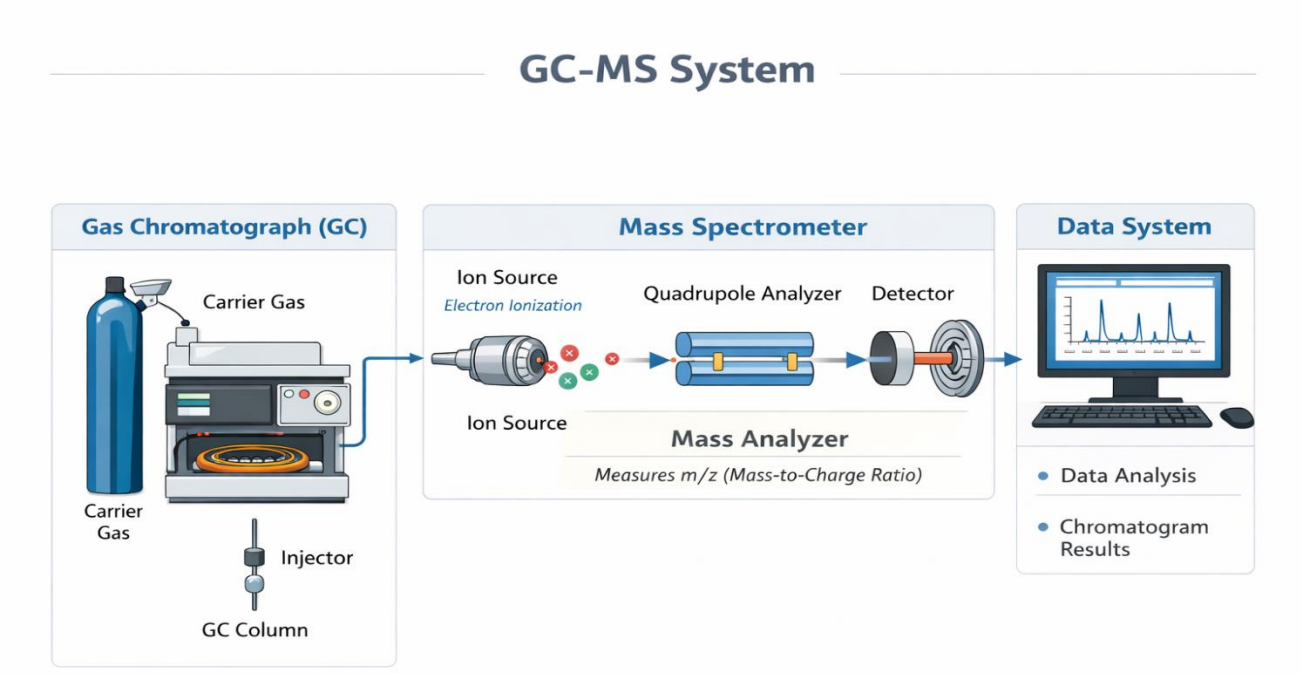

Figure 7: GC-MS System

In gas chromatography, the sample is vaporized and carried by an inert carrier gas, commonly helium, through a capillary column coated with a stationary phase. Compounds within the mixture separate based on their volatility and interaction with the stationary phase, resulting in different retention times for each component (Wang *et al.*, 2019). These separated compounds then enter the mass spectrometer where they are ionized, typically by electron impact ionization. The ionization process causes the molecules to fragment into charged ions that generate characteristic mass spectra. These fragmentation patterns serve as unique fingerprints for individual compounds, enabling accurate identification (Sparkman *et al.*, 2019).

Mass spectra produced during GC–MS analysis are compared with reference spectral libraries such as those maintained by the National Institute of Standards and Technology (NIST) or Wiley databases. These libraries contain thousands of spectra of known compounds, allowing researchers to identify unknown compounds by matching spectral patterns and retention data (Nielsen, 2017; Stashenko & Martínez, 2020).

7.2 GC–MS Analysis of Antimicrobial Phytochemicals

GC–MS has become an essential tool in the analysis of plant-derived antimicrobial compounds. Many phytochemicals with antimicrobial activity, such as essential oils, terpenes, phenolics, aldehydes, and fatty acids, are volatile or semi-volatile and therefore suitable for GC–MS analysis. The technique allows for the identification of these bioactive compounds in plant extracts, microbial metabolites, and natural products (Wang *et al.*, 2019; Kataoka, 2018).

Several studies have used GC–MS to characterize essential oils and low-molecular-weight compounds responsible for antimicrobial activity. For example, GC–MS analysis of plant extracts has revealed numerous bioactive compounds including terpenoids, phenolic compounds, and fatty acids that contribute to antibacterial and antifungal properties (Nielsen, 2017; Tranchida *et al.*, 2020; Oualdi *et al.*, 2025).

In microbial and plant metabolite studies, GC–MS has successfully identified dozens of volatile compounds associated with antimicrobial activity (Bordin *et al.*, 2021). A study investigating rhizobacterial metabolites reported the detection of 68 different compounds including tridecane, acetic acid esters, and aromatic hydrocarbons, several of which demonstrated inhibitory effects against pathogenic microorganisms such as Bacillus cereus and Pseudomonas aeruginosa (Qiu *et al.*, 2020).

Similarly, GC–MS analysis of plant extracts and essential oils has identified antimicrobial constituents such as cinnamaldehyde, terpineol, sesquiterpenes, and diterpenes, which exhibit significant antibacterial and antifungal activity against both Gram-positive and Gram-negative pathogens (Kataoka *et al.*, 2018).

The identification of these compounds is typically achieved through spectral library matching using databases such as NIST and Wiley, which compare unknown mass spectra with thousands of previously characterized compounds. This approach allows researchers to determine the molecular formula, molecular weight, and structural features of antimicrobial phytochemicals present in complex mixtures (Cajka and Fiehn, 2017).

7.3 Strengths and Limitations of GC–MS

GC–MS is widely regarded as one of the most sensitive and selective analytical techniques for chemical profiling (Cagliero *et al.*, 2018). One of its major advantages is its high sensitivity, allowing detection of compounds at very low concentrations (Zhang *et al.*, 2024). The technique also provides high compound specificity because each compound produces a unique fragmentation pattern that can be matched with reference databases for accurate identification. These characteristics make GC–MS particularly valuable in natural product research, drug discovery, and environmental analysis (Mondello *et al.*, 2022; Qi *et al.*, 2023). Another advantage of GC–MS is its ability to analyze complex mixtures and identify numerous compounds in a single analytical run. In phytochemical studies, the technique can detect dozens of metabolites from plant extracts, providing comprehensive chemical profiling that helps researchers

understand the bioactive constituents responsible for antimicrobial activity (Zhang *et al.*, 2024). Despite its advantages, GC–MS also has some limitations. The technique is primarily suitable for volatile and thermally stable compounds. Non-volatile or thermally unstable compounds cannot be directly analyzed by GC–MS and may require chemical derivatization to improve volatility and thermal stability before analysis (Kind and Fiehn, 2017). Additionally, the instrumentation can be expensive and requires skilled personnel for operation and data interpretation (Mondello *et al.*, 2022).

Nevertheless, due to its high sensitivity, reliability, and compound identification capability, GC–MS remains a widely used technique for the characterization of antimicrobial phytochemicals and natural product metabolites in pharmaceutical and biomedical research (Lyngby *et al.*, 2026).

8. Liquid Chromatography–Mass Spectrometry (LC–MS)

8.1 Principle of LC–MS

Liquid Chromatography–Mass Spectrometry (LC–MS) is a powerful analytical technique that combines the separation capability of liquid chromatography (LC) with the detection and identification capability of mass spectrometry (MS) (**Figure 8**). This hyphenated technique is widely used in pharmaceutical, environmental, and natural product research for the analysis of complex mixtures, particularly compounds that are polar, thermally unstable, or non-volatile (Niessen, 2017).

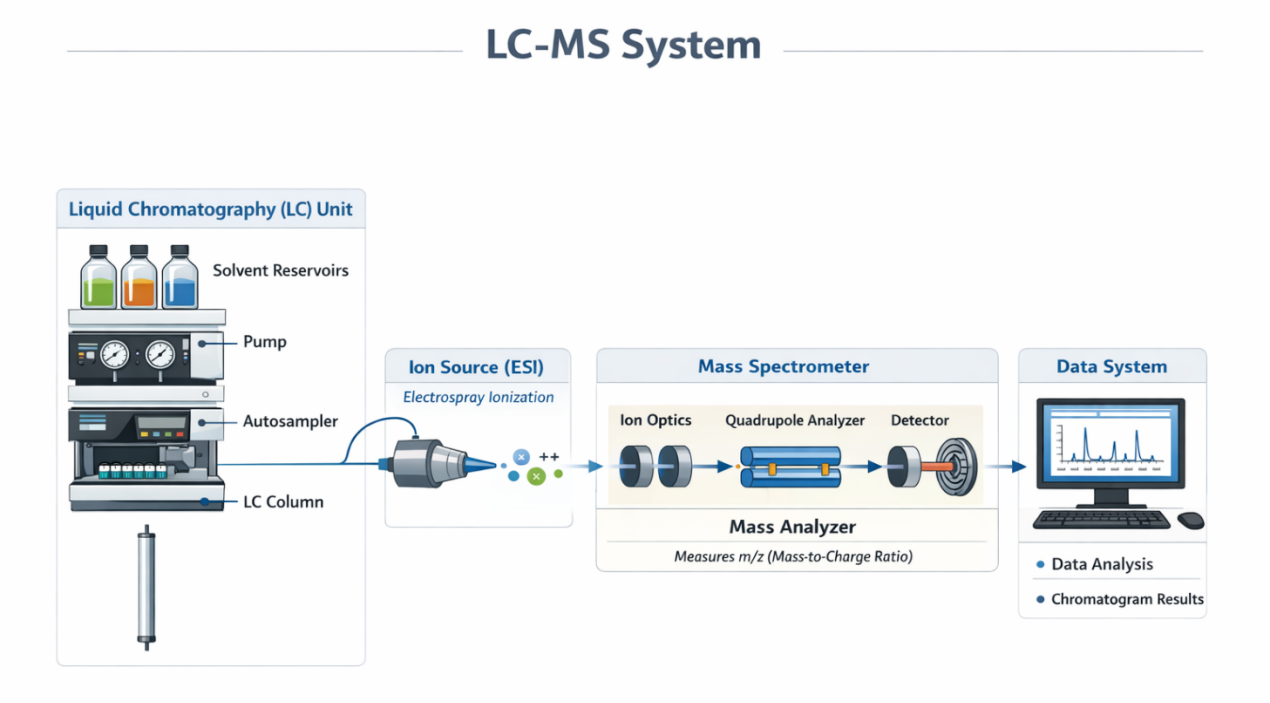

Figure 8: LC-MS System

In LC–MS analysis, compounds are first separated in the liquid chromatography system based on their interactions with the stationary phase and the mobile phase. High-performance liquid chromatography (HPLC) columns, often containing modified silica particles such as C18 stationary phases, enable the

separation of compounds according to polarity, hydrophobicity, and other physicochemical properties (Li *et al.*, 2020). After chromatographic separation, the eluting compounds are introduced into the mass spectrometer where they are ionized and analyzed according to their mass-to-charge ratio (m/z).

A critical component of LC–MS is the ionization interface, which converts neutral molecules into charged ions suitable for mass analysis (Pit, 2022). The most commonly used ionization techniques are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Both are atmospheric-pressure ionization methods that allow the direct introduction of liquid effluent from the chromatographic column into the mass spectrometer without disrupting the vacuum system (Zhang *et al.*, 2021).

Electrospray ionization is particularly effective for highly polar and large biomolecules because it produces multiply charged ions through the formation of charged droplets from the LC effluent. In contrast, APCI involves vaporization of the LC eluate followed by ionization through chemical reactions initiated by a corona discharge, making it more suitable for moderately polar and thermally stable compounds (Kaufmann, 2023; Li *et al.*, 2024).

8.2 LC–MS in Phytochemical Identification

LC–MS has become an essential tool in phytochemical research for the identification and characterization of plant-derived bioactive compounds (Zhang *et al.*, 2018). Many phytochemicals, including flavonoids, alkaloids, glycosides, and phenolic compounds, are polar and thermally unstable, making them difficult to analyze using gas chromatography. LC–MS provides an effective alternative because it allows direct analysis of these compounds in their native form without the need for derivatization (Wolfender *et al.*, 2019).

In phytochemical studies, LC–MS is commonly used for metabolite profiling, enabling the detection and identification of multiple compounds in plant extracts simultaneously (Kaufman, 2018). The technique provides accurate mass measurements that can be used to determine molecular weights and predict molecular formulas of unknown compounds. This high mass accuracy is particularly important in metabolomics and natural product research, where researchers aim to characterize complex mixtures of secondary metabolites (Li et al., 2020).

Furthermore, tandem mass spectrometry (LC–MS/MS) plays an important role in structural elucidation. In this technique, selected precursor ions are fragmented into product ions in a collision cell, producing characteristic fragmentation patterns that reveal structural information about the compound (Farag *et al.*, 2017). These fragmentation spectra help differentiate compounds with similar molecular masses and allow researchers to determine functional groups and structural features of phytochemicals (Li *et al.*, 2020).

Because of these capabilities, LC–MS is widely used to analyze plant metabolites such as flavonoids, phenolic acids, terpenoids, and alkaloids, many of which exhibit antimicrobial, antioxidant, and

pharmacological activities. The technique is therefore crucial in the discovery of bioactive natural products and the characterization of medicinal plant extracts (Cajka and Fiehn, 2017; Tranchida *et al.*, 2023).

8.3 Advantages and Limitations of LC–MS

One of the major advantages of LC–MS is its broad compound coverage. Unlike gas chromatography, which is mainly limited to volatile and thermally stable compounds, LC–MS can analyze a wide range of polar, non-volatile, and thermally labile molecules. This capability makes it particularly useful in pharmaceutical analysis, metabolomics, proteomics, and natural product chemistry (Pitt, 2019; Tranchida *et al.*, 2023).

Another advantage is the high sensitivity and selectivity of the technique. LC–MS can detect compounds at very low concentrations while simultaneously providing structural information through mass spectral analysis. Additionally, the use of tandem mass spectrometry (LC–MS/MS) enhances compound identification and improves analytical accuracy in complex mixtures (Niessen, 2018).

Despite these advantages, LC–MS also has several limitations. The instrumentation is expensive and requires highly trained personnel for operation and data interpretation (Zhang *et al.*, 2021). The technique also involves complex sample preparation and method optimization to minimize matrix effects and ion suppression, which can affect analytical accuracy. Furthermore, maintenance and calibration of LC–MS systems can be demanding due to the sophisticated interface between the chromatographic and mass spectrometric components (Wolfender *et al.*, 2020). Nevertheless, due to its high sensitivity, versatility, and capability for detailed structural analysis, LC–MS remains one of the most powerful analytical tools for phytochemical analysis and natural product research (Li *et al.*, 2020).

9. Correlation of Phytochemical Profiles with Antimicrobial Activity

A central goal of natural product research is to link chemical profiles of plant extracts with their antimicrobial properties. Establishing this correlation involves combining analytical chemistry with biological assays to connect specific phytochemicals with observed bioactivity (Farag *et al.*, 2017). Spectroscopic and chromatographic techniques such as FTIR, GC–MS, and LC–MS allow comprehensive profiling of plant metabolites, while bioassays (e.g., minimum inhibitory concentration tests, disc diffusion assays) quantify antimicrobial effects. Integrating both approaches enables identification of compounds responsible for antimicrobial effects and helps discriminate active from inactive constituents (Wolfender *et al.*, 2019)

Bioactivity-guided fractionation remains an effective strategy for correlating phytochemical composition with function. In this approach, crude plant extracts are fractionated based on polarity or chemical class, and individual fractions are evaluated for antimicrobial potency. Bioactive fractions are then chemically

profiled using analytical techniques such as GC–MS or LC–MS to identify candidate lead compounds. This targeted isolation strategy increases the likelihood of discovering potent antimicrobial agents and allows the elimination of inactive or antagonistic components early in the discovery pipeline (Cajka and Fiehn, 2017).

To handle the complexity of data generated from phytochemical profiling and antimicrobial assays, multivariate statistical methods such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) have become invaluable (Ghosh and Chakraborty, 2018). These chemometric tools help reduce dimensionality, visualize patterns, and identify correlations between chemical fingerprints and bioactivity data. PCA can distinguish groups of samples based on chemical similarity, highlighting metabolites associated with antimicrobial activity. HCA organizes samples into clusters based on overall chemical similarity, revealing natural groupings among extracts or fractions. The application of these techniques enhances objectivity in linking spectral and chromatographic data with biological outcomes and has been extensively used in metabolomics and natural product research (Li *et al.*, 2020; Shang and Li, 2021).

10. Applications in Antimicrobial Drug Discovery

10.1 Identification of Lead Compounds

The integration of phytochemical profiling with antimicrobial bioassays accelerates the identification of lead compounds with therapeutic potential. With advances in LC–MS/MS and high-resolution mass spectrometry, researchers can now detect low-abundance metabolites and accurately determine molecular formulas and fragmentation patterns that facilitate structural characterization (Farag *et al.*, 2017; Wolfender *et al.*, 2020). Lead compounds emerging from plant-based research have included flavonoids, terpenoids, alkaloids, and phenolic acids that exert significant antibacterial and antifungal effects against clinically relevant pathogens. These discoveries inform preclinical development and synthetic optimization efforts, contributing to the expansion of novel antimicrobial scaffolds (Li *et al.*, 2020).

10.2 Quality Control of Herbal Formulations

Analytical profiling is also essential in quality control of herbal medicines. Herbal products often suffer from batch-to-batch variability due to differences in plant source, harvest season, and processing methods (Wolfender *et al.*, 2019; Nouioura *et al.*, 2024). Techniques like LC–MS and GC–MS can detect marker compounds that validate authenticity and potency, while FTIR fingerprinting can be used as a rapid quality screen. Ensuring consistent phytochemical composition helps guarantee reproducible antimicrobial efficacy and reduces safety concerns associated with adulteration or degradation (Shang and Li, 2021).

10.3 Detection of Synergistic Antimicrobial Phytochemicals

Phytochemicals often act synergistically, where the combined effect of compounds is greater than the sum of individual activities (Ghosh and Chakraborty, 2018). Analytical profiling coupled with bioassays enables identification of such synergistic interactions by comparing chemical and biological data across fractions and their combinations. Multivariate models can further predict synergistic pairs by correlating co-occurrence of compounds with enhanced bioactivity (de Souza *et al.*, 2022).

11. Challenges and Future Perspectives

11.1 Complexity of Plant Matrices

Plant extracts contain thousands of metabolites spanning diverse chemical classes, posing a significant analytical challenge (Huber *et al.*, 2019). Overlapping chromatographic signals, low-abundance compounds, and structurally similar isomers complicate compound separation and identification. Even advanced techniques such as LC–MS/MS cannot always fully resolve complex mixtures without extensive sample preparation or orthogonal separation strategies (Salem *et al.*, 2020).

11.2 Data Interpretation and Compound Identification Challenges

High-throughput profiling generates large datasets that require sophisticated bioinformatics tools for interpretation. Accurate compound annotation remains a bottleneck due to limited spectral library coverage and structural ambiguities. Novel compounds often lack reference spectra, necessitating de novo structural elucidation, which is time-intensive and requires expertise in mass spectrometric interpretation (Gika et al., 2019)

11.3 Need for Standardized Analytical Protocols

Variations in sample preparation, chromatographic conditions, and ionization protocols can introduce analytical variability (Scalbert et al., 2020). Standardized methodologies are needed to ensure reproducibility within and across laboratories, enabling meaningful comparisons of phytochemical profiles and their associated bioactivities. Standard operating protocols for LC–MS, GC–MS, and FTIR utilized in antimicrobial studies would improve cross-study consistency and aid method validation (Perez de Souza *et al.*, 2019).

Integration with Metabolomics and In Silico Tools

Future research will benefit from integrating analytical profiling with metabolomics and computational prediction tools (Scalbert *et al.*, 2020). Metabolomics approaches use untargeted and targeted strategies to comprehensively profile biological samples while advanced chemometric models improve correlation between profiles and phenotypes. In silico tools such as molecular docking and machine learning models

can predict antimicrobial activity based on chemical structure, prioritizing target compounds for experimental validation (Silva *et al.*, 2025).

12. Future Role in Combating Antimicrobial Resistance

As antimicrobial resistance continues to pose a global health threat, analytical techniques that accelerate lead discovery and screening are increasingly valuable. By identifying novel bioactive phytochemicals, elucidating mechanisms of action, and enabling rapid quality control of therapeutic formulations, integrated analytical strategies will play a pivotal role in developing next-generation antimicrobials and supporting stewardship efforts (Garcia-Segura, 2021; Silva *et al.*, 2025).

13. Conclusion

FTIR, GC–MS, and LC–MS represent complementary analytical platforms essential for the identification and characterization of antimicrobial phytochemicals. Together, they provide a comprehensive understanding of plant-derived metabolites, linking chemical composition to bioactivity. FTIR offers rapid screening of functional groups, GC–MS excels in profiling volatile compounds, and LC–MS enables detailed analysis of polar and thermolabile constituents, including flavonoids, alkaloids, and phenolic acids. The integration of these techniques with bioactivity-guided fractionation, chemometric analysis, and metabolomics enhances the discovery of potent antimicrobial agents and supports the identification of synergistic phytochemical interactions. Despite challenges such as complex plant matrices, analytical variability, and data interpretation, advancements in standardization, high-resolution instrumentation, and computational tools promise improved reproducibility and efficiency. By accelerating lead compound discovery, elucidating mechanisms of action, and enabling quality control, these analytical strategies play a pivotal role in advancing plant-based antimicrobials and addressing the global challenge of antimicrobial resistance.

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