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Indian Medicinal Plants in Antimicrobial Drug Discovery: Phytochemical Diversity, Structure-Activity Relationships, Advanced Analytical Characterization, and Therapeutic Potential

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ABSTRACT: Multidrug-resistant organisms are a major public health concern, as antimicrobial resistance (AMR) has become one of the greatest threats worldwide, prompting researchers to search for new therapeutic agents that can inhibit multidrug-resistant organisms. Indian medicinal plants are a treasure trove of structurally diverse bioactive phytochemicals such as phenolic compounds, flavonoids, alkaloids, terpenoids, coumarins, sulfur-containing compounds, tannins and glycosides, many of which have potent antimicrobial properties. This review comprehensively summarizes recent studies on the phytochemical diversity of Indian medicinal plants, highlighting their structure–activity relationships (SAR), molecular mechanisms of antimicrobial action, and applications against clinically significant bacterial and fungal pathogens. Advanced analytical methods such as GC-MS, LC-MS, UPLC-QTOF-MS, FTIR and one- and two-dimensional NMR spectroscopy have contributed to the accurate phytochemical characterization and quality assessment highlighted by the review. In addition, it covers various antimicrobial mechanisms, such as disruption of microbial cell walls and cell membranes, inhibition of DNA replication and protein synthesis, inhibition of efflux pumps, induction of oxidative stress, and biofilm suppression. Growing evidence supports the activity of natural products and nano-formulations against multidrug-resistant organisms such as methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum β-lactamase (ESBL) producers, Acinetobacter baumannii, Pseudomonas aeruginosa, and Candida auris. These encouraging results, however, come with many hurdles, such as phytochemical standardization, bioavailability, toxicity assessment, regulatory clearance, and industrialization. Prospects include metabolomics, artificial intelligence, nanotechnology, green extraction technologies, and translational research, all of which can help speed the development of safe and effective antimicrobial therapeutics from plants. In conclusion, Indian medicinal plants are a valuable and sustainable resource for developing antimicrobial agents of the future, holding significant potential to address the escalating issue of antimicrobial resistance.

1. INTRODUCTION

The spread of antimicrobial resistance (AMR) is one of the biggest challenges of the twenty-first century for public health, endangering the success of prevention and treatment of bacterial, fungal, viral, and parasitic diseases. The rapid emergence and spread of multidrug (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) pathogens have made it more difficult to treat conventional antimicrobial agents and have resulted in higher levels of morbidity, mortality, and longer hospital stays, along with higher healthcare costs [1]. In humans, animals, and agriculture, antibiotics have been widely prescribed and given, leading to the evolution of resistant microorganisms and the search for new antimicrobial agents with novel mechanisms

of action. The structural diversity of natural products, especially medicinal plants, as a source of new bioactive compounds that can combat resistant pathogens, has renewed scientific interest. India is one of the richest stores of medicinal plant biodiversity, with over 7,000 medicinal species used in traditional systems such as Ayurvedic, Siddha, Unani, and Folk medicine [2].

For centuries, these medicinal plants have been used for the treatment of infectious diseases, inflammatory disorders, metabolic illnesses, and various chronic illnesses. They are believed to be most effective because of the wide range of secondary metabolites they contain, such as phenolic compounds, flavonoids, alkaloids, terpenoids, coumarins, tannins, glycosides, saponins, essential oils, and sulfur-containing compounds [3]. These phytochemicals have outstanding structural diversity and wide-ranging biological properties, including antimicrobial, antioxidant, anti-inflammatory, antiviral, antifungal, immunomodulatory, and anticancer effects. Their ability to act on multiple microbial pathways makes them more complex and less likely to develop microbial resistance than traditional antibiotics. However, advances in phytochemistry, analytical chemistry, metabolomics, molecular biology, and pharmaceutical sciences have significantly improved understanding of the antimicrobial potential of medicinal plants, as shown in Figure 1.1 [4].

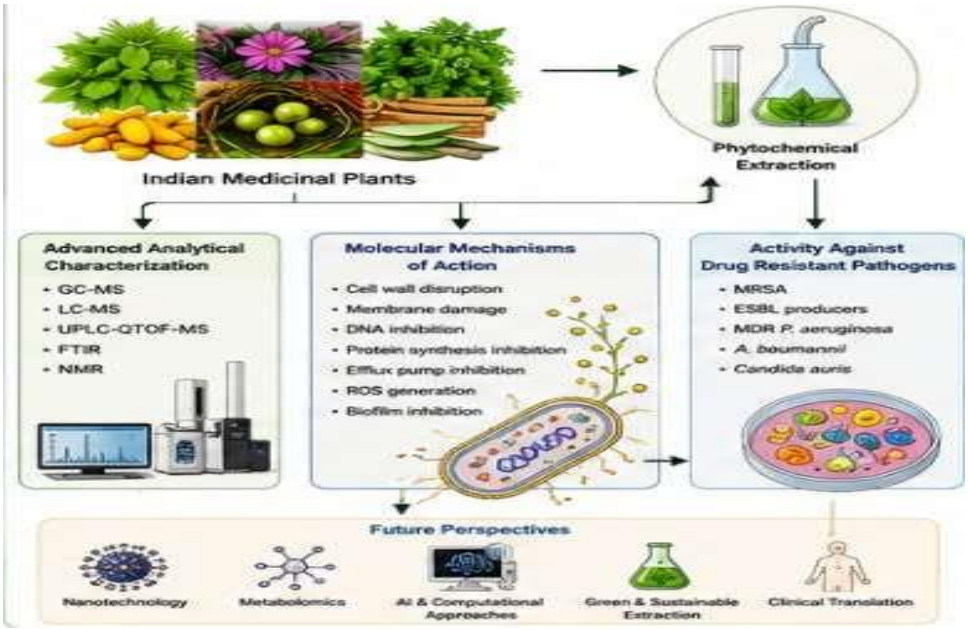


Figure 1.1 Overview of antimicrobial agents from Indian medicinal plants: from phytochemical extraction to future perspectives against AMR

The identification, isolation, and structural characterization of bioactive phytochemicals have been achieved with the use of modern analytical technologies like gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS), Fourier transform infrared spectroscopy (FTIR), and two- and one-dimensional nuclear magnetic resonance (2D/1D NMR) spectroscopies. These sophisticated analytical devices enable quality control, standardization and structure–activity relationship (SAR) studies, which help expedite the development of new antimicrobial lead molecules for pharmaceutical products [5].

Many experiments have shown that phytochemicals found in plants have antimicrobial activity by different means and are complementary to each other. These include disruption of microbial cell walls and membranes, inhibition of nucleic acid and protein synthesis, interference with essential metabolic pathways, induction of oxidative stress via production of reactive oxygen species (ROS), inhibition of bacterial efflux pumps, and prevention/disruption of bacterial biofilm formation. This multitarget action makes phytochemicals potent against Gram-positive and Gram-negative bacteria, fungi, and clinically significant multidrug-resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum β-lactamase (ESBL)- producing Enterobacteriaceae, carbapenem-resistant Pseudomonas aeruginosa, Acinetobacter baumannii, and the emerging fungal pathogen Candida auris. Moreover, the plant extracts have been observed to exhibit a synergistic effect with

conventional antibiotics, indicating their potential as antibiotic adjuvants to regain the antimicrobial susceptibility of resistant bacteria [6].

Despite these promising signs, several scientific and translational hurdles remain. Due to geographical origin, husbandry, maturity, extraction, and analytical procedures, the variability in phytochemical composition may be a source of non-reproducibility and non-standardization. Furthermore, the lack of proper toxicological studies, low aqueous solubility, low bioavailability, inadequate pharmacokinetic characterization, and lack of well-designed clinical trials remain challenges in the successful clinical translation of plant-derived antimicrobial agents. To overcome these challenges, a multi-disciplinary approach will be necessary, which includes the use of green technologies for extraction, metabolomics, nanotechnology-based medicine delivery systems, artificial intelligence for drug discovery, computational modelling, and rigorous clinical testing. This review provides a detailed overview of the antimicrobial potential of Indian medicinal plants, summarizing their phytochemical diversity, structure–activity relationships, analytical characterization methods, and molecular mechanisms of action [7]. It also reviews the antimicrobial activity of plants against multidrug-resistant pathogens, recent developments in phytochemical research and the use of nanotechnology in formulating antimicrobial agents, and challenges and future opportunities for developing safe, effective, and sustainable plant-based antimicrobial therapeutics. In this context, the review also aims to explore the scientific basis for discovering and developing next-generation antimicrobial agents from Indian medicinal plants by integrating and correlating contemporary knowledge from phytochemistry, microbiology, pharmaceutical sciences, and translational research [8].

2. PHYTOCHEMICAL DIVERSITY OF INDIAN MEDICINAL PLANTS:

India is one of the countries with the highest number of custodians of medicinal plants in the world, with thousands of species being used in traditional Indian medicine systems such as Ayurvedic, Siddha, and Unani medicine. These plants have several secondary metabolites, including alkaloids, flavonoids, phenolic acids, tannins, terpenoids, coumarins, lignans, glycosides, saponins, and essential oils. This phytochemical diversity is a major reason medicinal plants are naturally free of pathogens and environmental stressors, and most of their therapeutic value is attributed to it. This diversity in chemical structure and biological activity makes Indian medicinal plants an excellent source for discovering novel antimicrobial agents and leads for pharmaceutical development [9]. Figure 1.2 shows the major phytochemical constituents and their activities.

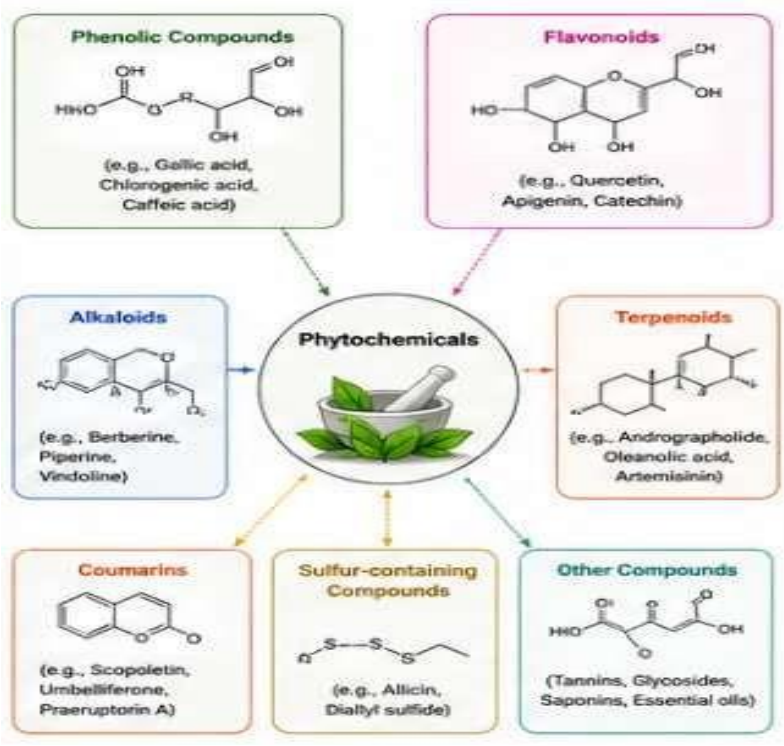


Figure 1.2 Classification of major phytochemical constituents and their representative compounds

2.1 Phenolic compounds

Phenolic compounds are a diverse group of plant secondary metabolites playing crucial roles in plant physiology, growth, development, and defence mechanisms. These compounds are predominantly produced via the phenylpropanoid pathway and are classified into several groups, including flavonoids, anthocyanins, tannins, coumarins, lignin derivatives, and phenolic acids. The authors found phenolic compounds to be crucial in plant defence against biotic stress (such as pathogen attack) and abiotic stress (such as salinity, drought, and ultraviolet radiation). Flavonoids have good antioxidant activity because they reduce the production of Reactive Oxygen Species (ROS), and lignin provides structural strength and protection against environmental stresses and pathogens. Further, tannins, coumarins, and anthocyanins are protective compounds that are toxic and bitter to herbivores and insects. The review also highlighted the close relationship between the biological activity of polyphenols and their redox properties. Polyphenols easily oxidize and reduce, such that they can act as antioxidants by donating electrons and scavenging free radicals [10].

They primarily exert their antioxidant activity through single-electron transfer (SET), hydrogen-atom transfer (HAT), and metal-chelation mechanisms, by scavenging ROS and decreasing oxidative stress. Both the number and the position of hydroxyl groups attached to the aromatic rings have a significant effect on the antioxidant activities of polyphenols. The compounds that have catechol and gallol structures show better antioxidant activity because of the greater stabilization of phenoxyl radicals by resonance. Antimicrobial relevance: it should be noted that the redox activity of polyphenols can be used to produce Reactive Oxygen Species (ROS), which induce oxidative stress in microbial cells [11]. When ROS accumulation is excessive, multiple damages occur in the cell, such as DNA oxidation, lipid oxidation, protein oxidation, membrane disruption, and metabolic dysfunction. The antimicrobial activity of ROS is multifaceted, involving several cellular targets, making polyphenol-based materials an interesting approach to inhibit pathogenic microorganisms and combat antimicrobial resistance. Numerous examples of the antibacterial, antifungal, and antiviral effects of catechol- and polyphenol-based materials were summarized, based on ROS generation, disruption of cell membranes, DNA damage, enzyme inactivation, and synergistic actions with metal ions and other antimicrobial agents [12].

2.2 Flavonoids

Flavonoids are among the most important antimicrobial compounds of plants and have been reported to have antibacterial activity by several mechanisms. In the above-mentioned study, flavonoids were specifically mentioned as inhibitors of bacterial efflux pumps, which allows them to increase the concentration of toxic compounds and antibiotics in the cell. The authors noted that flavonoids are potent efflux pump inhibitors and specifically mentioned that apigenin has been known as an efflux pump inhibitor in the past [13]. Flavonoids inhibit bacterial efflux systems that expel ethidium bromide, antibiotics, and other toxic molecules, resulting in poorer bacterial survival. Moreover, orientin and isoorientin enhanced the accumulation of ethidium bromide in both antibiotic-sensitive and resistant strains of Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus, suggesting that they inhibit bacterial membrane transport systems. The authors proposed that the flavonoids have other antimicrobial mechanisms as well, such as inhibition of protein synthesis, interference with cell wall synthesis, disruption of metabolic pathways, interference with nucleic acid synthesis, inhibition of membrane function, and impairment of membrane integrity. The results confirm that flavonoids have multifunctional antimicrobial activity, targeting not only membrane-associated processes but also essential bacterial enzymes [14].

2.3 Alkaloids

The antibacterial effect of berberine and palmatine on methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Staphylococcus aureus (MDRSA) strains was evaluated. Out of all the phytochemicals, berberine was found to be the most active antibacterial compound and was found to have a synergistic effect against ciprofloxacin in the study. The authors indicated that berberine's antimicrobial activity may be strongly linked to its inhibitory effects on bacterial efflux pumps, which lead to greater accumulation of antimicrobial agents and toxic compounds in the intracellular environment [15].

Real-time ethidium bromide accumulation assays showed that berberine enhanced ethidium bromide accumulation within the cell, suggesting the inhibition of efflux-mediated resistance mechanisms. Moreover, the study showed that berberine works synergistically with antibiotics, which could be due to its ability to inhibit the antibiotic efflux pump NorA in bacteria, thereby increasing antibiotic concentrations in the bacterial cell. Apart from efflux pump inhibition, the authors reviewed previous reports that berberine has antibacterial properties by various mechanisms, such as inhibition of DNA replication, inhibition of

RNA transcription, inhibition of protein synthesis, inhibition of enzyme activity, and disruption of the cell surface of bacteria. Together, these actions make berberine and related alkaloids effective against resistant bacterial pathogens, and they may be used as adjuncts to conventional antibiotic therapy [16].

2.4 Terpenoids

Terpenoids are secondary metabolites of plants that are important in nature and are known to have strong antimicrobial activity. The study indicated that terpenoids play a role in plant defence mechanisms and also demonstrated their antimicrobial activity against various microorganisms. Among the terpenoids tested, andrographolide and its C-14 ester derivatives showed potential as antibacterial agents, with minimum inhibitory concentration (MIC) values between 4 and 16 μg/mL. Membrane integrity and cell viability assays were conducted to explore how it works. Andrographolide derivatives increased bacterial cell permeability when tested using fluorescence staining, indicating disruption of cell membrane integrity. In addition, SEM images showed well-defined, opaque, and rigid Escherichia coli cells when untreated, and blurred, deflated, and translucent cells when treated. Based on these observations, the antimicrobial activity of andrographolide derivatives was hypothesized to disrupt bacterial cell membranes, increasing membrane permeability and causing cellular damage. The authors suggest that andrographolide monoester derivatives have greater antimicrobial activity and could be used to inhibit bacterial cells, since they act on the bacterial cell [17].

2.5 Coumarins

Coumarins are the major active compounds found in Peucedanum praeruptorum, and they can be divided into simple coumarins, furanocoumarins, and pyranocoumarins. In the study conducted by Harborne & Williams in 1994 total of 17 coumarin compounds were quantitatively analyzed, of which three are simple coumarins (umbelliferone, isofraxidin and ostenol), six furanocoumarins (marmesin, psoralen, xanthotoxin, bergapten, oxypeucedanin and imperatorin), and eight pyranocoumarins (qianhucoumarin D, qianhucoumarin A, peucedanocoumarin II, pteryxin, praeruptorin A, praeruptorin B, praeruptorin E and decursin). The authors mentioned that the most significant phytochemical and pharmacologically active compounds in P. praeruptorum were coumarin derivatives, of which pyranocoumarins are the most abundant. In plants growing for 1, 2, and 3 years, the total content of coumarin and the total content of pyranocoumarin were found to increase with an increase in the growth period. P. praeruptorum A and B were found to be significant bioactive compounds, while praeruptorin B was observed to increase more prominently with an increase in cultivation time. Additionally, temporal gene expression changes were found to affect coumarin accumulation by regulating the coumarin biosynthetic pathway, as shown by transcriptomic analysis. The study also highlighted the biological significance of coumarins: furanocoumarins have been linked to plant defence mechanisms, and pyranocoumarins have significant pharmacological activities, including antimicrobial, anti-inflammatory, anticancer, and neuroprotective effects. The authors suggested that the bioactive coumarins are likely to increase with prolonged culture and concluded that the therapeutic potential of P. praeruptorum can be improved by prolonged cultivation [18].

2.6 Sulphur-Containing Compounds

The biological activity and the therapeutic value of sulfur-containing compounds have led to their growing interest. According to the review, the plant-synthesized sulfur nanoparticles (SNPs) have shown good physicochemical characteristics, such as high surface areas, redox properties, and comparatively low toxicity. Flavonoids, phenolics, alkaloids, and saponins containing plant extracts are used as reducing and stabilizing agents in the synthesis of sulfur nanoparticles, which help in the formation of nanoparticles and inhibit the aggregation of nanoparticles. Cannabis sativa, Allium sativum (garlic), Citrus limon, and Citrus sinensis are some medicinal plants that have been used to successfully synthesize sulfur nanoparticles. The authors noted that the biological activity of sulfur nanoparticles is enhanced by their nanosize, improved surface properties, and greater interaction with cell components. Moreover, previous studies mentioned in the review showed antibacterial activity in plant-mediated sulphur nanoparticles, which may be due to their redox properties, increased cellular interaction, and the presence of bioactive phytochemicals on the nanoparticle surface [19].

1. Structure–Activity Relationship (SAR) of Antimicrobial Phytochemicals:

SAR studies aim to understand the bioactivity of phytochemicals and how specific structural elements contribute to that activity. Knowledge of SAR is essential for understanding the functional groups and molecular properties involved in

antimicrobial activity and for maximizing the activity of bioactive molecules. During the process of hydroxylation, methoxylation, glycosylation, lipophilization, changes in size, and/or the presence of aromatic rings, many phytochemicals can interact with the targets of the microbes. SAR analysis is crucial for designing efficient and effective antimicrobial agents, predicting biological activity, and developing phytochemical-based therapeutics against drug-resistant pathogens [20]. The chemical structure was found to significantly affect the antibacterial activity of the phytochemicals in the study. The authors reported that the antimicrobial activity of flavonoids is structure-dependent, with hydroxylation patterns often increasing antimicrobial activity and some methoxylation patterns decreasing biological activity. Several flavonoids were found in the active extracts, such as quercetin, kaempferol, luteolin, rutin, catechin, and epicatechin; all of which exhibited antibacterial potential, disrupting membranes and cell walls, inhibiting protein and nucleic acid synthesis, interfering with signal transduction, suppressing efflux pumps, and reducing the production of virulence factors and the formation of biofilms [21].

The previous studies have also highlighted that the better antimicrobial activity of the hydroethanolic extracts and enzyme-assisted extracts was correlated with a higher yield of flavonoids and other phenolics with favourable structural characteristics for antimicrobial activity. So, the authors conclude that hydroxylation is a beneficial functional group for antibacterial activity, while certain methoxy substitution patterns could harm activity, thus emphasizing the significance of SAR in elucidating the antimicrobial activity of phytochemicals from plants [22].

Biological and antimicrobial properties of phytochemicals are significantly affected by their structural features. The authors conclude that long-chain fatty acids (LCFAs), including palmitic acid, have antimicrobial effects mediated by membrane disruption, while PUFAs, such as linoleic acid, have antimicrobial activity mediated by integrating into cellular membranes and modulating biological processes. The study also revealed aromatic hydrocarbons and aromatic ring-containing compounds such as p-cymene, toluene, o-xylene, cumene, and benzoquinone derivatives, which are known for their antimicrobial activity and the biological properties of plant extracts and seed oils. Phenolic acids include sinapic acid and 3-feruloylquinic acid, which have hydroxyl and methoxy groups on aromatic rings that help give them antioxidant and antimicrobial properties. The SAR analysis indicated that hydroxyl and methoxy groups drive positive effects on biological activity through electron delocalization and stabilization of reactive species, while glycosylated structures, such as polydatin, improved solubility and bioavailability. Specific structural components such as aromatic ring substitution, lipophilic fatty-acid chains, hydroxyl-rich aromatic systems, and glycosylated moieties were found to be responsible for the antimicrobial and therapeutic activities found in extracts and oils from the neem, black seed, and baobab, respectively [23].

Case Studies of Alkaloids, Flavonoids, and Terpenoids

Phytochemical screening of selected medicinal plants highlighted compounds such as alkaloids, flavonoids, terpenoids, tannins, saponins, glycosides, phenolic compounds, and steroids. Previous studies have highlighted the importance of phytochemicals are said to be responsible for the therapeutic and pharmacological activities of medicinal plants of these, flavonoids showed antioxidant and anti-inflammatory properties, and alkaloids exhibited antimicrobial and analgesic properties. Antiviral and anti-inflammatory properties were claimed for terpenoids, and they were found in all investigated medicinal plant species. The study also highlighted that the alkaloids and tannins have antimicrobial properties, while flavonoids and phenolic compounds have significant roles in antioxidant defence. The presence of these phytochemicals in medicinal plants such as neem, tulsi, turmeric, aloe vera, and ashwagandha supports their traditional applications and therapeutic uses [24].

4. Analytical Characterization of Bioactive Compounds

Determination of the structure of isolated bioactive plant compounds is one of the fundamental steps in the identification and elucidation of plant-based medicines. Use of advanced analytical techniques to determine the composition, structure, functional groups, purity, and physicochemical properties of phytochemicals. Methods that provide detailed information, which is necessary for identification and quality control of compounds, include chromatography, mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy, infrared spectroscopy, and analytical techniques mentioned in Table 1.1. Analytical characterization helps to evaluate the biological activities correctly, aids plant extract standardization, and aids in the formulation of safe and effective therapeutic agents derived from plants [25].

Table 1.1 Analytical Techniques for the Identification and Characterization of Bioactive Compounds

Technique	Purpose	Information Obtained
GC-MS	Volatile compounds	Compound identification
LC-MS	Polar metabolites	Molecular mass
UPLC-QTOF-MS	High-resolution profiling	Metabolomics
FTIR	Functional groups	Chemical bonds
NMR	Structure elucidation	Molecular structure

4.1 GC-MS Profiling

This paper can only be used indirectly to review the antimicrobial activity of natural plants since the primary purpose of this paper was the phytochemical screening and analytical characterization of the Arachis hypogaea extracts using the GC-MS technique, which is not the primary method for testing antimicrobial activity. The study revealed that the polar ethanolic extract exhibited phenolics, flavonoids, tannins, saponins, sterols, and terpenoids with many identified bioactive compounds by GC-MS, while the non-polar oil exhibited sterols and terpenoids with many identified bioactive compounds by GC-MS. Major constituents in the oil fraction were oleic acid, n-hexadecanoic acid, tridecanoic acid, and 9,12-octadecadienoic acid derivatives, while the ethanolic fraction had a wider diversity of compounds like linoelaidic acid, 5-hydroxymethylfurfural, hexadecanoic acid derivatives, and octadecanoic acid. These compounds have been extensively associated with antimicrobial, antioxidant, and anti-inflammatory activities, and the paper could be used to suggest that Arachis hypogaea has great bioactive potential, but not been proven to have antimicrobial activity by microbial assays. The important point is that GC-MS profiling may help in the identification of candidate antimicrobial constituents and in the chemical basis for future antibacterial or antifungal evaluation, but cannot claim antimicrobial efficacy [26].

4.2 LC-MS and metabolomics:

Metabolomic characterization using liquid chromatography-mass spectrometry (LC-MS) gives a highly sensitive and specific method to characterize complex phytochemical architectures of medicinal plant extracts. For example, a new study has employed the LC-MS-2020 system to effectively characterize the bioactive methanolic extract of Ephedra alata. The separation was done by chromatographic methods with a C18 analytical column and a gradient mobile phase of (A) 0.1% formic acid in water, (B) 0.1% formic acid in methanol. To provide better identification of phenolic and other secondary metabolites, mass spectrometric analysis was carried out using electrospray ionisation (ESI) in negative mode $[M-H]^-$ within a mass range of 50 to 1500 m/z. The metabolomic profiling effectively identified a wide range of pharmacologically relevant flavonoids and glycosylated flavonoids by comparing the retention times, UV spectral data, and precursor/fragment mass data with reference standards and literature databases. Specifically, the highest amounts were measured for quercetin 3-D-galactoside (28.487%) and for 3,3',4,4',5,7-pentahydroxy-6-methoxyflavanonol-3-O-glucoside-5-O-glucuronic acid (10.74%), followed by other relevant active compounds like kaempferol (5.29%), rutin (2.43%), epigallocatechin (5.29%), naringin (0.38%) and apigenin 7-rutinoside (1.13%). These examples illustrate how advanced analytical screening of natural plant extracts in negative-mode LC-ESI-MS can serve as a cornerstone for reviewing plant extracts to determine the phytochemical blueprints that underlie downstream therapeutic, antioxidant, and biological properties [27].

4.3 HPLC and UPLC-QTOF -MS:

Liquid chromatography-mass spectrometry (LC-MS) and advanced ultra-high-performance liquid chromatography (UHPLC) frameworks are important tools to analyze the physicochemical and biological architectures of highly potent plant extracts and derivatives, as described in the literature. Reversed-phase RP-UHPLC systems with C18, C8, and phenyl stationary phases are used to systematically capture structural complexities using anisotropic lipophilicity mapping in the chromatographic isolation of plant secondary metabolites and specialized compounds, such as biologically active steroidal oximes. Retention parameters obtained across these stationary phases provide insight into the direct impact of intramolecular changes (such as isomers, molecular shape changes, local steric accessibility, and local aromatic stacking) on the downstream pharmacokinetics, target-binding affinity, and overall antimicrobial potency of an extract. These experimental separation techniques, combined with a high-resolution Quadrupole Time-of-Flight mass spectrometer (UPLC-QTOF-MS), enable the uncompromised elucidation of multiple trace bioactive elements simultaneously. Moreover, by applying these highly sensitive chromatographic runs and multi-dimensional mobile-phase modulations (such as matching protic (methanol) and aprotic (acetonitrile) mobile phases), it is possible to separate highly lipophilic structural fragments from hydrophilic scaffolds. The metabolomic workflow then produces a mathematically supported, rigorous blueprint that links the organism's detailed phytochemical makeup to its biological activity against pathogenic target lines, using methods such as Hierarchical Cluster Analysis (HCA), PCA, and Artificial Neural Networks (ANN) [28,29].

4.4 FTIR spectroscopy:

FTIR-ATR (Fourier Transform Infrared Spectroscopy-Attenuated Total ~~Reflectance) is~~Reflectance) is an important non-destructive analytical technique for profiling the various intramolecular and intermolecular chemical bonds found in natural plant matrices, based on its analytical characterization obtained from the reference literature. FTIR-ATR spectroscopic analysis of the methanolic extract of the leaves of Lepisanthes rubiginosa was employed and was able to identify the complex phytochemical composition responsible for its remarkable antimicrobial activity against various Gram-positive and Gram-negative bacterial pathogens within the range 4000–400 cm^{-1}. In particular, a wide, intense absorption band at about 3351.43 cm^{-1}, corresponding to the {O-H} stretching vibration, confirms that the extract is rich in hydroxylated compounds such as alcohols, phenols, sugars, and flavonoids, making it a bioeconomically efficient extract. The presence of asymmetric and symmetric {C-H} stretching bands at 2923.78 and 2852.65, respectively, confirms the presence of basic alkane structures, while the localized peak at 1631.22 is due to {C=O} stretching vibrations typical of important carbonyl structures such as ketones, aldehydes, or carboxylic acids. Further, aromatic skeleton markers are seen in the {C-C} stretching at 1415.82, and multiple explicit {C-O} stretching frequencies at 1256.54 -1158.56, 1, 1073, and 1035.81 indicate the presence of a high concentration of esters, ethers, polysaccharides, glycosides, and phenolic constituents. The out-of-plane bending vibrations, as the last step, are mapped comprehensively in the spectroscopic blueprint provided by FTIR-ATR analysis, thereby systematically establishing the structural identity of the secondary metabolites found in the plant extracts, which is particularly relevant to their potential therapeutic and antimicrobial effects [30].

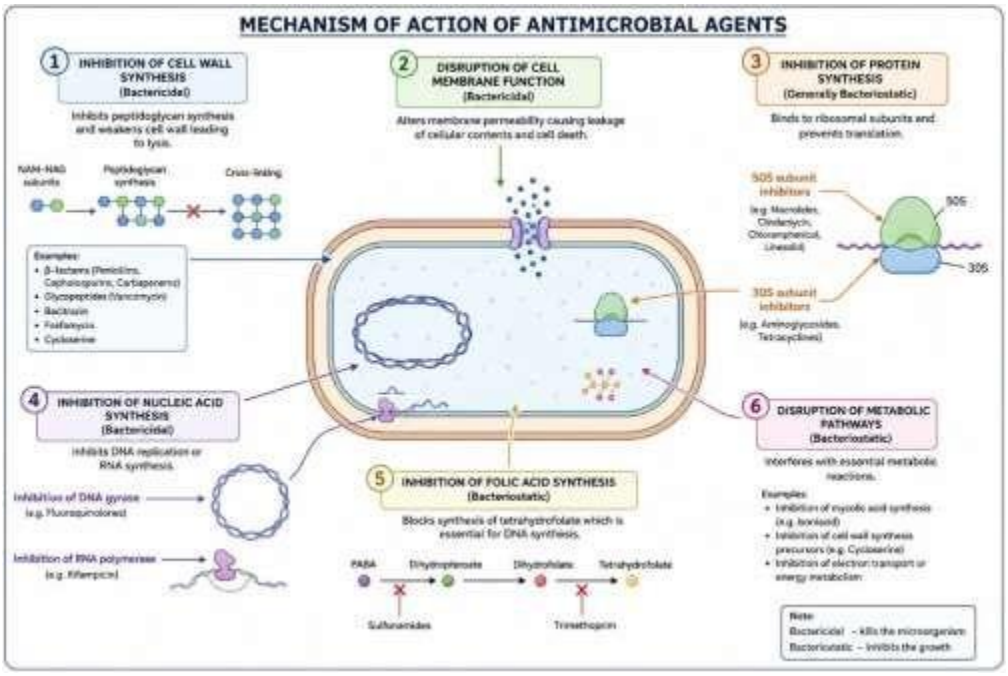
4.5 NMR (1D and 2D)

One-dimensional (1D) and two-dimensional (2D) Nuclear Magnetic Resonance (NMR) spectroscopy are essential tools for establishing the exact stereochemical and structural blueprints of bioactive plant secondary metabolites that relate to their downstream antimicrobial activity. For medicinal flora, especially the most dominant genera in the families, e.g., Lamiaceae, the intensive NMR profiling (including BB, APT, and DEPT experiments) allows for systematically deciphering complex polycyclic carbon skeletons such as abietanes, neo-clerodanes, ent-kaurane diterpenes, as well as structurally diverse flavonoids and phenolics. For instance, 1D DEPT experiments are routinely used to map total carbon budgets and to resolve specific functions, for example, between the methyl, methylene, and methine domains in highly customized isopropyl or functionalised ring systems. Advanced 2D NMR allows determination of homonuclear and heteronuclear connectivity evidence and is essential when complex structural rearrangements or ring-cleavages (such as formation of 4,5-seco-5,10-friedo-abietanes) occur in structural architectures encountered in unique antimicrobial isolates [31]. Homonuclear correlation spectroscopy (COSY) can map direct vicinal and long-range scalar proton interactions, and heteronuclear single quantum coherence/heteronuclear multiple quantum coherence (HSQC/HMQC) and heteronuclear correlation (HETCOR) can correlate protons directly with their corresponding carbons. Natural product chemists use heteronuclear multiple bond correlation (HMBC), FLOCK, or selective insensitive nuclei enhanced by polarisation transfer (SINEPT/INEPT) sequences to detect long-range (two- or three-

bond-away) heteronuclear connectivities in space and bond to precisely determine the connectivity of important biological functional groups to macrocycles. Lastly, the nearest proximity and the absolute stereochemistry of chiral centers in these antimicrobial complexes are finally mapped by the difference of Nuclear Overhauser Effect spectroscopy (NOE/NOESY). This method combines these ultra-resolving 1D and 2D NMR spectra with additional biochemical data from mass spectrometry, providing a rigorously validated chemical profile that connects an accurate structural configuration to a plant's defensive biochemical properties against micro-pathological lines [32].

The mechanism of antimicrobial action :

Understanding these mechanisms helps assess the antimicrobial activity of phytochemicals and/or design effective antimicrobial treatments. The antimicrobial activity of bioactive compounds from Indian medicinal plants has multiple targets and pathways, making it difficult for resistance to develop against them. They can disrupt microbial cell walls and cell membranes, inhibit nucleic acid and protein synthesis, interfere with essential metabolic processes, cause oxidative stress, inhibit efflux pump activity and prevent biofilm formation. They work in complex ways, making them very effective against a broad spectrum of bacteria, fungi, and MDR pathogens. Understanding of such mechanisms can be used for drug candidate identification, optimization of their therapeutic activity, and for the development of new plant-based antimicrobial products to fight AMR [33].



5.1 Cell Wall Disruption

The study showed that, primarily, the antimicrobial activity of the vine tea extract (VTE) and its main bioactive compound, dihydromyricetin (DMY), is linked to disruption of the bacterial cell wall and cell membrane homeostasis in Staphylococcus aureus. Proteomic analysis of differentially expressed proteins in both the DMY-treated and VTE-treated groups showed that most proteins were associated with pathways known to be involved in cell wall and membrane biology. Proteins involved in the degradation of fatty acids, glyceride metabolism, and cell wall synthesis were enhanced in DMY, while proteins involved in the acquisition of heme/iron and the degradation of the cell wall were enhanced in VTE. Lipidomic analysis also showed membrane lipid remodelling featuring increased unsaturation of fatty acids and decreased length of acyl chains. Furthermore, treatment with DMY and VTE resulted in significantly decreased levels of bacterial terpenol lipids, which are essential in cell wall biosynthesis. It was hypothesized that decreased terpenol levels would slow the transmembrane transport of Park nucleotide precursors and hamper glycan incorporation into cell walls at expansion sites, which would, in turn, slow cell wall synthesis and reduce bacterial growth. Together, the results showed that both DMY and VTE have antibacterial effects through

membrane-lipid remodelling, altered cell wall/cell membrane homeostasis, disruption of cell wall biosynthesis, and ultimately by inhibiting the growth of S. aureus [34].

5.2 Cell Wall Inhibition

The study found that Lonicera japonica polyphenols exhibited antibacterial effects against Cronobacter sakazakii and caused significant changes in metabolic pathways involved in bacterial growth and survival. Metabolomic analysis revealed that polyphenols altered amino acid metabolism, carbohydrate metabolism, energy metabolism, nucleotide metabolism, and membrane-related processes. The authors reported that pathways responsible for maintaining cellular structure and physiological functions were targeted by polyphenol treatment, resulting in the suppression of bacterial growth. In addition, the study suggested that antimicrobial phytochemicals might disrupt key cellular processes, causing structural damage and inhibiting bacterial growth. These results agree with the use of polyphenols from plants as antimicrobial substances with the ability to change the basic functions essential for the survival of bacteria [35].

5.3 DNA Damage and Replication Interference

Gunderson and Segall (2005) examined the antimicrobial activity of peptides that trap the Holliday structure and showed that the antimicrobial activity of these peptides correlates with the accumulation of DNA damage and the disruption of DNA repair processes. The authors measured DNA damage (nicks, gaps, stalled or collapsed replication forks, and DSBs) using the TUNEL assay, which detects free 3' hydroxyl DNA ends. DNA damage increased with increasing peptide doses, particularly in peptide-treated bacterial cells, as shown by increasing numbers of TUNEL-labeled cells. Time-course experiments also showed that after peptide treatment, DNA damage accumulated over time, suggesting interference with DNA maintenance mechanisms. The study also revealed that peptide treatment induced the bacterial SOS response, a characteristic of DNA damage stress. Additionally, the peptides showed synergistic effects with ultraviolet (UV) radiation and mitomycin C, DNA-damaging agents, suggesting that peptides could interfere with DNA repair/replication related processes. The authors showed that trapping Holliday junctions and branched DNA intermediates blocks normal DNA repair pathways, resulting in DNA break accumulation, replication fork collapse, inhibition of DNA replication, and, ultimately, bacterial cell death [36].

5.4 Protein Synthesis Inhibition

The study showed that the antimicrobial peptide B7-005 has antibacterial activity by inhibiting bacterial protein synthesis. The in vitro Protein Synthesis assay using purified ribosomes from the ESKAPE pathogens and Escherichia coli indicated that B7-005 was active against all the tested bacteria, with varying levels of inhibition among the organisms. Further, toe-printing analysis showed that B7-005 inhibits the initiation-to-elongation transition of the ribosome in the early phase of ribosome-mediated protein biosynthesis. According to the authors, this is akin to interaction with B7-005 in the ribosome exit tunnel, which leads to inhibition of bacterial translation. E. coli and Klebsiella pneumoniae are the most inhibited bacteria and the most susceptible to the peptide. The results indicated that protein synthesis is one of the most important mechanisms involved in the antibacterial activity of B7-005 and can be its main mechanism of action or act synergistically with membrane permeabilization, depending on the bacterial species studied [37].

5.6 Efflux Pump Inhibition

The resistance-modifying ability of the hydrazone HDZH1,4BENZ was investigated for S. aureus strains with the efflux pumps NorA and MepA. The compound showed no inherent antibacterial potency (MIC > 1024 μg/mL) but was a potent inhibitor of bacterial efflux systems and boosted antibiotic activity. The authors found that efflux pumps are part of bacterial resistance mechanisms, which act to lower the amount of antibiotics and other toxic substances inside bacteria and actively expel those substances out of cells. In modulation assays, HDZH1,4BENZ decreased the MICs of ethidium bromide, norfloxacin, and ciprofloxacin in efflux pump-expressing strains, suggesting inhibition of efflux activity and enhanced intracellular accumulation of antimicrobial compounds. The hydrazone showed activity against two efflux pumps, the NorA efflux pump, which belongs to the Major Facilitator Superfamily (MFS), and the MepA efflux pump, which falls into the Multidrug and Toxic Compound Extrusion (MATE) category. The molecular docking results also indicated a high affinity of the hydrazone towards the MepA transporter with a binding energy of −8.1 kcal/mol, exceeding that of chlorpromazine and the other reference compounds. The authors concluded that HDZH1,4BENZ was an efflux pump inhibitor that could restore antimicrobial susceptibility by blocking the efflux of such antimicrobial agents from bacterial cells [38].

5.7 Biofilm Disruption

Mechanism of disruption and eradication

Previous studies have shown that antimicrobial and host defence peptides (HDPs) have strong antibiofilm activity by disrupting biofilm formation, maintenance, and survival. One of the pathways identified by the authors is the interaction with the bacterial signalling nucleotide (p)ppGpp that regulates the stringent stress response, an important pathway in biofilm development. It has been demonstrated that the antibiofilm peptide IDR-1018 selectively binds to ppGpp, which results in its degradation inside bacterial cells. This interaction inhibited the bacterial stress response, leading to the prevention, eradication, and dispersal of preformed biofilms. The review also highlighted that HDPs could interact with different biofilm components, such as nucleotides, proteins, and other cellular targets related to biofilm stability and persistence, which could break them up. The authors have found targeting the intracellular signaling pathways involved in biofilm regulation to be a feasible approach towards controlling and eradicating biofilms [39].

6. ANTIMICROBIAL ACTIVITY OF INDIAN MEDICINAL PLANTS:

Being used in many traditional medicines for centuries, Indian medicinal plants are known as valuable sources of antimicrobial agents and have been used in traditional medicine to treat infectious diseases. Based on the presence of various bioactive phytochemicals, including alkaloids, flavonoids, phenolics, tannins, terpenoids, coumarins, saponins, and essential oils, these plants are considered to have antimicrobial properties. The compounds are found to possess inhibitory activity against different pathogenic microorganisms like Gram-positive and Gram-negative bacteria, fungi, and drug-resistant microorganisms. Phytochemicals derived from medicinal plants have various modes of action, such as disrupting microbial cell membranes, inhibiting nucleic acid and protein synthesis, interfering with metabolic pathways, inhibiting efflux pumps, and inhibiting microbial biofilms. Because of their broad-spectrum antimicrobial activity, potential low toxicity, and ability to break resistance in multidrug-resistant infections, medicinal plants in India have played an important role in discovering new drugs for centuries [40].

6.1 Plant-Derived Antimicrobial Agents

Both Andrographis paniculata and Jatropha curcas showed high antibacterial activity against the clinical isolates of Klebsiella pneumoniae and Klebsiella quasipneumoniae in the study. The authors reported that the antimicrobial activities of these medicinal plants are linked to bioactive phytochemicals obtained by extraction with different solvents. Methanol extracts showed the highest antibacterial activity and were also found to extract the flavonoids, phenolic compounds, and diterpenoids (andrographolide) efficiently [41]. The authors suggest that these phytochemicals have antimicrobial action in several ways, such as disrupting bacterial membranes, inhibiting nucleic acid synthesis, affecting quorum sensing systems, and suppressing bacterial biofilm formation. Moreover, the activity observed in hexane extracts indicated that the terpenoids and essential oils may also play a role in the antibacterial activity by disrupting membrane integrity and permeability. The study also reported that the antibacterial activity of A. paniculata was generally higher than that of J. curcas, possibly due to the presence of andrographolide and other related diterpenes with activity against multidrug-resistant Gram-negative bacteria [42].

6.2 MIC-Based Comparative Evaluation

The study compared the antimicrobial activity of selected medicinal plant extracts and showed that there are differences in the antimicrobial effects between the different plant species and solvent extraction. The authors reported that antimicrobial activity is affected by plant species, extraction method, solvent type, and the microorganism tested. Filipendula vulgaris and Lythrum salicaria were the species with the best antimicrobial activity among those studied, and phenolic compounds and flavonoids were determined as the main constituents responsible for antimicrobial activity. Comparative MIC data from the literature showed that L. salicaria had significant antibacterial activity against various microorganisms such as Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, and Salmonella enteritidis, with reported MIC ranges from 0.625 to 5 mg/mL depending on the microorganism. Teucrium chamaedrys and Teucrium montanum, however, exhibited inconsistent antimicrobial properties in various experiments; the MIC varied depending on the extract and the microorganism. The authors also noted that Gram-positive bacteria were more susceptible than Gram-negative bacteria, which they attributed to differences in bacterial cell-wall structure [43].

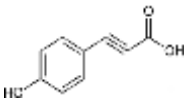
6.3 Pathogen Coverage Spectrum

The study established the wide range of antimicrobial activity of medicinal plants against Gram-positive and Gram-negative bacteria, with the susceptibility level differences noted between bacteria and medicinal plant species. The antimicrobial activity of extracts was mainly attributed to phenolic compounds and flavonoids, particularly in Filipendula vulgaris and Lythrum salicaria, with the highest antimicrobial potential, the authors reported. The extracts from the plants investigated were tested against some clinically important bacteria: Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes, Salmonella Typhimurium, Pseudomonas aeruginosa, and other bacteria as reported by past researchers. L. salicaria had the widest spectrum of pathogen activity, with the ability to inhibit Micrococcus lysodeikticus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus, Salmonella enteritidis, and Salmonella Typhimurium, as well as several fungal species [44]. The authors also observed that Gram-positive bacteria tended to be more susceptible than Gram-negative bacteria, which they attributed to differences in cell wall structure and permeability. Overall, the results suggest that extracts from these medicinal plants are active against various pathogenic microorganisms, indicating broad-spectrum antimicrobial activity [45].

7. DEVELOPMENT OF RESISTANCE TO ANTIBIOTICS

Antibiotic resistance is the ability of bacteria to survive and grow in the presence of an antibiotic that would normally kill them or stop their growth. Activity against Drug-resistant pathogens and antibiotic resistance is given in Table 1.2

Table 1.2 Activity against Drug-resistant pathogens

Resistant Pathogen	Active Phytochemical	Plant name	References
MRSA	Berberine 	Berberis aristata (common plant source of berberine)	Gorniak et al., 2019; Cushine & Lamb, 2005
ESBL-producing E. coli	ZnO nanoparticles	Phoenix dactylifera (root extract)	Naser et al., 2021
MDR P. aeruginosa	Plant extracts	Cave-derived Streptomyces/actinobacteria	Rai et al., 2014
Acinetobacter baumannii	p-Coumaric acid 	Cinnamic acid rich medicinal plants	Attia et al., 2026
Candida auris	Terpenoids	Essential oil bearing medicinal plants	Santana et al. / relevant C. auris terpenoid study

7.1 MRSA

The antimicrobial activity of $Fe(tropo)_3$ was examined against drug-resistant strains of S. aureus, such as methicillin-resistant S. aureus (MRSA), vancomycin-intermediate S. aureus (VISA), and mupirocin- and fusidic acid-resistant strains. The authors reported that $Fe(tropo)_3$ showed strong antibacterial activity against susceptible and resistant strains of S. aureus and inhibited

MRSA growth. The compound also showed activity against MRSA, which is resistant to widely used topical antibiotics. Moreover, $Fe(tropo)_3$ was significantly effective at reducing bacterial burden in a murine wound infection model using the Xen36 strain of S. aureus, suggesting its therapeutic potential against resistant staphylococcal infections. The antibacterial effect of $Fe(tropo)_3$ has been correlated to ROS generation inside the bacteria, causing the death of the cell, according to the authors. The study found that $Fe(tropo)_3$ is a promising antimicrobial drug that could be used to treat infections caused by MRSA and other antibiotic-resistant bacteria of the genus S. aureus [46].

7.2 ESBL-Producing Bacteria

The antimicrobial properties of the green-synthesized zinc oxide nanoparticles (ZnO-NPs) synthesized using Phoenix dactylifera root extract were assessed for various clinically important and MDRpathogens. Previous studies havefound ZnO-NPs to have a wide-spectrum effect on both Gram-positive and Gram-negative bacteria, including resistant ones. Specifically, ZnO-NPs were effective against extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae (and so were active against carbapenem-resistant P. aeruginosa (CRPA). Disc diffusion assays showed an inhibition zone diameter of ~19.6 mm for ESBL-negative strains of E. coli and K. pneumoniae, and MIC analysis showed that resistant strains such as P. aeruginosa needed a higher concentration to inhibit. P. aeruginosa with CRPS had an MIC of 310 μg/mL, while lower MIC values were noted for the more susceptible organisms. Based on the results, the authors suggested that ZnO-NPs have an excellent antibacterial potential against drug-resistant pathogens and could be used as an alternative antimicrobial agent in the era of antimicrobial resistance [47].

7.3 Resistant Pseudomonas aeruginosa

The study aimed to explore the antimicrobial properties of actinobacteria isolated from the caves against multidrug-resistant (MDR) pathogens such as (MDR) P. aeruginosa , which are clinically important. According to the authors, some of the actinobacterial isolates showed good antibacterial activity against MDR bacterial strains. MDR P. aeruginosa was selected as one of the tested pathogens as it is a clinically important pathogen and is resistant to multiple antibiotics. Screening showed that selected cave actinobacteria produced bioactive metabolites with inhibitory activity against the growth of MDR pathogens, including P. aeruginosa. The active isolates were molecularly identified, and most were of the genus Streptomyces, a well-known producer of antimicrobial products [48].

7.4 Acinetobacter baumannii

The activity of p-coumaric acid (p-CA) against imipenem-resistant extensively drug-resistant (IXDRAb) isolates obtained from cancer patients. The authors reported that all the isolates were carbapenem-resistant and had a strong ability to form biofilms, and that p-Coumaric acid had measurable antibacterial activity against IXDRAb and, more importantly, potentiated the activity of imipenem. Treatment with subinhibitory concentrations of p-CA significantly decreased the imipenem MIC in almost all resistant strains, suggesting resistance modulation. In addition to its antibacterial activity, p-CA was also able to significantly inhibit the growth of biofilms, and the established biofilm biomass was reduced, which was correlated with decreased extracellular polymeric substance (EPS) production, cell-surface hydrophobicity, and metabolic activity. Further, gene expression analysis showed that key genes involved in biofilm production, such as bfmR, abaI, bap, csuE, and pgaB, were downregulated, suggesting a multitarget effect against biofilm production. The in vivo pneumonia model results confirmed the in vitro findings: the combination reduced bacterial load in the lungs, histopathology showed a better outcome, and survival improved compared with imipenem alone. The authors recommend that p-coumaric acid could be a natural phenolic adjuvant with added value as an antimicrobial resistance and biofilm inhibitor in the case of XDR-AB [49].

7.5 Candida auris

The considerable ability of C. auris to develop pan-antifungal resistance strongly underscores the need for new therapeutic avenues, as specific and acquired molecular mutations are seen across all major classes of antifungal drugs. Target site mutations of the ERG11 gene, such as Y132F and K143R, which occur in many genes across the different clades, commonly cause conventional triazole resistance by altering the structure of important hydrogen-bonding networks near the catalytic pocket, in addition to the upregulation of ATP-binding cassette (ABC) transporters, such as Cdr [50]. From this perspective, natural extracts and phytocompounds appear to represent a promising paradigm based on multiple targets, as they can overcome such evolutionary adaptations. Plant secondary metabolites, including terpenoids, phenolics, and EOs, exert broad-spectrum

pressure on the fungal cell, as opposed to monotherapeutic synthetic drugs, which require specific active pocket coordination. These natural agents physically intercalate into the plasma membrane, disrupting lipid homeostasis and increasing intracellular reactive oxygen species (ROS) accumulation; importantly, they also inhibit or downregulate the efflux systems responsible for clinical isolates' fluconazole resistance. Using multi-component botanical complexes instead of single-target therapeutics targets different physiological vulnerabilities of C. auris and provides a potential avenue to overcome structural protection against standard antifungal treatments in current strains [51].

8. CHALLENGES AND LIMITATIONS

Though Indian medicinal plants hold great promise as a source of novel antimicrobial agents, a number of challenges and limitations remain in the development and clinical application of such plants. The major challenge is the lack of standardization of the plant collection, authentication, extraction methods, identification of phytochemicals, and antimicrobial testing methods. There are many different extraction solvents, extraction methods, parts of the plant used, and assay methods, which often lead to variable results and comparisons between studies. Moreover, the phytochemical content of medicinal plants can also vary significantly depending on geographical location, climate conditions, soil properties, plant maturity, harvesting season, and storage conditions. These batch-to-batch variations affect the concentrations of bioactive compounds and may have a major impact on antimicrobial activity and reproducibility [52].

One of the most significant drawbacks is the lack of assessment of toxicity and safety. While many medicinal plants have been used for centuries, toxicological studies are usually limited, and comprehensive studies are lacking; the majority of toxicological investigations have been targeted towards in vitro antimicrobial properties. Their progression towards clinical use is still limited due to a lack of information on acute toxicity, chronic toxicity, genotoxicity, pharmacokinetics, and long-term safety. Moreover, antimicrobial activity and structure–activity relationships (SARs) of many phytochemicals are not completely understood, limiting the optimization of their biological activity and the prediction of therapeutic activity. Some antimicrobial compounds from plants also exhibit low bioavailability, are rapidly metabolized, and have low aqueous solubility, limiting their therapeutic potential [53]. There are also challenges to the large-scale production and commercialisation of phytochemicals. Low natural abundance of active compounds, seasonal availability of plant materials, long cultivation time, and extraction, purification, and quality control issues can make industrial-scale production complex. Furthermore, a consistent phytochemical profile and biological activity are a big challenge. The available clinical evidence of efficacy for plant-based antimicrobial agents is also poor, with the majority of evidence available being based on laboratory studies and animal models, with well-designed clinical trials in humans being relatively scarce. Regulatory restrictions also pose a challenge for developing such products; regulatory procedures for herbal medicines and plant-based antimicrobial products differ between countries, and the lack of standardized guidelines for quality control, efficacy evaluation, safety assessment, and product registration is a restriction in the way of commercialization and clinical use [54]. Another concern is the possibility of herb–drug interactions that could affect the metabolism, effectiveness, or safety of a standard antimicrobial agent, as well as other drugs. But these interactions are not yet well-studied. Lastly, plant-based antimicrobials act on multiple cellular targets and are not as likely to develop resistance as traditional antibiotics, but there is the potential for microbial adaptation and resistance emergence. Hence, ongoing surveillance, research on mechanisms of action, and long-term trials are needed to assess the long-term efficacy of natural products against antimicrobial resistance [55].

9. FUTURE PERSPECTIVES

Mechanism-based and translational approaches are the future direction of antimicrobial research from Indian medicinal plants, by moving away from the traditional approach of antimicrobial screening. The identification of specific microbial targets and elucidation of molecular mechanisms of antimicrobial activity of phytochemicals will be facilitated by phytochemical characterization, metabolomics, proteomics, and molecular docking. Special focus should be given to structure-activity relationship (SAR) studies to enhance the antimicrobial activity of flavonoids, alkaloids, terpenoids, coumarins, and other bioactive phytochemicals. Furthermore, the ability of many phytochemicals to inhibit efflux pumps, disrupt biofilms, and resensitize the multidrug-resistant bacteria suggests that such compounds may be used as adjuvants to combination therapy. A new delivery system has been invented using nanotechnology that can help stabilize, enhance bioavailability, and target the delivery of plant-based antimicrobial agents, including nanoparticles, nano-emulsions, hydrogels, and electrospun nanofibers. With the ability of green extraction techniques, powerful analytical tools such as LC-MS, UPLC-QTOF-MS, and NMR, artificial intelligence, and machine-learning tools, the process of identification and development of new antimicrobial leads from the

vast medicinal plant diversity of India could be accelerated. In vivo validation, toxicity studies, pharmacokinetic analysis, and clinical trials should be emphasized in future studies, aiming to close the gap between laboratory results and clinical use in the future. These multi-disciplinary strategies may be the key to revolutionizing the use of medicinal plant-based phytochemicals as effective and sustainable solutions to address the global issue of antimicrobial resistance and the growing public health challenge of infectious diseases.

10. CONCLUSION

Antimicrobial resistance has become a growing threat that necessitates the development of novel therapeutic approaches that do not involve conventional antibiotics. Indian medicinal plants provide an exceptional source of a wide variety of phytochemicals with diverse chemical structures and have broad-spectrum antimicrobial activity with several modes of action; hence, the development of resistance is minimized. The development of phytochemical isolation, analytical characterization and structure–activity relationship studies has greatly aided understanding of the therapeutic potential of plant-derived chemicals. Modern analytical techniques such as GC–MS, LC–MS, UPLC–QTOF–MS, FTIR, and NMR spectroscopy can extensively characterize bioactive components and identify antimicrobial leads. In addition, phytochemicals have antimicrobial properties such as membrane disruption, inhibition of nucleic acid synthesis, inhibition of protein synthesis, inhibition of efflux pumps, induction of oxidative stress, and suppression of biofilms, thereby holding potential for multidrug-resistant organisms.

The advent of novel nanotechnology-based formulations, green extraction techniques, metabolomics, and the use of artificial intelligence in drug discovery has opened avenues for improved effectiveness, bioavailability, and clinical potential of plant-derived antimicrobials. However, considerable challenges remain, including standardizing extraction procedures, ensuring reproducible phytochemical profiling, conducting comprehensive toxicological assessments, conducting pharmacokinetic investigations, and designing clinical trials. A multidisciplinary approach will be crucial to overcome these challenges and translate laboratory discoveries into clinical therapies. In summary, Indian medicinal plants have a tremendous scope for being a sustainable source of next-generation antimicrobial agents and provide a promising approach towards combating the global problem of antimicrobial resistance and advancing pharmaceutical innovation for the future.

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AUTHOR CONTRIBUTIONS

Payal Chauhan: Conceptualization, Investigation.

Dr. Bal Krishan: Methodology, Laboratory Analysis, Supervision, Writing-Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Ethics Statement

Ethical approval was not required for this study.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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