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ANTIMICROBIAL ACTIVITY OF PIPER NIGRUM AND SOLANUM NIGRUM AND THEIR SYNERGISTIC EFFECT AGAINST PSEUDOMONAS AERUGINOSA AND PROTEUS VULGARIS

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ABSTRACT

The escalating prevalence of antimicrobial resistance (AMR) among pathogenic bacteria such as *Pseudomonas aeruginosa* and *Proteus vulgaris* has prompted a renewed focus on plant-derived antimicrobials as potential therapeutic alternatives. This review critically evaluates the antimicrobial activity of two widely used medicinal plants, *Piper nigrum* which is also known as black pepper and *Solanum nigrum* that goes by the common name of black nightshade, with a specific emphasis on their individual and synergistic effects against *P. aeruginosa* and *P. vulgaris*. Both plants are rich in bioactive phytochemicals such as piperine, alkaloids, flavonoids, and glycoalkaloids, which have demonstrated antimicrobial efficacy through diverse mechanisms including disruption of bacterial membranes, inhibition of protein and DNA synthesis, and induction of oxidative stress. The literature surveyed includes in vitro studies employing various extracts—aqueous, ethanolic, methanolic, and essential oils—evaluated for minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), and zones of inhibition. Furthermore, evidence supporting synergistic interactions between *P. nigrum* and *S. nigrum* is discussed, highlighting enhanced antibacterial activity when used in combination, as indicated by fractional inhibitory concentration (FIC) indices and checkerboard assays. Despite promising results, the translation of these findings into clinical practice is limited by a lack of in vivo and toxicological studies. This review underscores the potential of these plants as complementary agents in antimicrobial therapy, particularly against multidrug-resistant pathogens, and advocates for further pharmacological and clinical investigations to validate their efficacy and safety. The synergistic approach involving phytocompounds offers a promising avenue for novel antimicrobial drug development.

KEYWORDS: Piper nigrum, Solanum nigrum, Antimicrobial activity, Pseudomonas aeruginosa, Proteus vulgaris, synergistic effect, phytochemicals.

1. INTRODUCTION

One of the most urgent problems facing contemporary medicine is the rising worldwide health danger posed by antimicrobial resistance (AMR). The extensive and often improper use of antibiotics has resulted in the creation of multidrug-resistant (MDR) bacterial strains, therefore greatly complicating the treatment of infectious illnesses (Ventola, 2015). Among the notorious MDR pathogens, Pseudomonas aeruginosa and Proteus vulgaris are particularly concerning due to their intrinsic resistance mechanisms and adaptive capabilities, which allow them to survive in hospital environments and resist most conventional antibiotics (Santajit & Indrawattana, 2016). Usually affecting immunocompromised people, P. aeruginosa is a Gram-negative, opportunistic bacterium often linked with nosocomial infections. Reduced outer membrane permeability, efflux pumps, and the synthesis of β-lactamases (Lister et al., 2009) comprise its resistance strategies. Comparably, P. vulgaris, a member of the Enterobacteriaceae family, shows resistance to many medication classes, including aminoglycosides and cephalosporins (Sood & Gupta, 2012) and is routinely linked with urinary tract infections and wound infections. The ongoing increase in diseases brought on by these pathogens has sparked once again interest in the investigation of substitute antimicrobial compounds, especially those produced from medicinal plants.

For millennia, plants have been a classic source of medicinal medicines; modern pharmacological research has shown their ability to produce bioactive molecules with antibacterial, antioxidant, and anti-inflammatory effects. Among the different medicinal plants, *Piper nigrum* which is also known as black pepper and

Solanum nigrum that goes by the common name of black nightshade have received substantial interest owing to their distinct phytochemical profiles and purported antibacterial activities.

Belonging to the Piperaceae family, Piper nigrum is extensively used in traditional medicine all throughout Asia and has a broad spectrum of biological actions including antibacterial, antifungal, and anti-inflammatory properties. The major bioactive ingredient, piperine, together with other alkaloids and essential oils, has been demonstrated to break bacterial cell walls, hinder DNA replication, and interfere with quorum sensing in pathogenic bacteria (Gorgani et al., 2017; Srinivasan, 2007). Various in vitro investigations have revealed the efficiency of P. nigrum extracts against Gram-negative pathogens including P. aeruginosa, indicating its potential as a therapeutic adjuvant (Manoharan et al., 2022).

Usually used in Ayurvedic and Chinese medicine for its hepatoprotective, anti-inflammatory, and antibacterial properties, Solanum nigrum is a member of the Solanaceae family. It includes various essential phytochemicals, including solanine, solasodine, flavonoids, and phenolic compounds, which contribute to its antibacterial effect. These molecules are well-known to produce oxidative stress, denaturation of proteins, and bacterial membrane permeability increase, therefore compromising bacterial viability (Kumar et al., 2020).

In recent years, research has increasingly concentrated on examining the synergistic effects of plant-derived chemicals. Synergy occurs when the combined action of two agents provides a higher impact than the sum of their separate effects. This idea is especially relevant in antimicrobial treatment, since synergistic combinations may lower the minimum inhibitory concentration (MIC) of each antibiotic, limit toxicity, and avoid the establishment of resistance (Wagner & Ulrich-Merzenich, 2009). Studies combining P. nigrum and S. nigrum extracts have demonstrated potential synergistic effects against resistant strains of P. aeruginosa and P. vulgaris, generally displaying greater zones of inhibition and lower MIC values when compared to solo extracts.

Given the critical need for innovative antimicrobial methods and the encouraging early evidence on the combined effectiveness of Piper nigrum and Solanum nigrum, a complete study of their antibacterial potential is relevant and necessary. This study attempts to outline the phytochemical contents, modes of action, and antibacterial activities of these plants, with a special focus on their synergistic interactions against P. aeruginosa and P. vulgaris. By critically assessing current in vitro and in vivo investigations, this report intends to emphasize the therapeutic potential of these botanicals in treating AMR and suggest future research objectives to allow their clinical implementation.

2. OVERVIEW OF THE PLANTS

2.1. *Piper nigrum* (Black Pepper)

Piper nigrum L., usually referred to as black pepper, is a perennial climbing vine belonging to the family Piperaceae. It is endemic to the Western Ghats of India and extensively farmed in tropical places throughout Asia, including Vietnam, Indonesia, and Sri Lanka (Parthasarathy et al., 2008). The plant produces little spherical fruits called as peppercorns, which are picked at different ripening stages and processed to provide black, white, or green pepper. These forms are not just culinary classics but also fundamental to traditional medicinal systems. Phytochemically, P. nigrum is a rich source of alkaloids, essential oils, and phenolic compounds. The principal bioactive alkaloid is piperine, which adds to the pungency of black pepper and contains around 5–9% of the dried fruit (Gorgani et al., 2017). Piperine demonstrates numerous pharmacological effects including antibacterial, antioxidant, anti-inflammatory, and bioenhancing actions. In addition, the essential oil fraction includes sabinene, β-caryophyllene, limonene, and pinene, which are known for their antibacterial properties against a broad range of pathogens (Dorman & Deans, 2000).

Figure 1: Depiction of *Piuper nigrum.*

Traditionally, P. nigrum has been utilized in Ayurvedic and Unani medicine to handle respiratory diseases, gastrointestinal troubles, and as a general health tonic. It also serves as a bioavailability enhancer by blocking drug-metabolizing enzymes and improving gastrointestinal absorption of co-administered medicines (Khajuria et al., 2002). Recent microbiological investigations have showed that piperine and P. nigrum extracts display substantial antibacterial activity against Gram-negative pathogens, including Pseudomonas aeruginosa, suggesting their potential as supplementary antimicrobials (Manoharan et al., 2022).

2.2. *Solanum nigrum* (Black Nightshade)

Solanum nigrum L., often known as black nightshade, belongs to the Solanaceae family and is extensively dispersed over Asia, Africa, and Europe. It is an annual or short-lived perennial plant that grows up to 1 meter in height and has little white flowers and globular, purplish-black berries. It is historically utilized in several indigenous systems of medicine, including Ayurveda, Siddha, and Traditional Chinese Medicine (Ghosh & Maiti, 2021). The plant is recognized for its rich composition of bioactive secondary metabolites, including glycoalkaloids such as solanine and solasodine, which demonstrate powerful antibacterial and cytotoxic properties (Chandra et al., 2012).

Figure 2: Depiction of *Solanum nigrum.*

Other ingredients include flavonoids, tannins, saponins, and phenolic acids, which together contribute to its pharmacological actions such as hepatoprotection, anti-inflammatory activity, and antibacterial effectiveness (Kumar et al., 2020). Solanum nigrum has been historically used for treating liver disorders, ulcers, asthma, and skin illnesses. Its fresh leaf juice is widely eaten to ease symptoms of fever, urinary tract infections, and inflammation. The antibacterial activity of its aqueous and ethanolic extracts has been established against numerous bacterial strains, including P. aeruginosa and P. vulgaris (Raghavendra et al., 2016). The various phytoconstituents of S. nigrum are considered to damage microbial membranes, inhibit enzymatic systems, and promote oxidative stress inside bacterial cells, leading to microbial death. Collectively, Piper nigrum and Solanum nigrum provide a rich mix of phytochemicals with documented ethnomedicinal significance and shown antibacterial action. Their synergistic potential against drug-resistant bacterial strains needs further exploration in the context of innovative phytotherapeutic development.

Table 1: Classification of phytochemical with their biological activities.

Plant Name	Phytochemical	Class	Biological Activities
Piper nigrum	Piperine	Alkaloid	Antimicrobial, anti-inflammatory, antioxidant, bioenhancer
	β-Caryophyllene	Sesquiterpene	Anti-inflammatory, antimicrobial
	Sabinene	Monoterpene	Antibacterial, antifungal
	Limonene	Monoterpene	Antioxidant, antimicrobial
	Flavonoids	Polyphenols	Antioxidant, antibacterial
	Tannins	Polyphenols	Antibacterial, astringent
	Essential oils	Volatile compounds	Antimicrobial, digestive stimulant
Solanum nigrum	Solanine	Glycoalkaloid	Antimicrobial, cytotoxic
	Solasodine	Glycoalkaloid	Antimicrobial, anti-inflammatory
	Flavonoids (e.g., quercetin)	Polyphenols	Antioxidant, anti-inflammatory, antimicrobial
	Tannins	Polyphenols	Antibacterial, antifungal
	Saponins	Glycosides	Antimicrobial, antifungal, cholesterol-lowering
	Phenolic acids	Phenolics	Antioxidant, antibacterial
	Ascorbic acid (Vitamin C)	Antioxidant	Immune-boosting, antimicrobial synergy

3. Mechanisms of Antimicrobial Action

The antibacterial effectiveness of plant-based bioactive chemicals originates from their capacity to target many locations inside microbial cells, therefore minimizing the likelihood of resistance development. Phytochemicals in *Piper nigrum* which is also known as black pepper and *Solanum nigrum* that goes by the common name of black nightshade display different modes of action that contribute to their Inhibitory effectiveness against multidrug-resistant (MDR) bacteria the likes of Pseudomonas aeruginosa and Proteus vulgaris.

3.1. Disruption of Cell Wall and Membrane Integrity

One of the key mechanisms of action is the destruction of the microbial cell wall or membrane integrity. Piper nigrum includes piperine and essential oils such as caryophyllene and sabinene, which have been demonstrated to intercalate into the lipid bilayer, producing enhanced permeability and loss of cytoplasmic contents. This breakdown leads to osmotic imbalance and ultimately cell death (Dorman & Deans, 2000; Ghoshal et al., 1996). Similarly, in Solanum nigrum, saponins and steroidal glycoalkaloids such as solanine and solasodine interact with sterol components of the membrane, altering its structure and promoting pore formation (Chanda & Rakholiya, 2011).

3.2. Inhibition of DNA and Protein Synthesis

Another significant antibacterial mechanism includes the suppression of nucleic acid and protein production.

Piperine has been shown to block topoisomerase I and II enzymes, interfering with DNA replication and transcription (Jayaraman et al., 2011). Moreover, S. nigrum flavonoids and alkaloids show antibacterial action via binding to ribosomal subunits, impeding translation and protein creation. This broad-spectrum effect disrupts important metabolic activities and decreases bacterial growth (Al-Snafi, 2015).

3.3. Induction of Oxidative Stress

Phytochemicals may also produce oxidative stress in microbial cells by creating reactive oxygen species (ROS). In P. nigrum, piperine and terpenoids have been demonstrated to boost intracellular ROS levels, damaging DNA, lipids, and proteins, therefore resulting to apoptosis-like cell death (Srinivasan, 2007). Similarly, S. nigrum polyphenolic chemicals induce ROS generation, affecting antioxidant defense systems in pathogens (Sharma et al., 2013). This pro-oxidant activity is particularly efficient against gram-negative bacteria with thicker peptidoglycan coatings, such as P. aeruginosa and P. vulgaris.

3.4. Evidence-Based Mechanistic Studies

Several in vitro and in vivo investigations corroborate these pathways. A research by Ghosh et al. (2011) indicated that ethanolic extracts of P. nigrum induced substantial membrane damage in E. coli and P. aeruginosa, validated using electron microscopy and propidium iodide uptake tests. Similarly, S. nigrum extracts have showed dose-dependent inhibition of DNA gyrase and elevated ROS production in Proteus spp., as indicated by DNA fragmentation tests (Naz & Bano, 2012). Collectively, these data show the diverse antimicrobial actions of Piper nigrum and Solanum nigrum, including membrane disruption, inhibition of biosynthetic processes, and oxidative damage. Such broad-spectrum, multi-target activities make these plants strong candidates for development into phytotherapeutic medicines, especially against resistant bacterial strains.4. Antimicrobial Resistance and Clinical Relevance of Target Pathogens.

3.5. Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative, rod-shaped opportunistic pathogen that is ubiquitous in the environment and a frequent cause of hospital-acquired infections, particularly in immunocompromised individuals. It is implicated in ventilator-associated pneumonia, urinary tract infections, bacteremia, and wound infections (Pang et al., 2019). This pathogen is notorious for its intrinsic resistance to multiple antibiotics due to a variety of mechanisms including efflux pumps (e.g., MexAB-OprM), decreased outer membrane permeability, and production of β-lactamases (Moradali et al., 2017). Furthermore, it can form biofilms on medical devices and host tissues, further protecting it from antibiotics and the host immune system. The World Health Organization (WHO) has listed carbapenem-resistant P. aeruginosa as a critical priority pathogen for the development of new antimicrobials (WHO, 2017).

3.6. Proteus vulgaris

Proteus vulgaris, another Gram-negative facultative anaerobe, belongs to the Enterobacteriaceae family. It is commonly found in soil, water, and the gastrointestinal tract. Although it is a part of the normal flora, it can act as an opportunistic pathogen, particularly in nosocomial settings. It is frequently associated with wound infections, and septicemia (Armbruster et al., 2018). P. vulgaris exhibits multiple resistance mechanisms such as extended-spectrum β-lactamase (ESBL) production, efflux pump activity, and aminoglycoside-modifying enzymes, which limit therapeutic options (Sastry et al., 2021).

3.7. Need for Plant-Based Alternatives

The global rise in antimicrobial resistance (AMR) necessitates the search for novel antimicrobials with different mechanisms of action. Plant-derived phytochemicals offer a promising avenue due to their structural diversity and multitarget potential. Many plant-based compounds, including alkaloids, flavonoids, and essential oils, have demonstrated antimicrobial effects via disruption of microbial membranes, inhibition of efflux pumps, and interference with nucleic acid synthesis (Cowan, 1999). Given the phytochemical richness of Piper nigrum and Solanum nigrum, their application against drug-resistant strains of P. aeruginosa and P. vulgaris is of high clinical relevance. Studies have increasingly demonstrated that combinations of plant extracts or isolated phytochemicals can exert synergistic effects when used together or in conjunction with antibiotics. Such synergy not only enhances antimicrobial efficacy but may also reduce the emergence of resistance and the required dosage of conventional drugs (Hemaiswarya et al., 2008). This forms the basis for investigating the combined antimicrobial potential of P. nigrum and S. nigrum against MDR pathogens.

Table 2: Key Phytochemicals of Piper nigrum and Solanum nigrum, Their Antimicrobial Mechanisms, and Target Organisms.

Plant Species	Major Phytochemicals	Proposed Antimicrobial Mechanisms	Target Microorganisms	References
Piper nigrum	Piperine, β-Caryophyllene, Sabinene	Inhibition of efflux pumps, membrane disruption, protein binding	P. aeruginosa, E. coli, S. aureus	Gorgani et al., 2017; Manoharan et al., 2022
	Limonene, Pinene, Flavonoids	ROS generation, inhibition of DNA gyrase	Proteus vulgaris, Klebsiella pneumoniae	Dorman & Deans, 2000
Solanum nigrum	Solanine, Solasodine	Membrane pore formation, enzyme inhibition, oxidative stress induction	P. aeruginosa, P. vulgaris, Salmonella spp.	Chandra et al., 2012; Raghavendra et al., 2016
	Flavonoids, Tannins, Saponins	Metal chelation, bacterial enzyme inactivation, anti-quorum sensing	E. coli, Bacillus subtilis, S. typhi	Kumar et al., 2020

5. Evidence of Synergistic Antimicrobial Activity of *Piper nigrum* and *Solanum nigrum*

Rationale for Synergism

The greater effect of a combination of drugs than the sum of the drugs is called synergism and this principle is particularly important in combating multidrug-resistant (MDR) organisms, where monotherapy often fails due to various resistance mechanisms. The combination of *Piper nigrum* and *Solanum nigrum* is hypothesized to be synergistic due to the complementary nature of their phytochemicals. While *P. nigrum* contains compounds like piperine and essential oils that disrupt bacterial membranes and inhibit efflux pumps, *S. nigrum* offers glycoalkaloids and flavonoids that enhance oxidative stress and inhibit quorum sensing (Manoharan et al., 2022; Raghavendra et al., 2016).

5.2. Experimental Studies Supporting Synergy

Several in vitro studies have demonstrated enhanced antimicrobial activity when plant extracts are used in combination. For instance, Shahid et al. (2021) observed a significant reduction in the minimum inhibitory concentration (MIC) values of both *P. nigrum* and *S. nigrum* extracts when tested in combination against *Pseudomonas aeruginosa*. Similarly, checkerboard assays conducted by Kumar et al. (2020) showed a fractional inhibitory concentration index (FICI) of <0.5, indicating strong synergism against *Proteus vulgaris*. Time-kill assays further confirmed enhanced bactericidal effects within 6–8 hours post-treatment.

5.3. Mechanistic Insights into Synergism

The observed synergy may result from.

- **Membrane permeabilization** by *P. nigrum* compounds that facilitates the intracellular entry of *S. nigrum* glycoalkaloids.
- **Combined inhibition of efflux mechanisms** and metabolic enzymes, leading to accumulation of toxic metabolites in bacteria.
- **Dual oxidative stress induction**, overwhelming bacterial antioxidant defenses.

  This multifactorial approach simultaneously disrupts various microbial pathways, thereby reducing the potential for resistance development and increasing the likelihood of effective pathogen clearance (Hemaiswarya et al., 2008).

5.4. Synergy with Conventional Antibiotics

Beyond the inter-plant synergy, both *P. nigrum* and *S. nigrum* have demonstrated potential in enhancing the efficacy of conventional antibiotics. Piperine, for instance, has been shown to restore the activity of ciprofloxacin and tetracycline against resistant strains of *P. aeruginosa* (Khajuria et al., 2002). The combination of phytochemicals with antibiotics may help lower therapeutic doses, reduce side effects, and delay the onset of resistance.

5.5. Implications for Phytotherapeutic Development

The documented synergistic interactions highlight the potential for developing standardized polyherbal formulations. Such combinations may serve as adjunct or alternative therapies, particularly for infections caused by MDR organisms such as *Pseudomonas aeruginosa* and *Proteus vulgaris*. Further validation through in vivo models and clinical trials is required before translation to human applications.

Experimental Approaches for Evaluating Antimicrobial and Synergistic Activity.

5.6. Extraction and Preparation of Plant Materials

The evaluation of antimicrobial activity begins with the collection, authentication, and processing of plant materials. Dried fruits of *Piper nigrum* and aerial parts (leaves and berries) of *Solanum nigrum* are commonly used. The plant material is dried under shade and ground into fine powder. Extraction is performed using solvents such as ethanol, methanol, or aqueous solutions via maceration or Soxhlet extraction (Duraipandiyan et al., 2006). The extracts are filtered and concentrated under reduced pressure using rotary evaporation, and stored at 4°C for further analysis.

5.7. Phytochemical Screening and Quantification

Qualitative and quantitative analysis of phytochemicals is crucial for standardization. Standard phytochemical screening methods are used to detect the presence of alkaloids, flavonoids, terpenoids, tannins, and glycoalkaloids (Harborne, 1998).

The techniques such as GC-MS, LC-MS and HPLC are employed to identify the compounds like piperine and solanine (Chandra et al., 2012).

5.8. Antimicrobial Activity Assays

The antimicrobial potential of individual and combined plant extracts is assessed against clinical and standard strains of *Pseudomonas aeruginosa* and *Proteus vulgaris*.

- **Agar well diffusion and disc diffusion methods** are used for preliminary screening to measure zones of inhibition (CLSI, 2023).
- **Minimum inhibitory concentration (MIC)** and **minimum bactericidal concentration (MBC)** are determined using the broth microdilution method.
- **Time-kill kinetics assays** assess bactericidal activity over time, indicating the rate and extent of microbial killing.

5.9. Synergistic Activity Evaluation

To evaluate synergy between plant extracts or with antibiotics.

- **Checkerboard assay** is widely used to determine the fractional inhibitory concentration index (FICI). A FICI ≤0.5 indicates synergism, >0.5–1.0 indicates additivity, and ≥2.0 indicates antagonism (Odds, 2003).
- **Time-kill synergy testing** monitors microbial counts at different time points after treatment with extract combinations.
- **Isobologram analysis** may also be used for advanced synergy assessment.

5.10. Biofilm Inhibition and Quorum Sensing Studies

Given the role of biofilms in antimicrobial resistance, the effects of the plant extracts on biofilm formation and quorum sensing are also investigated.

- **Crystal violet assay** quantifies biofilm biomass.
- **Microscopic imaging (e.g., SEM or CLSM)** visualizes structural changes in biofilms.
- **Reporter gene assays** and inhibition of signal molecules (e.g., AHLs) assess quorum sensing activity (Rutherford & Bassler, 2012).

5.11. Statistical Analysis

All experimental results are subjected to statistical analysis using software like SPSS or GraphPad Prism. Data are expressed as mean ± SD, and statistical significance is determined using ANOVA or t-tests, with $p < 0.05$ considered significant.

6. DISCUSSION AND FUTURE PERSPECTIVES

The rising frequency of antibiotic resistance among clinically relevant organisms such as Pseudomonas aeruginosa and Proteus vulgaris poses a critical public health problem. Conventional antibiotics are typically rendered ineffective by multidrug resistance mechanisms, including efflux pumps, biofilm development, and enzymatic degradation. In this context, plant-derived phytochemicals provide potential options, especially when employed in synergistic combinations. This study consolidates current information on the antibacterial activities of Piper nigrum and Solanum nigrum, emphasizing their individual and synergistic activity against resistant infections. The bioactive components of P. nigrum, especially piperine and essential oils, exert their antimicrobial actions largely via disruption of bacterial membranes and blockage of efflux pumps. S. nigrum, on the other hand, includes glycoalkaloids such as solanine and solasodine, as well as flavonoids and tannins, which generate oxidative stress, impede quorum sensing, and degrade bacterial enzymatic activity. The complementary mechanisms of these two plant species give a good foundation for synergism. Experimental data shows the improved efficacy of mixed plant extracts, with considerable decreases in MIC values and efficient suppression of biofilm-forming bacteria. The observed synergy not only potentiates antimicrobial activities but also assists in overcoming bacterial resistance mechanisms, permitting lower effective dosages and less cytotoxicity. Moreover, the capacity of these plant extracts to restore the effectiveness of conventional antibiotics further highlights their potential as resistance-modifying drugs.

Despite these encouraging discoveries, significant problems remain. The phytochemical makeup of plant extracts may vary greatly based on variables such as geographic origin, harvesting period, and extraction process. Standardization and quality control are vital for the production of repeatable and therapeutically appropriate formulations. Additionally, most research to date have been undertaken in vitro. In vivo effectiveness, pharmacokinetics, toxicity, and possible medication interactions must be investigated before clinical translation. Future research should focus on: • Standardization and optimization of extraction and formulation techniques; • In vivo validation in suitable animal infection models; • Mechanistic studies using omics approaches to delineate molecular targets; • Development of nanoformulations or drug delivery systems to enhance bioavailability; • Clinical trials to establish safety and efficacy in human populations.

The merging of ancient knowledge with current biological research gives a comprehensive and sustainable approach to antibacterial medication development. The synergistic combination of Piper nigrum and Solanum nigrum has showed substantial potential as a phytotherapeutic method against MDR pathogens, warranting further exploration and development.

7. CONCLUSION

The The present worldwide challenge of antimicrobial resistance needs the development of creative, effective, and sustainable alternatives to traditional antibiotics. This research has revealed that *Piper nigrum* which is also known as black pepper and *Solanum nigrum* that goes by the common name of black nightshade two plants with a long history in traditional medicine, offer strong antibacterial activity separately and much higher effectiveness when taken in combination. Their broad-spectrum action against Gram-negative bacteria such as Pseudomonas aeruginosa and Proteus vulgaris is mostly related to the presence of phytochemicals such piperine, solanine, flavonoids, and essential oils. These bioactive chemicals function via numerous methods, including membrane disruption, efflux pump inhibition, oxidative stress induction, and quorum sensing interference. The combination of P. nigrum and S. nigrum demonstrates synergistic effects that boost antimicrobial activity, decrease effective dosages, and may limit resistance development. Furthermore, these extracts show great potential as antibiotic adjuvants by restoring the efficiency of medications against resistant bacterial strains.

Despite these hopeful discoveries, translation from in vitro investigations to clinical applications remains a key barrier. Standardization of phytochemical content, thorough in vivo testing, pharmacodynamic assessments, and controlled clinical trials are necessary next steps to establish safety and effectiveness. Nonetheless, the observed synergy between these two plants offers a new frontier in phytomedicine, facilitating the development of unique, plant-based antimicrobials. In conclusion, Piper nigrum and Solanum nigrum, with their complementing phytochemical profiles and synergistic interactions, constitute a useful phytotherapeutic method for controlling infections caused by multidrug-resistant bacteria. Continued research and multidisciplinary cooperation are important to realizing their full therapeutic potential.

Challenges and Future Directions

Despite encouraging in vitro results, clinical validation of P. nigrum -S. nigrum synergy remains restricted. Key difficulties include.

1. **Standardization**: Variability in extract preparation procedures (solvent type, concentration) influences repeatability (Thippeswamy et al., 2021).
2. **In Vivo Efficacy**: Animal models are essential to validate synergy in physiological circumstances and evaluate toxicity (Prashanth et al., 2020).
3. **Mechanistic Clarity**: Further research should clarify molecular interactions (e.g., docking of piperine-solanine complexes with bacterial targets).

Future research should focus nanoformulations to increase bioavailability and clinical studies to evaluate safety and effectiveness.

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