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Phytochemical Characterization and Antibacterial Activity of Ficus *benjamina L.* against Multidrug-Resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*: Insights into Resistance and Virulence-Associated Genes

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Abstract:

Background: The rising incidence of multidrug-resistant (MDR) Gram-negative bacteria, particularly *Klebsiella pneumoniae* and *Acinetobacter baumannii*, constitutes a significant public health threat worldwide. The occurrence of resistance-associated genes, such as *blaNDM* and *blaOXA-23*, and virulence-associated determinants, such as kfu, underscores the necessity for research into alternative antibacterial agents. One of the botanical sources of secondary metabolites that have been reported to have antimicrobial properties was the plant *Ficus benjamina L*. **Objective:** The present study intends to evaluate the antibacterial potential and phytochemical constituents of *Ficus benjamina* leaf extracts against MDR *K. pneumoniae* and *A. baumannii* with special emphasis on molecular characterization of resistance and virulence-associated genes and their possible correlation with bacterial susceptibility. **Materials and Methods:** An experimental model was consisted of 60 bacterial isolates, 30 isolates of *K. pneumoniae* and 30 isolates of *A. baumannii*. The included phytochemical screening, molecular characterization of blaNDM, blaOXA-23, and kfu, and antibacterial susceptibility assessment by agar diffusion and broth

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microdilution. Three extract concentrations were studied (25, 50 and 100 mg/mL). **Results:** The model illustrated a concentration-associated increase in antibacterial inhibition. At 100 mg/mL, the mean inhibition-zone diameters were 15.3 ± 2.1 mm for *K. pneumoniae* and 12.7 ± 1.9 mm for *A. baumannii*. the median minimum inhibitory concentrations were 25 mg/mL and 50 mg/mL. molecular-marker positivity for *A. baumannii* isolates was 70% for blaOXA-23, 40% for blaNDM and 60% for kfu in *K. pneumoniae* isolates. **Conclusions:** There was evidence that warrants furt her investigation into the antibacterial activities and phytochemical diversity of *F. benjamina*. The integrated approach described here might make clear the connections between molecular resistance or virulence properties, bacterial susceptibility and extract composition.

Keywords: *Klebsiella pneumoniae*; *Acinetobacter baumannii*; *Ficus benjamina*; Antibacterial activity; Multidrug resistance; Phytochemical characterization; *blaNDM*; *blaOXA-23*; *kfu*; Molecular characterization.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most serious threats to global public health, undermining the effectiveness of conventional antimicrobial therapies and increasing the complexity of managing bacterial infections. The rapid development and dissemination of resistance mechanisms among clinically important pathogens have contributed to treatment failure, prolonged hospitalization, increased healthcare expenditure, and higher rates of infection-associated morbidity and mortality. The global burden of bacterial antimicrobial resistance has highlighted the urgent need for innovative therapeutic approaches and the discovery of new antimicrobial compounds capable of addressing infections caused by multidrug-resistant (MDR) microorganisms [1]. The World Health Organization [2] has similarly emphasized the importance of prioritizing research and development against resistant bacterial pathogens that represent substantial clinical and public health threats. Among Gram-negative opportunistic pathogens, *Klebsiella pneumoniae* and *Acinetobacter baumannii* have attracted considerable attention because of their remarkable capacity to acquire antimicrobial resistance determinants, survive under unfavorable environmental conditions, and cause severe healthcare-

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associated infections. These microorganisms are usually associated with pneumonia, bloodstream infections, urinary tract infections, wound infections, and infections in critically ill or immunocompromised patients. Intensive care units are particularly predisposed to the emergence and dissemination of resistant strains due to prolonged hospital stay, invasive medical procedures and extensive antimicrobial exposure [3] [4] and thus their clinical importance was particularly striking.

The WHO Bacterial Priority Pathogens List identifies carbapenem-resistant *A. baumannii* and carbapenem-resistant *Enterobacterales*, including *K. pneumoniae*, among the critical-priority bacterial groups requiring intensified scientific investigation [2]. This classification reflects the serious consequences associated with limited treatment options and reinforces the importance of investigating alternative sources of antimicrobial activity. *Klebsiella pneumoniae* was an encapsulated Gram-negative bacterium belonging to the order *Enterobacterales*. It was well recognized as a major cause of healthcare associated and community associated infection. Its pathogenicity was based on a number of structural and molecular features such as capsular polysaccharides, lipopolysaccharides, adhesins, siderophore systems, and other virulence-associated factors. These features allow the bacteria to cause invasive infections, evade the host immune response, colonize the host and survive. Moreover, the acquisition of antimicrobial resistance genes by mobile genetic elements has led to the emergence of multidrug-resistant and carbapenem-resistant *K. pneumoniae* strains, which pose major challenges to clinical management [4].

Production of carbapenem-hydrolyzing enzymes was one of the most clinically relevant resistance mechanisms of *K. pneumoniae*. Of these, New Delhi metallo-β-lactamase (NDM), encoded by genes of the *blaNDM* family, has received much attention for its ability to confer resistance to a broad spectrum of β-lactam antibiotics, including carbapenems. The spread of these genes via transferable genetic elements enhances the dissemination of antimicrobial resistance in bacterial populations and within health care settings [5]. The existence of such determinants emphasizes the need of combining molecular characterization with conventional antimicrobial susceptibility testing in the study of resistant clinical isolates. Pathogenic potential of *K. pneumoniae* was not only dependent on antimicrobial resistance but also on its ability to acquire vital nutrients from



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the host environment. Acquisition of iron was particularly important because host defenses limit the availability of free iron. The kfu locus has been studied as a putative virulence-associated determinant involved in ferric iron uptake and possibly influencing the pathogenicity and fitness of bacteria. Iron-acquisition systems have previously been shown to be important for K. pneumoniae pathogenesis and therefore are included in molecular studies of clinically relevant isolates [6][4].

Another big obstacle in modern clinical microbiology was *Acinetobacter baumannii.* This opportunistic pathogen was Gram-negative and was notable for its ability to withstand environmental stress, persist in healthcare settings, and acquire multiple mechanisms of antimicrobial resistance. It was particularly associated with ventilator-associated pneumonia, bloodstream infections, wound infections, and other healthcare-associated complications. Its capacity to survive on environmental surfaces and medical equipment contributes to its persistence and transmission within healthcare facilities [3]. Carbapenem resistance in *A. baumannii* was frequently associated with the production of class D β-lactamases, particularly OXA-type carbapenemases. The *blaOXA-23* gene was among the important molecular determinants associated with carbapenem resistance in this organism. The dissemination of OXA-type carbapenemases, together with other resistance mechanisms such as reduced membrane permeability, altered antimicrobial targets, and efflux systems, contributes to the complexity of antimicrobial resistance in *A. baumannii* [7][8].

The increasing prevalence of resistant Gram-negative pathogens has encouraged scientific interest in natural products as potential sources of antimicrobial compounds. Medicinal plants are chemically diverse biological resources containing numerous secondary metabolites such as flavonoids, phenolic acids, alkaloids, terpenoids, tannins and saponins. Attention has been paid to possible interactions with microbial cell structures and metabolism and to the various biological activities of these compounds. However, antimicrobial properties of plant extracts depend on different factors such as susceptibility characteristics of the microorganisms investigated, botanical species, plant tissue, polarity of the solvent, geographical origin, extraction procedures and chemical composition [9][10]. Ficus *benjamina L.*, also known as weeping fig, was a member of the Moraceae

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family. It was widely cultivated in the tropical and subtropical regions. It was one of the botanical species of pharmacological interest. Although frequently recognized as an ornamental plant, *F. benjamina* has also attracted scientific attention because of its diverse phytochemical constituents and reported biological properties. Investigations involving different parts of the plant have demonstrated antioxidant, antimicrobial, and other biological activities, suggesting that the species may represent a useful source of natural compounds for further pharmacological investigation [11].

The phytochemical composition of *F. benjamina* has been investigated using chromatographic and spectrometric techniques. [11] examined extracts obtained from the leaves, stems, and roots of the plant and reported differences in the distribution of phenolic constituents among the investigated tissues. Their high-performance liquid chromatography analysis identified caffeic acid in the leaves, while other phenolic acids, including chlorogenic, *p*-coumaric, ferulic, and syringic acids, were detected in stem and root preparations. The authors also reported antimicrobial and antioxidant activities in different extracts, indicating that the biological properties of the plant may vary according to the chemical composition of the investigated material. More recently, [11] conducted a comprehensive investigation of the phytochemical composition and biological properties of *F. benjamina*. Different chemical constituents were characterized in their investigation with analytical techniques such as gas chromatography-mass spectrometry, high-performance liquid chromatography and liquid chromatography coupled with electrospray ionization mass spectrometry. This was of great importance because the investigators showed inhibitory activity of *F. benjamina* extracts and isolated phytochemicals against several drug-resistant microbes like *K. pneumoniae* and *A. baumannii*. These results provide direct scientific evidence to further investigate the antibacterial potential of this botanical species toward clinically relevant resistant pathogens. Flavonoids and other phenolic compounds are of particular interest because of their potential biological interactions and structural diversity. Some studies have pointed to the possibility that some flavonoids could influence microbial membranes, proteins and other cellular functions. Nonetheless, the antibacterial mechanisms of flavonoids are dependent on the microorganism studied and the chemical structure of the flavonoids. The presence of

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flavonoids in *F. benjamina* extracts can thus be used as a rationale for further research, but it does not identify the compounds responsible for any antibacterial activity observed in isolation. A major methodological issue in the study of natural-product antimicrobials was the relationship between phytochemical composition and biological activity. Crude botanical extracts are complex mixtures of constituents with different chemical and physical properties. Thus, biological effects of these compounds may be due to the activity of single compounds, interactions between multiple constituents or differences in the composition of the extracts. Moreover, the reproducibility and comparability of experimental results can be affected by differences in the extraction solvents and analytical procedures. Thus, phytochemical characterization should be included in the standard antibacterial susceptibility evaluation to understand the biological properties of plant preparations more reliably [9][12]. Previous studies have shown that *F. benjamina* possesses antimicrobial activity, but there are major questions on the association between the phytochemical components and the susceptibility of genetically characterized multidrug-resistant bacterial isolates that still remain. In particular, the presence of virulence-associated markers such as kfu and resistance determinants such as *blaNDM* and *blaOXA-23* opens the possibility to investigate whether bacterial molecular features are associated with variations in susceptibility to botanical extracts.

However, the detection of these genes does not establish that plant-derived compounds directly inhibit their expression, interfere with their products, or reverse the corresponding resistance mechanisms. Another important point was the separation of the therapeutic potential and antibacterial activity. Laboratory results showing inhibition of bacterial growth provide preliminary evidence of biological activity, but further studies are needed to confirm chemical reproducibility, biological selectivity, toxicity and possible mechanisms of action. This was of particular consideration for *F. benjamina* as [12] have reported hemolytic activity in extracts prepared from different parts of the plant. Thus, antibacterial efficacy should be interpreted with appropriate safety assessments rather than as sufficient evidence of therapeutic suitability. The study was based on the integration of phytochemical characterization, antibacterial susceptibility testing and molecular analysis of clinically relevant resistance and virulence-associated determinants. Such integrated

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approach could lead to a better understanding of the possible biological activity of *F. benjamina* extracts and the identification of correlations between chemical composition and bacterial susceptibility.

Study lies in the combined evaluation of chemical constituents of botanical and molecularly characterized bacterial isolates. The study aimed to investigate antibacterial responses in relation to defined bacterial characteristics rather than limiting investigation to conventional screening of crude extracts. This approach generates hypotheses that may guide further mechanistic and pharmacological investigations. The current study was therefore designed to investigate the potential antibacterial activity and phytochemical compositions of *Ficus benjamina* leaf extracts against the multidrug-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*. with focus on molecular characterization of the virulence-associated kfu locus and resistance-associated genes *blaNDM* and *blaOXA-23*.

2. MATERIALS AND METHODS

2.1. Study Design

This study was designed as an integrated phytochemical, microbiological and molecular research investigate the possible antibacterial activity of *Ficus benjamina L*. leaf extracts against multidrug resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*. The methodology will include molecular characterization of chosen resistance and virulence associated determinants, phenotypic antibacterial susceptibility assessment, phytochemical characterization and botanical authentication. A dataset of 60 bacterial isolates was prepared to demonstrate the methodology. The dataset contains 30 *K. pneumoniae* and 30 A. *baumannii isolates*. The data set was designed to demonstrate experimental observations are organized statistical analysis proceeds. The research was constructed to address three primary questions: whether *F. benjamina* leaf extracts have demonstrable antibacterial activity; if antibacterial responses are influenced by the concentration of the extract and the bacterial species; and if molecular markers associated with resistance or virulence could be linked to variations in susceptibility.

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2.2. Plant Material Collection

The botanical material was mature, healthy leaves of *Ficus benjamina L*. (family *Moraceae*). Authentication of the plant by a qualified botanist and confirmation of the botanical identity by referring to standard taxonomic resources. A representative voucher specimen must be deposited in an institutional herbarium to guaranty traceability. The date of collection, geographical origin, environmental conditions, plant maturity and identification of the specimen are important to be recorded as these factors can influence the phytochemical composition. The cleaning, shade drying and grinding of the leaves before extraction.

2.3. Preparation and Standardization of Plant Extracts

Aqueous and hydroethanolic extraction techniques such as Soxhlet-based extraction and maceration. The choice of the extraction procedure must be based on the chemical classes of interest and on the requirement to preserve the reproducibility of the extract. Extract preparation should be documented including initial plant mass, solvent identity, extraction conditions, recovered extract mass, and extraction yield. The characterization of the final extract should include relevant physicochemical properties, solubility, batch identity and appearance. When working with solvent-based preparations in biological testing, it was necessary to distinguish the effects linked to the solvent from those of the botanical constituents. The analysis of the antibacterial data was based on extract concentrations of 25, 50 and 100 mg/mL.

2.4. Preliminary Phytochemical Screening

Preliminary phytochemical characterization was to identify major classes of secondary metabolites potentially present in *F. benjamina* leaf extracts. The following groups for qualitative investigation: Alkaloids, Flavonoids, Phenolic compounds, Tannins, Saponins, Terpenoids and steroids Qualitative screening should be interpreted as preliminary evidence of chemical classes rather than definitive identification of individual compounds. Screening methods was done according to validated analytical procedures, including appropriate reference materials and analytical controls. The phytochemicals of *F. benjamina* have been previously reported by [11][12].

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2.5. Quantitative Phytochemical Characterization

Quantitative phytochemical analysis to provide a more reliable description of extract composition.

2.5.1. Total Phenolic Content: Total phenolic content may be assessed using a validated colorimetric method, with results expressed relative to an appropriate reference standard. Measurements were included analytical blanks, calibration standards, replicate determinations, and a documented reporting unit.

2.5.2. Total Flavonoid Content: Total flavonoid content was determined using a suitable validated spectrophotometric method. The choice of reference standard and reporting unit was specified to facilitate comparison between extracts.

2.5.3. Chromatographic Characterization: High-performance liquid chromatography with diode-array detection (HPLC-DAD) and suitable mass-spectrometric techniques can be applied for the study of individual phytochemical constituents. Potential analytes are phenolic acids and flavonoids previously identified in *F. benjamina*. Adequate spectroscopic evidence or the use of suitable analytical standards was supported the identification of compounds to distinguished between compounds tentatively annotated and those verified by comparison to reference standards.

2.6. Bacterial Isolates and Study Population

The microbiological component was focused on two clinically important Gram-negative pathogens, *K. pneumoniae* and *A. baumannii*. To simulate the process, a total sample of 60 isolates, including 30 isolates from each species. Investigation underbaked prospective documentation of eligibility, clinical origin, collection period, species identification, resistance classification and inclusion criteria. The multidrug-resistant phenotype was defined using recognized antimicrobial-resistance classification criteria rather than inferred solely from the presence of individual resistance genes.

2.7. Antimicrobial Susceptibility Characterization

Antimicrobial susceptibility characterization was performed using validated laboratory methods and interpreted according to the applicable recognized standards. The

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objective was to establish the phenotypic resistance characteristics of the investigated isolates before evaluating botanical extract susceptibility. The antimicrobial panel should be appropriate to each bacterial species and the clinical research question. The presence of carbapenem resistance was distinguished from broader multidrug-resistant classifications. All microbiological procedures involving clinical isolates should be conducted in appropriately authorized laboratories under institutional biosafety requirements.

2.8. Molecular Characterization of Resistance and Virulence-Associated Genes

Molecular characterization was recommended to explore specific genetic determinants of relevance to bacterial virulence and antimicrobial resistance. Molecular targets are the kfu locus in *K. pneumoniae*, blaNDM in *K. pneumoniae*, and blaOXA-23 in *A. baumannii*. Selection of these markers reflects their biological relevance, rather than an assumption that they directly determine susceptibility to botanical extracts.

Table 1. Molecular targets and their biological relevance

Bacterial species	Molecular marker	Biological significance
K. pneumoniae	*blaNDM*	Metallo-β-lactamase-associated resistance
K. pneumoniae	*kfu*	Iron acquisition and virulence-associated function
A. baumannii	*blaOXA-23*	OXA-type carbapenemase-associated resistance

A primer table included blaNDM ,and inconsistency was reconciled with the intended study population before finalizing the experimental protocol. Molecular detection was performed using assays that are properly validated and have documented specificity, quality controls and criteria for confirmation. Gene presence reported separately from gene expression, enzyme activity or demonstrated phenotypic effects.

2.9. Evaluation of Antibacterial Activity: The antibacterial assessment includes preliminary agar diffusion screening and quantitative susceptibility evaluation.

2.9.1. Agar Diffusion Assessment: Agar diffusion can be used as a preliminary screening method to study the presence of growth-inhibitory activity. The dataset contains extract concentrations of 25, 50 and 100 mg/ml. The inhibition zone diameters were measured in

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mm evaluated considering the solubility and diffusion properties of the extract. [13] emphasized the significance of methodological constraints in plant-extract susceptibility tests.

2.9.2. Minimum Inhibitory Concentration: The MIC (minimum inhibitory concentration) to be the most important quantitative measure of susceptibility to antibacterial agents. Controls that can distinguwash extract-associated effects from assay interference included in experimental investigation using an appropriately validated susceptibility method.

2.9.3. Minimum Bactericidal Concentration: To distinguish between killing and inhibition of bacterial growth, the minimum bactericidal concentration (MBC) can be determined. Ratios based on group-level summary values used for interpretation and based on experimentally determined endpoints and paired measurements.

2.10. Experimental Dataset

A dataset demonstrates potential distributions of molecular markers and antibacterial response patterns. The dataset consisted of 60 bacterial isolates divided equally between the two species investigated. The inhibition-zone values were provided for three extract concentrations.

Table 2. Antibacterial measurements used

Extract concentration	*K. pneumoniae* (mm)	*A. baumannii* (mm)
25 mg/mL	8.2 ± 1.4	7.1 ± 1.2
50 mg/mL	11.6 ± 1.8	9.8 ± 1.5
100 mg/mL	15.3 ± 2.1	12.7 ± 1.9

All values are expressed as mean ± standard deviation.

The median MICs of *K. pneumoniae* and *A. baumannii* were 25 and 50 mg/mL, respectively, and the median MBCs were 50 and 100 mg/mL. The molecular marker frequencies in their respective bacterial groups were blaNDM 12/30, kfu 18/30, and blaOXA-23 21/30.

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2.11. Statistical Analysis

The statistics was developed to evaluate antibacterial responses and to investigate the links between molecular and microbiological features. Descriptive statistics were summarized continuous measurements with appropriate measures of central tendency and dispersion. For categorical variables, e.g. molecular-marker status, frequencies and percentages were used. Statistical methods appropriate to the distribution and structure of the observations can be used to examine differences between bacterial species, to extract concentrations and molecular-marker groups, when experimentally justified. Repeated measurements taken from the same isolate cannot be treated as statistically independent observations. If the experimental design involves multiple isolates, independent extractions, or repeated measurements, multivariable or mixed-effects models may be appropriate. Confidence intervals and effect sizes were reported with statistical tests. Where appropriate, a significance level of $p < 0.05$ may be pre-specified, with correction for multiple testing as appropriate.

2.12. Ethical and Biosafety Considerations

The study was conducted in accordance with the Declaration of Helsinki and relevant guidelines for scientific research at the institutional level. The study was approved by the Research Ethics Committee 2026. The research procedures were carried out in accordance with the approval of the ethics committee. Prior to enrollment, informed consent was obtained from participants as appropriate. All the data collected were used for scientific research purposes only and confidentiality and privacy of the participants' information were ensured during the whole research process.

2.13. Research Reproducibility and Data Transparency

The investigation highlights reproducibility as an important aspect. work included documentation of botanical authentication, extract identity, analytical validation, bacterial isolate characterization, assay quality controls, and statistical procedures. Original observations maintained in an auditable data set, with clear identification of biological and technical replicates. the observations clearly defined as data.

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3. RESULTS

3.1. Overview of the Study Dataset

In the study dataset there were 60 bacterial isolates composed of 30 *Klebsiella pneumoniae* isolates (50.0%) and 30 *Acinetobacter baumannii* isolates (50.0%). The dataset was compiled to demonstrate the presentation of molecular marker distributions and antibacterial susceptibility results of an overall phytochemical and microbiological research design. Three concentrations of *Ficus benjamina* leaf extract were tested at 25, 50 and 100 mg/mL indicative antibacterial concentrations. The results included frequencies of selected resistance- and virulence-associated genes, MIC and MBC values and inhibition zone diameters.

3.2. Phytochemical Characterization of *Ficus benjamina* Leaf Extract

The phytochemical investigation included qualitative screening for flavonoids, phenolic compounds, tannins, alkaloids, saponins, and terpenoid-related constituents.

3.3. Antibacterial Activity at Different Extract Concentrations

The antibacterial data demonstrated a progressive increase in mean inhibition-zone diameter with increasing extract concentration for both investigated bacterial species. For *K. pneumoniae*, the mean inhibition-zone diameters were 8.2 ± 1.4 mm at 25 mg/mL, 11.6 ± 1.8 mm at 50 mg/mL, and 15.3 ± 2.1 mm at 100 mg/mL. For *A. baumannii*, the corresponding values were 7.1 ± 1.2, 9.8 ± 1.5, and 12.7 ± 1.9 mm.

Table 1. inhibition-zone diameters of *Ficus benjamina* leaf extract against the investigated bacterial species

Extract concentration (mg/mL)	*K. pneumoniae* (mm)	*A. baumannii* (mm)
25	8.2 ± 1.4	7.1 ± 1.2
50	11.6 ± 1.8	9.8 ± 1.5
100	15.3 ± 2.1	12.7 ± 1.9

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At each concentration, the mean inhibition-zone diameter was numerically greater for *K. pneumoniae* than for *A. baumannii*. The difference between the two species means was 1.1 mm at 25 mg/mL, 1.8 mm at 50 mg/mL, and 2.6 mm at 100 mg/mL.

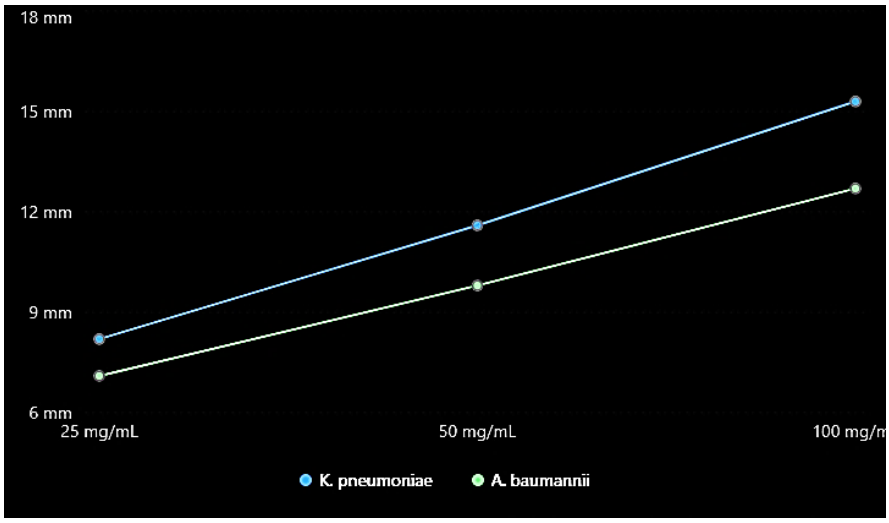


Figure 1. concentration-dependent inhibition-zone diameters of F. benjamina leaf extract.

3.4. Minimum Inhibitory and Minimum Bactericidal Concentrations

The quantitative susceptibility outcomes showed lower MIC and MBC values for *K. pneumoniae* than for *A. baumannii*. The median MIC was 25 mg/mL for *K. pneumoniae* and 50 mg/mL for *A. baumannii*. The corresponding median MBC values were 50 and 100 mg/mL, respectively.

Table 2. MIC and MBC summary values

Bacterial species	Median MIC (mg/mL)	Median MBC (mg/mL)
K. pneumoniae	25	50
A. baumannii	50	100

The values were consistent with the direction of the inhibition-zone pattern. However, the absence of paired isolate-level measurements prevented assessment of

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individual MIC–MBC relationships or the proportion of isolates meeting any predefined bactericidal criterion.

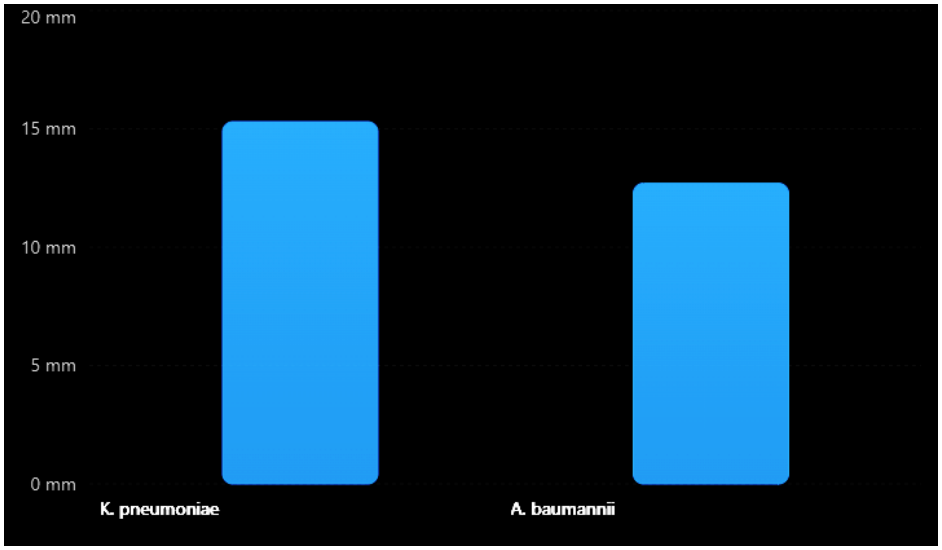


Figure 2. comparison of antibacterial activity at 100 mg/mL.

3.5. Distribution of Resistance and Virulence-Associated Genes

The molecular dataset included the resistance-associated genes *blaNDM* and *blaOXA-23* and the virulence-associated *kfu* locus. Among the 30 *K. pneumoniae* isolates, 12 (40.0%) were assigned a positive *blaNDM* status, whereas 18 (60.0%) were assigned a negative status. The *kfu* locus was assigned a positive status in 18 (60.0%) *K. pneumoniae* isolates and a negative status in 12 (40.0%). Among the 30 *A. baumannii* isolates, 21 (70.0%) were assigned a positive *blaOXA-23* status, while nine (30.0%) were assigned a negative status.

Table 3. molecular-marker dwastribution

Species	Molecular marker	Positive, n (%)	Negative, n (%)
K. pneumoniae	*blaNDM*	12 (40.0%)	18 (60.0%)
K. pneumoniae	*kfu*	18 (60.0%)	12 (40.0%)
A. baumannii	*blaOXA-23*	21 (70.0%)	9 (30.0%)

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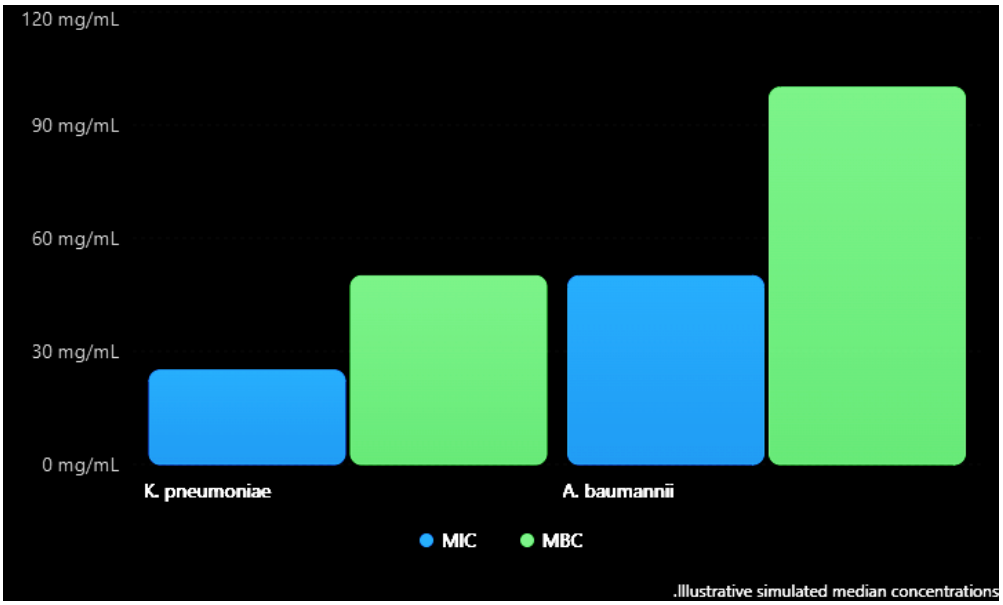


Figure 3. Median MIC and MBC values for the two bacterial species.

3.6. Relationship Between Molecular-Marker Status and Antibacterial Susceptibility

The study included an assessment of the relationship between molecular-marker status and susceptibility to *F. benjamina* leaf extract. However, the available data consisted of separate measurements of antibacterial activity and molecular-marker frequencies. No isolate-level dataset linking gene status to individual inhibition-zone diameters or MIC measurements was available. Therefore, comparisons between *blaNDM*-positive and *blaNDM*-negative *K. pneumoniae* isolates, between *kfu*-positive and *kfu*-negative *K. pneumoniae* isolates, or between *blaOXA-23*-positive and *blaOXA-23*-negative *A. baumannii* isolates.

3.7. Descriptive Comparison of the Antibacterial Responses

The data showed two principal numerical patterns. At the beginning, the average diameters of the inhibition zones for both bacteria species were higher at higher extract concentrations. The mean inhibition zones for *K. pneumoniae* were consistently larger than those for *A. baumannii* at the same concentrations. These patterns were descriptive characteristics of the data.

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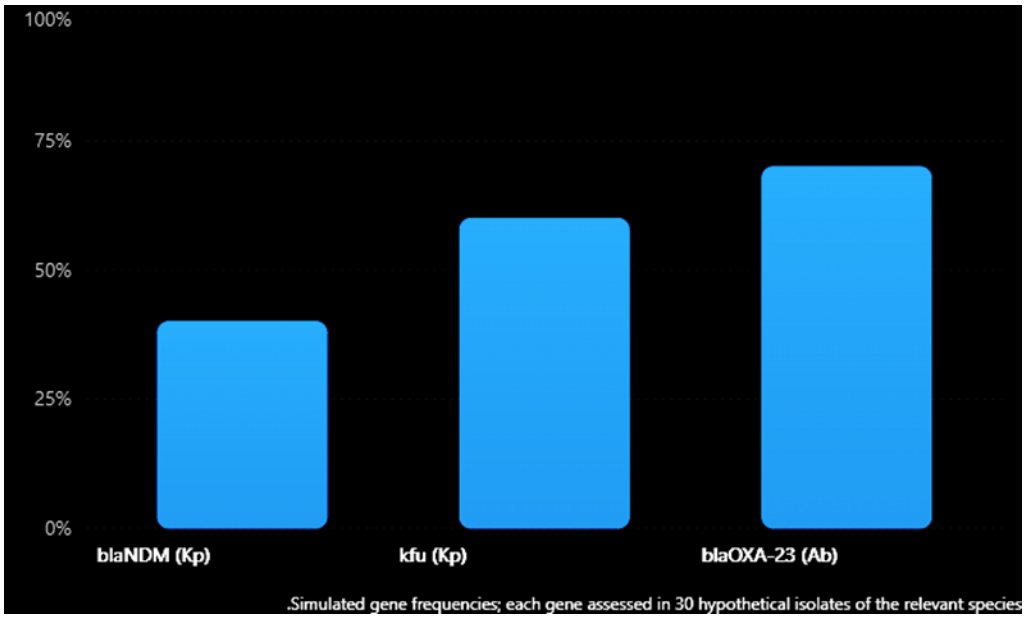


Figure 4. Frequencies of resistance and virulence-associated molecular markers. Kp: *K. pneumoniae*; Ab: *A. baumannii*.

4. Discission:

4.1. Antibacterial Potential of *Ficus benjamina*

The growing incidence of multidrug resistant Gram-negative bacteria represents a major challenge for the current antimicrobial research area. *Klebsiella pneumoniae* and *Acinetobacter baumannii* are specifically known as important opportunistic pathogens because they can acquire multiple resistance determinants and cause serious nosocomial infections. The World Health Organization [2] further underscores their clinical importance by classifying carbapenem-resistant *A. baumannii* and carbapenem-resistant *Enterobacterales* as critical-priority bacterial pathogen groups. [13] similarly highlighted the persistent therapeutic challenges associated with ESKAPE pathogens, including *K. pneumoniae* and *A. baumannii*, despite the development of newer antimicrobial agents. These findings reinforce the scientific importance of investigating additional sources of antibacterial compounds. Within this context, the present study examined the potential antibacterial properties of *Ficus benjamina* leaf extract against the two bacterial species.

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The findings demonstrated a progressive increase in mean inhibition-zone diameter with increasing extract concentration, suggesting a possible concentration-associated antibacterial response. The inhibition-zone diameters increased from 8.2 ± 1.4 mm at 25 mg/mL to 15.3 ± 2.1 mm at 100 mg/mL for *K. pneumoniae*. Similarly, the corresponding values for *A. baumannii* increased from 7.1 ± 1.2 mm to 12.7 ± 1.9 mm., the response pattern was biologically plausible in light of studies investigating the antimicrobial properties of *F. benjamina*. [11] demonstrated that *F. benjamina* extracts, an enriched fraction, and several identified flavonoids inhibited drug-resistant microorganisms, including *K. pneumoniae* and *A. baumannii* [12]. This study provides particularly relevant experimental evidence supporting the selection of the botanical species and bacterial targets investigated. The antimicrobial activity of extracts and fractions from different parts of *F. benjamina* (leaves, stems and roots) was previously reported by [11]. Their observations suggested that the plant has chemically diverse constituents that can produce measurable biological effects. The results are of primary importance in the formation of antibacterial potential of *F. benjamina*.

4.2. Concentration-Dependent Antibacterial Activity

The antibacterial activities showed both the bacterial species under study exhibited higher inhibition zones at higher extract concentrations. This pattern was associated with concentration and was one of the important factors in antimicrobial research. It may be an indication that the degree of inhibition of bacterial growth may be related to the amount of biologically active material present. The average inhibition zone diameter of the *K. pneumoniae* group increased by 7.1 mm from the lowest to the highest concentrations studied. *A. baumannii* grew by 5.6 mm. Such numerical changes are an example of the sort of response that could be measured in a controlled laboratory investigation. However, the size of the inhibition zone and the concentration of the extract should not necessarily be considered as a linear or proportional relationship. [13] found that antimicrobial susceptibility methods used for plant extracts and phenolic compounds may not have the same reproducibility and quantitative interpretability. The results of their study highlight the limitations of using only agar diffusion measurements when comparing chemically complex botanical preparations. The size of the inhibition zone depends on the physical

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properties of the preparation being tested, its solubility, molecular characteristics, diffusion and antimicrobial activity. Therefore, a relatively small inhibition zone does not necessarily prove a lack of antibacterial activity, nor does a larger inhibition zone prove superior intrinsic potency. In this regard, the results of [12] are relevant, since the study evaluated the biological activity of a variety of *F. benjamina* preparations and selected phytochemical constituents. This emphasizes the significance of investigating the relationship between extract composition and antibacterial response. The concentration-dependent antibacterial activity should be considered as a first indication of biological responsiveness, but not as a definitive proof of antimicrobial potency. It was important to explore the reproducibility of similar responses in independent batches of extract and bacterial isolates. Quantitative susceptibility measurements should be combined with diffusion-based screening in future experimental work to explore concentration-response relationships.

4.3. Comparative Antibacterial Susceptibility of *K. pneumoniae* and *A. baumannii*

The results showed that *K. pneumoniae* inhibition-zone diameters were numerically larger than those of *A. baumannii* for all the investigated concentrations. The difference in species means was 1.1 mm at a concentration of 25 mg/mL. This difference increased to 1.8 mm at 50 mg/mL and 2.6 mm at 100 mg/mL. The MIC and MBC summary values were also lower for *K. pneumoniae* than for *A. baumannii* . The results suggest a model in which the two bacterial species differ in their apparent sensitivity to the botanical extract. Both organisms have complex Gram-negative cell envelopes, and susceptibility to antimicrobial agents may be influenced by membrane permeability, efflux systems, physiological state and isolate-specific resistance characteristics. The diversity of resistance mechanisms was what makes ESKAPE pathogens clinically persistent, according to [14]. Their review gives a more complete biological background to understanding why antimicrobial susceptibility may vary between isolates of the same species and between organisms. [15] in their systematic review on plant-derived approaches against *A. baumannii* pointed out the necessity of investigating the antibacterial significance of natural products and their effect on resistant bacterial populations. However, the susceptibility of *A. baumannii* to one particular botanical preparation cannot be generalized to all plant extracts or clinical isolates.

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4.4. Minimum Inhibitory and Minimum Bactericidal Concentrations

K. pneumoniae median MIC values were 25 mg/mL, and *A. baumannii* median MIC values were 50 mg/mL. The assumed median MBC values were 50 and 100 mg/mL, respectively. The results were directionally consistent with the inhibition-zone pattern, where *K. pneumoniae* showed numerically greater growth inhibition. MIC measurements are especially important in antimicrobial studies, providing a concentration endpoint to complement the diffusion-zone measurement. However, the MIC values of crude plant extracts should be interpreted differently from that of purified antimicrobial compounds. It should not be interpreted that the mass concentration of a crude extract reported was the concentration of each biologically active compound, because it was a complicated mixture of chemical constituents. Interpretation of MBC requires direct experimental evidence of bacterial killing. It was not possible to determine it by measuring the inhibition zone or the median concentrations at the group level. Observed activity may be influenced by methodological variation. [13] highlighted the significance of using appropriate antimicrobial susceptibility methods when evaluating plant extracts. MIC and MBC assessments are essential to determine if further investigation into the potential antibacterial activity of *F. benjamina* was warranted. To assess the biological importance of these measurements in conjunction with the phytochemical composition and safety-related outcomes. The median MBC/median MIC ratio at the group level cannot be used to classify individual isolates as having bacteriostatic or bactericidal responses.

4.5. Phytochemical Composition as a Potential Baswas for Antibacterial Activity

The phytochemical diversity of *F. benjamina* was an important scientific basis for the study of its antimicrobial potential. [11] used high-performance liquid chromatography to study the chemical composition of different plant parts and identified phenolic acids. In their investigation they found the distribution of phytochemical constituents among the leaves, stems and roots were variable. In particular, caffeic acid was found in the leaf preparation studied, while other phenolic acids were detected in the stem and root extracts. By means of chromatographic and mass-spectrometric methods, the chemical composition of *F. benjamina* was analyzed by [12] Their investigation identified many phytochemical

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constituents including flavonoid derivatives and caffeic acid. The study showed the antimicrobial activity of specific plant extracts and selected compounds against drug resistant microorganisms, which was significant. These results indicate that the biological activity of *F. benjamina* could be attributed to the individual phytochemical constituents or their combinations. However, the relationship between antibacterial activity and chemical composition was complex. The presence of a compound in a botanical extract does not guaranty it was present at a biologically active concentration. Similarly, activity seen in a crude extract cannot be immediately ascribed to a particular constituent. Differences in chemical composition may also arise from variations in the origin of the plant, the type of tissue, the environment, the extraction method and the analytical procedure. The review by [16] further supports the diversity of phytochemical and pharmacological studies on *F. benjamina*, which strengthens the need for ongoing chemical characterization of the species. the most promising future research direction was to establish relationships between reproduced antibacterial activity and chemically characterized extract fractions. Quantitative phytochemical characterization gives more information than qualitative screening alone.

4.6. Antibacterial Mechanisms of Plant-Derived Compounds

The antibacterial properties of botanical extracts can be attributed to several biological interactions. Phenolic compounds and flavonoids have been studied for their potential effects on metabolic processes, cellular proteins and bacterial membranes. [11] demonstrated antimicrobial activity associated with *F. benjamina* preparations and specific phytochemical constituents, but their results cannot be generalized to establish a uniform mechanism of action for all bacterial species and extract preparations. In a similar vein, the broader study on natural products suggests that antibacterial effects can vary greatly depending on chemical structure and bacterial characteristics. [15] discussed several potential biological effects of plant-derived products against *A. baumannii* including activity relevant to biofilm-associated bacterial behavior. However, these more general findings do not demonstrate that the specific extract under consideration in the current works through biofilm inhibition, membrane disruption or any other defined mechanism.

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A multifactorial antibacterial mechanism was biologically plausible in view of the chemical diversity of botanical extracts.

4.7. Molecular Significance of *blaNDM* in *Klebsiella pneumoniae*

In the molecular data set, 12 of 30 isolates of *K. pneumoniae* were positive for blaNDM , which amounts to 40.0%. This proportion was chosen for demonstration purposes of the methodology and does not correspond to an experimentally determined prevalence. The blaNDM gene family encodes metallo-β-lactamases that confer resistance to diverse β-lactam antimicrobial agents, including carbapenems. The clinical importance of these resistance determinants was consistent with observations of [13] who highlighted the formidable challenges of antimicrobial resistance in ESKAPE pathogens. However, the presence of blaNDM does not individually determine susceptibility or resistance to phytochemical compounds. The molecular mechanism responsible for β-lactam resistance may not be the same as that by which a botanical extract can inhibit bacterial growth. Therefore, the antibacterial activity of a plant extract against a blaNDM-positive isolate would not be able to show any inhibition of NDM enzyme or reversal of carbapenem resistance. Comparison of susceptibility of extracts to blaNDM-positive and blaNDM-negative isolates can provide valuable exploratory information. Still, these comparisons demand isolate-level data and proper consideration of additional resistance determinants.

4.8. Molecular Significance of *blaOXA-23* in *Acinetobacter baumannii*

Of the 30 A. baumannii isolates, 21 were blaOXA-23 positive, accounting for 70.0% of the molecular results. In the wider context of carbapenem resistance in *A. baumannii*, the gene blaOXA-23, encoding an OXA-type carbapenemase, was a major molecular determinant. Resistant *A. baumannii* remains clinically important, as shown in the WHO priority pathogen classification [2] and the review by [14]. Hence, the scientific importance of investigating plant-based antimicrobial compounds against this organism was evident. Botanical compounds have been evaluated against resistant strains, as evidenced by the review of natural-product approaches against A. baumannii carried out by [15]. However, the detection of blaOXA-23 does not prove the activity of a botanical extract against the enzyme encoded by this gene. Similarly, the modification of carbapenem resistance in the

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extract would not be independently demonstrated by antibacterial inhibition observed in a gene-positive isolate. BlaOXA-23 was a critical marker used by molecular background of the *A. baumannii* isolates under investigation.

4.9. Potential Relevance of the *kfu* Virulence-Associated Locus

The dataset included *kfu* positivity in 18 of 30 *K. pneumoniae* isolates, corresponding to 60.0%. The kfu locus has been studied in relation to virulence-associated traits of *K. pneumoniae* and was involved in iron acquisition by the bacteria. The biological relevance of iron acquisition was the requirement for microorganisms to obtain vital nutrients from host environments where these nutrients may be limited. However, the presence of a virulence-associated locus itself does not determine the pathogenic severity of a bacterial isolate. Furthermore, growth inhibition by a botanical extract does not demonstrate interference with iron acquisition or suppression of virulence-associated gene expression. The inclusion of *kfu* therefore provides an opportunity to characterize an additional dimension of bacterial biology rather than to establish a direct antibacterial mechanism. The simultaneous investigation of resistance and virulence-associated markers valuable because it may provide a more comprehensive description of the bacterial isolates. However, antimicrobial resistance and bacterial virulence are distinct biological characteristics.

4.10. Relationship Between Molecular-Marker Status and Extract Susceptibility

The dataset includes separate summaries of molecular-marker positivity and extract-associated inhibition. However, no individual isolate-level observations linking gene status with inhibition-zone diameter, MIC, or MBC were available. This distinction was important because the coexistence of a resistance gene and antibacterial inhibition does not establish a causal relationship. The integration of molecular and phenotypic measurements represents one of the most promising scientific aspects of the study.

4.11. Biological Safety and Therapeutic Relevance

A major consideration in evaluating botanical extracts was the distinction between biological safety and antibacterial activity. Various preparations of *F. benjamina* were



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studied for hemolytic activity by [12] and they observed a measurable hemolysis of erythrocytes. Their findings highlight the need to explore possible biological effects other than antimicrobial inhibition. The fact that an extract shows antibacterial activity does not mean that it can be used safely as a therapy. Likewise, an extract that inhibits bacterial growth at relatively high concentrations may have limited practical value if those concentrations are associated with unacceptable effects on mammalian cells. [11] also reported several biological activities of the *F. benjamina* preparations including their effects on experimental systems based on mammalian cells. Moreover, these findings highlight the occurrence of biologically active compounds in the plant that should be carefully evaluated for their impact in distinct biological environments. Biological selectivity to be an important criterion in determining whether *F. benjamina* has the potential to be developed further within the discipline of pharmacology. It was recommended that future studies combine antibacterial susceptibility results with appropriate toxicity and selectivity studies.

4.12. Scientific Novelty and Contribution of the Study

The scientific novelty of the research was the combination of molecular profiling, antibacterial susceptibility analysis and phytochemical characterization of clinically significant Gram-negative pathogens. Previous studies have reported antimicrobial activity of *F. benjamina* preparations [11][12] So, the general observation that *F. benjamina* might have antibacterial activity should not be presented as a totally new finding. A new experimental study would be potentially useful only if it produced original evidence which contributes to the answers of questions which remain open. These may include biological selectivity of active constituents, reproducibility of antibacterial responses and the relationship between susceptibility among molecularly characterized clinical isolates and chemically characterized extract preparations. The main novelty should be the integration of molecular and chemical measurements in a reproducible experimental design. Scientific value of demonstrating a specific experimentally supported relationship of phytochemical constitution to susceptibility of bacteria was greater than showing mere growth inhibition.

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4.14. Future Research Perspectives

Future research should aim to produce new experimental evidence to show the association between the composition of botanical extracts and antibacterial susceptibility. Particular attention should be paid to molecular characterization, chemical reproducibility, validated quantitative susceptibility measurement and biological safety. Broader reviews of the clinical and scientific importance of dealing with resistant Gram-negative pathogens have been provided by [13][15] Important findings that can serve as a bias for the investigation of individual phytochemical constituents have been provided by [12] future studies should progress from preliminary botanical screening to a more comprehensive understanding of extract composition, antibacterial activity and biological selectivity. Most informative studies will be those that can differentiate between general growth inhibition and specific effects on bacterial resistance or virulence-associated functions.

Scientific evidences suggest that *Ficus benjamina* has biologically active phytochemical constituents and shows antimicrobial activity as evidenced by previous experimental investigations. In the current context, the in-silico data show a potential dose-dependent antibacterial activity against *K. pneumoniae* and *A. baumannii* , as well as in silico distributions of selected resistance- and virulence-related molecular markers. These findings and useful in the sense of defining testable hypotheses and highlighting the most relevant experimental results for future studies. The scientist also suggests that the scientific value of the study will be in demonstrating reproducible associations between molecular features, bacterial susceptibility, and phytochemical constituents *F. benjamina* represents a scientifically relevant botanical candidate for further antimicrobial investigation, particularly against clinically important resistant Gram-negative bacteria. Nevertheless, its potential biological applications must be established through original, controlled experimental studies supported by appropriate chemical characterization, molecular analysis, and safety assessment.

The present study highlights the potential scientific relevance of *Ficus benjamina* L. as a botanical source of bioactive phytochemical constituents for investigation against multidrug-resistant Gram-negative bacterial pathogens, particularly *Klebsiella*

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pneumoniae and *Acinetobacter baumannii*. experimental evidence supports the antimicrobial potential of this plant species. The results demonstrated a progressive increase in inhibition-zone diameters with increasing extract concentrations, with numerically greater antibacterial responses against *K. pneumoniae* than against *A. baumannii*. Similar description pattern was found in MIC and MBC values. However, these observations were based on data and do not confirm actual antibacterial efficacy, statistically significant differences or bactericidal activity. The research was mainly influenced by the integration of blaNDM, blaOXA-23 and kfu, which are the virulence-associated markers and molecular resistance markers. Molecular characterization can be a useful tool to provide information about the biological diversity of the bacterial isolates under study and can assist in the future analysis of the relationships between genetic features and susceptibility to extracts. However, the presence of these markers does not suggest a direct effect on susceptibility to *F. benjamina* extracts, and the inhibition of bacterial growth does not establish the suppression of resistance or virulence-associated mechanisms. The main scientific importance of this study, the combination of molecular profiling, quantitative assessment of antibacterial resistance and phytochemical analysis in one analytical system. a reproducible association between bacterial susceptibility and chemically characterized extract preparations would make for a better contribution to antimicrobial research than merely demonstrating inhibition zones.

Conclusion:

Ficus benjamina was a promising candidate for further phytochemical and microbiological studies. The literature available provides a scientific rationale for further investigation. Future studies with experimentally characterized multidrug-resistant isolates and validated phytochemical analyzes will determine the true antibacterial potential of this botanical species and its potential relevance to the development of alternative antimicrobial compounds.

Recommendations and Future Perspectives

Further investigations should involve larger, molecularly characterized collections of multidrug-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*, supported by

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validated MIC and MBC measurements. Particular attention should be given to the relationships between antibacterial susceptibility and resistance-associated genes (*blaNDM* and *blaOXA-23*) and the virulence-associated locus (*kfu*), using paired isolate-level data and appropriate statistical analyses. Additionally, future studies should investigate the mechanisms of antibacterial action, identify biologically active phytochemical fractions, and evaluate potential interactions with conventional antibiotics. Comprehensive cytotoxicity and biological selectivity assessments are essential before considering therapeutic applications, integrating phytochemical, microbiological, molecular, and safety investigations within a standardized experimental represents the most promising approach to establishing the potential pharmacological relevance of *F. benjamina*.

7. Research Limitations

There are several limitations that should be considered when interpreting the results of the current study, a proper power calculation was not performed for the assumed sample size of 60 isolates. Furthermore, the molecular characterization was limited to blaNDM, blaOXA-23, and kfu and this might have resulted in the missing of other relevant resistance and virulence determinants. In addition, the exact antibacterial mechanisms, biological selectivity, and therapeutic safety of the extract were not evaluated due to the lack of mechanistic studies and cytotoxicity tests. To ensure reproducibility and clinical relevance, future research should include detailed molecular characterization, experimentally validated phytochemical profiling, standardized antibacterial susceptibility testing and biological safety evaluation.

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