Antimicrobial Efficacy of Ethanolic Extracts of *Salvia officinalis* Leaves on *Proteus mirabilis.*

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Abstract

The study aimed to assess the antimicrobial efficacy of ethanolic extracts from *Salvia officinalis* leaves against *Proteus mirabilis* isolates, given the bacterium's significance in reoccurring cases of Urinary Tract Infections (UTIs) and resistance to conventional antibiotics. Sterile containers were used to obtain clean-catch urine samples from 20 hospitalized patients who are between the ages of 18-50 in Wukari. Spread-plate technique was used to culture samples on MacConkey agar for 24 hours at 32°C. Suspected colonies from a pure culture with a pale-smooth appearance and urease-positive were subjected to molecular identification of the *UreR gene* using Polymerase Chain Reaction (PCR). Following the respective evaporation of the crude ethanolic extracts of the dried and fresh leaves, 2.0mg, 4.0mg, 6.0mg, 8.0mg, and 10.0mg were dissolved into 10ml dimethyl-sulfoxide respectively. Antimicrobial susceptibility test was conducted using the Agar-well diffusion technique. The zones of inhibition (ZIB) measured for the dried leaves ranged from 12-26mm for the various ethanolic concentrations 2.0mg-10ml, 4.0mg-10ml, 6.0mg-10ml, 8.0mg-10ml, 10.0mg-10ml respectively, while the ZIB recorded for the fresh-leaves ranged from 10-24mm. No significant ZIB (≤3mm) was seen on the negative control which was the ethanol without extract. Ciprofloxacin 30µg was used as a positive control (≥29mm). Dried-leaf extracts of *Salvia officinalis* presented better antimicrobial activity on *Proteus mirabilis* compared to fresh-leaf extracts. The moisture content of fresh leaves was observed to favor microbial growth. Comparatively, both dried and fresh leaf extracts demonstrated moderate to strong antibacterial properties, falling within the effectiveness range of the positive control. Ultimately, this research successfully demonstrated the potential of *Salvia officinalis* as a medicinal plant in treating infections caused by *Proteus mirabilis.*

Keywords: Keywords: Antimicrobial resistance, Plant extract, Proteus mirabilis, Salvia officinalis, UTI

1. Introduction

Urinary Tract Infections (UTIs) are a prevalent health concern globally, with *Proteus mirabilis* emerging as a commonly isolated pathogen in these infections [1]. Its notoriety lies in its ability to cause persistent and recurrent UTIs, particularly in hospitalized patients. *Proteus mirabilis* is most prevalent in women aged 20 to 50 years [2]. Among otherwise healthy women, *Proteus mirabilis* causes 1% to 2% of UTIs, with *Escherichia coli* being more common [2]. In the context of hospital-acquired UTIs, *Proteus mirabilis* accounts for 5%, and its association with complicated UTIs, especially those linked to catheterization, increases substantially [2]. Overall, the role of *Proteus mirabilis* in UTI particularly among specific demographics and in healthcare settings is overwhelming. In specific demographics, such as elderly individuals or those with indwelling urinary catheters, *Proteus mirabilis* infections may be more prevalent due to factors such as urinary retention or compromised immune function [1]. Additionally, in healthcare settings, the use of invasive procedures or urinary catheters can increase the risk of UTIs caused by *Proteus mirabilis*, highlighting the importance of infection control measures and appropriate antimicrobial therapy [2].

Proteus mirabilis is a Gram-negative bacterium within the *Enterobacteriaceae* family [3]. It exhibits high motility, characterized by swarming growth on solid media owing to its flagellar movement [2]. The bacterium produces urease enzyme, leading to alkaline urine, stone formation, and increased urinary pH. It possesses virulence factors like fimbriae and enzymes that aid in adherence, motility, and evasion of the immune response [4]. While commonly found as a commensal in the gastrointestinal tract, *Proteus mirabilis* is implicated in opportunistic infections, particularly urinary tract infections (UTIs) [5]. It is associated with stone formation due to its urease activity, promoting struvite crystal precipitation in alkaline urine, thereby contributing to the pathogenesis of UTIs [2]. This bacterium possesses inherent resistance mechanisms that challenge conventional antibiotic therapies, posing a substantial clinical challenge [6]. These features underscore its significance in clinical settings posing challenges in devising effective treatment strategies.

One crucial component contributing to the pathogenicity of *Proteus mirabilis* is the *ureR* gene. This gene encodes the *UreR protein*, a transcriptional activator that enhances the bacterium's response to environmental signals, such as the presence of urea or changes in pH levels [7]. The presence of the *ureR*

gene in *Proteus mirabilis* enhances its ability to adapt to the urinary tract environment, promoting bacterial survival, colonization, and persistence [8]. In UTIs caused by *Proteus mirabilis*, the *ureR* gene enables the bacterium to regulate the expression of urease genes in response to urinary tract conditions [9]. In response to urea presence and acidic pH within human cells, the *UreR* protein activates transcription of urease genes, resulting in increased urease enzyme production [9]. This enzyme hydrolyzes urea to produce ammonia and carbonate ions, which alkalinize the urine and contribute to the formation of urinary stones and encrustations [9]. This can exacerbate UTI symptoms and contribute to the development of complications such as urinary obstruction and pyelonephritis [2]. In addition, Proteus mirabilis biofilms formed on the surface of urinary stones can serve as reservoirs for recurrent infections and contribute to treatment failure [8].

The escalating global issue of antibiotic resistance intensifies the difficulty in effectively treating infections caused by *Proteus mirabilis*. For instance, strains of *Proteus mirabilis* have exhibited resistance to commonly prescribed antibiotics such as cephalosporins (e.g., ceftriaxone, cefuroxime), penicillins (e.g., ampicillin, amoxicillin), fluoroquinolones (e.g., ciprofloxacin, levofloxacin), and trimethoprim/sulfamethoxazole, limiting the efficacy of these drugs in managing *Proteus mirabilis* infections [10, 11]. Additionally, emerging resistance to aminoglycosides (e.g., gentamicin) and the development of extended-spectrum beta-lactamases (ESBLs) in some strains further complicate treatment options, emphasizing the urgency in exploring alternative therapies or combination approaches to address this growing resistance issue [12]. The organism's adeptness at developing resistance to multiple antibiotics underscores the urgent need for alternative therapeutic strategies.

Amid this landscape, the exploration of natural compounds and medicinal plants gains prominence. *Salvia officinalis*, commonly known as sage, stands as one such botanical resource that has garnered attention for its diverse medicinal properties [13]. This perennial herbaceous plant has a rich history in traditional medicine, known for its antimicrobial, anti-inflammatory, and antioxidant characteristics [14]. Salvia officinalis, has been extensively studied for its antimicrobial properties, with numerous research articles investigating its efficacy against various microorganisms. In a study by [15][16], the antimicrobial activity of sage extract was evaluated against a panel of clinically relevant microorganisms, including S*taphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans,* and *Aspergillus niger*. The findings revealed significant inhibition of bacterial and fungal growth by sage extract, highlighting its potential as a natural antimicrobial agent. While sage extract exhibits broad-spectrum antimicrobial activity, its effectiveness against specific microorganisms may vary. Sage extract shows potent activity against Gram-positive bacteria like Staphylococcus aureus, but its efficacy against Gram-negative bacteria such as Escherichia coli may be comparatively lower due to differences in cell wall structure and permeability [15]. Gram-positive bacteria have a thinner cell wall, while Gram-negative bacteria possess an outer membrane that limits penetration by antimicrobial agents like sage extract [17]. Furthermore, the development of antimicrobial resistance to medicinal plant poses a significant challenge in the treatment of infectious diseases [18]. While sage extract may offer a natural alternative to conventional antimicrobial agents, the emergence of resistance mechanisms could potentially limit its long-term efficacy. Therefore, ongoing research is needed to explore synergistic interactions with existing antibiotics and strategies to mitigate the development of resistance. Hence, this study aims to explore the potential of ethanolic extracts of from *Salvia officinalis* leaves on *Proteus mirabilis* isolates obtained from hospitalized UTI patients.

2. Materials and Methods

2.1 Study Area

The experimental research was conducted in Wukari, Taraba State, Nigeria. Wukari serves as the administrative hub of Wukari Local Government Area (LGA), characterized by its vibrant community [19]. The predominantly language spoken by the populace is the Jukun language [19]. The major occupation of the population is agriculture. Nonetheless few others who are not gainfully employed in the civil service, engage in trading [19]. Wukari shares its borders with neighboring towns such as Takum to the northeast, Bali to the northwest, Donga to the southwest, and Ibi to the southeast, providing a dynamic socio-cultural environment for research endeavors [19]. Wukari is host to the Federal University Wukari, Wukari General Hospital. Additionally, the bustling Wukari Main Market serves as a vital center of commerce, facilitating trade and economic activities [19].

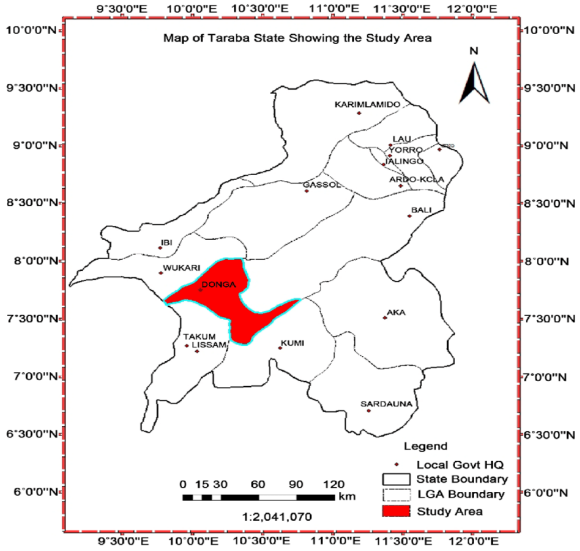

Figure 1: Study Area Source [20]

2.2 Methods

2.2.1 Study design

Study design

The study utilized a cross-sectional design to investigate the prevalence of urinary tract infections (UTIs) among hospitalized patients in Wukari, Taraba State, Nigeria. This study involved hospitalized patients aged between 18-50, with equal representation of males and females. Clean-catch urine samples were meticulously obtained from participants for subsequent laboratory analysis. Concurrently, the molecular identification of the *ure*R gene in Proteus mirabilis, was undertaken using polymerase chain reaction (PCR) amplification. Agarose gel electrophoresis confirmed the presence and size of amplified gene fragments, shedding light on the genetic characteristics and virulence potential of circulating *Proteus mirabilis* strains. Furthermore, the antimicrobial activity of ethanolic extracts from Salvia officinalis leaves against *Proteus mirabilis* was explored.

2.2.2 Study population

The study population consisted of hospitlised patients within the ages of 18 to 50, receiving treatment in hospital in Wukari, Taraba State.

2.2.3 Sample size

Given the research's focus on assessing the effect of *Salvia officinalis* on *Proteus mirabilis* isolates from hospitalized individuals, practical considerations and the research objectives guided the sample size. Hence, the sample size for this research comprises 20 consenting individuals, with equal representation of 10 males and 10 females.

2.2.4 Ethical clearance

Ethical approval was granted by the Research Ethics Committee of the Department of Microbiology, Federal University Wukari.

2.2.5 Inclusion criteria

The inclusion criteria encompassed individuals within the designated age range. Gender balance was maintained to obtain a comprehensive representation of both sexes in the sample. Exclusion criteria included patients who did not consent to participate in the study and those with comorbidities that could confound the study's outcomes.

2.2.6 Samples Collection and Transportation

Early morning mid-stream urine samples were aseptically collected from participates in sterile containers adhering to standard operating procedure (SOP) described by [21]. Each container was appropriately labeled with patient information. In other to maintain microbiological integrity and prevent overgrowth during transit to the laboratory, a gram of boric acid powder was added to each urine sample container upon collection [21].

2.2.7 Storage and Stability

Samples and reagents were handled meticulously following the SOP described by [21] to maintain integrity. Boric acid preserved urine samples. Prompt transportation to the lab prevented degradation. Reagents were stored per guidelines, and quality control ensured effectiveness, maintaining accuracy.

2.3 Laboratory Analysis

2.3.1. Isolation of Proteus mirabilis

The spread-plate technique was used to culture urine sample on MacConkey agar plates following the SOP described by [22]. Following incubation at 32°C for 24 hours, colonies exhibiting characteristic morphology were observed. Subsequently, suspected colonies were sub-cultured onto Nutrient Agar plates for further analysis. Selected colonies displaying an irregularly edged creamy-white appearance were identified for subsequent biochemical analysis. *Proteus mirabilis* ATCC 29906 served as a standard reference strain for proficiency testing and assay validation, ensuring the accuracy and reliability of microbiological assays.

2.3.2. Molecular Identification of *UreR* Gene:

Suspected colonies underwent molecular identification using Polymerase Chain Reaction (PCR) targeting the *ureR* gene following the SOP decribed by [23]. The DNA extraction from isolates was conducted using the boiling method to break down the cell wall and membrane and release genomic material. Subsequently, the extracted DNA was quantified using spectrophotometry to ensure sufficient yield and quality for downstream processes. The amplification of the *UreR* gene of *Proteus mirabilis* was conducted using a meticulously prepared master mix, comprising distilled water (35 µl), Taq DNA polymerase (1 µl), dNTPs (4 µl), buffer (5 µl), MgCl2 (3 µl), and DNA template (1 µl). Also, 0.5 µl each of forward primer (5'- GCTGAAGCTGCTATCGTCTC -3') and a reverse primer (5'- ATGGTGAAGACGGTTCTGGA -3'), (Table 1) were added to the master mix to give a final volume of 50 µl. The PCR reaction commenced with an initial denaturation step at 95°C for 3-5 minutes to separate the double-stranded DNA. This was followed by cycling phases involving denaturation at 95°C for 30 seconds to 1 minute, annealing of primers at 55-60°C for 30 seconds, and extension at 72°C for 30 seconds to 1 minute, repeated for 25 to 35 cycles to amplify the target DNA fragment. A final extension step at 72°C for 5-10 minutes was performed to ensure complete amplification. Amplicons were subjected to electrophoresis to confirm amplicon sizes using a 2% agarose gel prepared with tris-acetate-ethylenediaminetetraaceticacid (TAE) buffer. The size of amplicons was determined with the aid of a 100 bp marker. Electrophoresis was conducted at 100 volts for 30-45 minutes, allowing DNA fragments to migrate through the gel, separating based on size. Following completion, the gel was visualized under UV light or with a gel documentation system. The resulting band pattern was observed and compared to the DNA ladder to confirm the presence and size of the amplified UreR gene fragment specific to Proteus mirabilis.

2.3.3. Preparation of *Salvia officinalis* Extracts

The process began with the separate collection and processing of dried and fresh *Salvia officinalis* leaves. Crude ethanolic extracts were obtained by soaking the leaves in ethanol for 24 hours at room temperature following the SOP previously described by [24]. The soaking period facilitated the efficient extraction of bioactive compounds from the leaves into the solvent. Following the designated soaking period, the solvent was evaporated from both the dried and fresh leaves, resulting in concentrated solutions of the extract. Subsequently, the extract was separated from the leaves by filtration using filter paper and a funnel into a sterile container. Finally, the extracts were further prepared by dissolving specific amounts in 10ml of dimethyl-sulfoxide, creating a range of extract concentrations of (2.0mg, 4.0mg, 6.0mg, 8.0mg, 10.0mg) which was used for the assay.

2.3.4. Antimicrobial assay

The Agar-well diffusion technique was utilized as described by [25] to assess antimicrobial susceptibility of isolates to extracts of *Salvia officinalis*. Using pour plate techniques, one of the two isolates of *Proteus mirabilis* was randomly selected and inoculated onto two Mueller-Hinton agar plates. Each plate represented either dried leaf or fresh leaf extracts. Subsequently, 7 wells were carefully formed on the agar surface, into which various concentrations of *Salvia officinalis* extracts were accurately dispensed. To ensure experimental integrity, negative controls (ethanol without extract) and a positive control (Ciprofloxacin 30µg) were integrated into the setup. The negative control allowed evaluation of any inherent antimicrobial properties in the ethanol solvent without the extract, while Ciprofloxacin served as a benchmark for expected antimicrobial activity. This comprehensive approach facilitated a thorough assessment of the antimicrobial effects of Salvia officinalis extracts against Proteus mirabilis, supported by appropriate controls for result validation

2.4 Statistical Analysis

Data was processed and analyzed using Microsoft Excel. Also, Chi-square test was used to determine the significance of any research outcome.

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Table 1: Primer set for *ureR* gene

SN	Organism	Virulent gene primers	Base pair (bp) size	Source of primer
1	***Proteus mirabilis***	***UreR* Fw (5'-GGTGAGATTTGTATTAATGG -3')** ***UreR* Rev (5'-ATAATCTGGAAGATGACGAG -3').**	**225bp**	[26]

3.0 Results and Discussion

3.1 Results

3.1.1 Prevalence of Proteus mirabilis

The research findings shed light on significant disparities in urinary tract infection (UTI) positivity rates among participants across various age brackets (Table 2). *Proteus mirabilis* was isolated from two of the 20 urine samples collected, resulting in a prevalence of 10% (X^2 = 2.1667, df = 2, P= 0.035). The positive urine samples belong to female while no positive isolates were observed for males. In the 18-28 age group, one out of six female patients tested positive for UTIs, resulting in a positivity rate of 16.7%. Similarly, among females aged 29-39, one out of three patients tested positive, yielding a notably higher positivity rate of 33.3%. Remarkably, no positive cases were identified among female patients from other age categories and male patients across all age categories.

3.1.2. Identification of isolates

Cultural and biochemical analysis of isolates is presented in Table 3. The results indicated specific biochemical reactions, such as urease production. On MacConkey and Nutrient agar, the cultural analysis exhibited the growth of Proteus mirabilis colonies, aiding in identification based on their distinct morphological characteristics.

Table 2 Age Group and Sex of participants

	Hospitalised patients						
AGE GROUPS	**18 -28**		**29-39**		**40-50**		**Total**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	
Samples Examined	7	6	2	3	1	1	**20**
Positive Sample	0	1	0	1	0	0	**2**
Positive %	0	16.7	0	33.3	0	0	**10**

Key: M: Male, F: Female

Table 3. Morphological and biochemical characteristics of isolates

Isolate	Morphological Characteristic	Biochemical test								Gram stain
		CAT	GAS	MR	LAC	SUC	MAL	GLU	URE	
Proteus mirabilis	On MacConkey agar, the colonies appear pink with a swarming growth pattern, while on nutrient agar, they display larger, creamy-white colonies	+	+	-	-	-	+	+	+	*Negative rod*

Key: +: Positive, -: Negative, CAT: Catalase, URE: Urease, MAL: Maltose, LAC: Lactose, SUC: Sucrose, GLU: Glucose, GAS: Gas production, MR: Methyl red

3.1.3. Antimicrobial activity of *Salvia officinalis*

Table 3 reveals varying zones of inhibition (ZIB) for Salvia officinalis extracts against Proteus mirabilis. Specifically, dried leaf extracts exhibited ZIB ranging from 12 to 26mm across different ethanolic concentrations (2.0mg-10ml, 4.0mg-10ml, 6.0mg-10ml, 8.0mg-10ml, 10.0mg-10ml). In comparison, ZIB for fresh leaves ranged from 10 to 24mm. Notably, the negative control, represented by ethanol without extract, showed no significant ZIB (≤3mm), indicating the absence of inherent antimicrobial properties in the solvent alone. As a positive benchmark, Ciprofloxacin at 30µg demonstrated a substantial ZIB (≥29mm), reaffirming its efficacy as a known antimicrobial agent and a proven reference for assessing the efficacy of the extracts. These results emphasize the notable antimicrobial activity of Salvia officinalis extracts against Proteus mirabilis, with dried leaves exhibiting particularly enhanced inhibitory effects compared to fresh leaves, supporting the potential utility of this plant in the development of antimicrobial therapeutics.

Table 4 Antimicrobial effects of Salvia officinalis extracts on Proteus mirabilis isolate

Concentration	***Salvia officinalis***	
	Fresh leave	**Dried Leave**
2.0mg-10Ml	10mm	12mm
4.0mg-10mL	13mm	15mm
6.0mg-10mL	17mm	19mm
8.0mg-10mL	20mm	22mm
10.0mg-10mL	24mm	26mm

Key: mg: Milligram, mL: Millilitres, mm: Millimetre

3.1.4. *UreR* Gene Amplification

The agarose gel electrophoresis indicating PCR amplification products of 225 bp UreR gene in Proteus mirabilis isolates is shown in Figure 1. This indicates that the specific primers designed for the UreR gene effectively bound to the DNA of Proteus mirabilis and facilitated the amplification process. The visualization of distinct bands corresponding to the expected size of the UreR gene fragment confirms the presence of the gene in the isolates under study

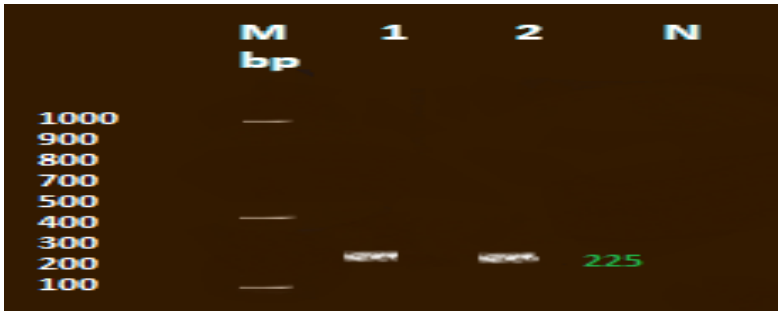

Figure 1: Agarose gel electrophoresis showing the UreR Gene in Proteus mirabilis
Lane M: 100 bp marker, Lane N: Negative control, Lane 1-5: amplicons

3.2 Discussion of Results

In the study of 20 urine samples collected from volunteers, *Proteus mirabilis* was isolated from 10% of the samples. Notably, the positive isolates were exclusive to female participants, with a prevalence rate of 20% among females. In contrast, no positive isolates of *Proteus mirabilis* were observed among male volunteers. This finding is consistent with that of [27] which observed a 20.7% prevalence of *Proteus mirabilis* from clinical isolates. While the sample size is limited, these findings emphasize the importance of considering gender-specific factors in the study of microbial prevalence in urinary samples and warrant further exploration with larger and more diverse cohorts to validate and generalize the observed trends. The observed variations in zones of inhibition (ZIB) denote the varying degrees of antimicrobial efficacy exhibited by *Salvia officinalis* leaf extracts against *Proteus mirabilis*. This experimentation showcased differing inhibitory

effects between dried and fresh leaf extracts, with dried variants generally demonstrating larger ZIB compared to their fresh counterparts across a spectrum of concentrations (ranging from 2.0mg-10ml to 10.0mg-10ml). According to [28], dried leaf extracts have consistently shown better antimicrobial activity against clinical bacterial isolates. The negligible ZIB of ≤3mm in the negative control, comprising solely ethanol without extract, signifies the absence of inherent antimicrobial properties in the solvent alone, thereby affirming the specificity of the observed effects. Ciprofloxacin at 30µg, employed as a positive benchmark, showcased a substantial ZIB (≥29mm), validating the methodology used and highlighting the potent inhibitory capacity of the antibiotic. The effectiveness of Ciprofloxacin at 30µg against isolates of *Proteus mirabilis* has been demonstrated in another study by [29]. Nonetheless, antimicrobial resistance to Ciprofloxacin by over 30% of clinical isolates of *Proteus mirabilis* has been documented in a study by [30].

The concentration-dependent nature of the antimicrobial activity observed in the *Salvia officinalis* extracts signifies a crucial aspect of their potential as natural antimicrobial agents. The experiment demonstrated that as the concentration of *Salvia officinalis* extracts increased, there was a proportional increase in the inhibitory effect on *Proteus mirabilis* isolates, as evidenced by larger zones of inhibition. This concentration-dependent trend suggests that the bioactive compounds responsible for the antimicrobial activity are present in higher concentrations in the extracts at elevated doses [31]. The increasing effectiveness with higher concentrations implies a dose-response relationship, reinforcing the idea that the concentration of these bioactive compounds plays a crucial role in inhibiting the growth of *Proteus mirabilis* isolates. Concentration dependent effectiveness of extracts has been previously reported by [32]. The observed concentration-dependent antimicrobial potency of *Salvia officinalis* extracts has significant implications for potential applications. Firstly, it suggests that these extracts could be used as a natural alternative to synthetic antimicrobial agents. Natural antimicrobials are often preferred due to their perceived safety, lower likelihood of inducing resistance, and potential for broader acceptance in various applications, such as food preservation [33]. Moreover, the concentration-dependent response opens avenues for the controlled utilization of *Salvia officinalis* extracts in different contexts. Tailoring the concentration of the extract could potentially optimize its antimicrobial efficacy for specific applications. For instance, in food preservation, where inhibiting microbial growth is essential, the use of *Salvia officinalis* extracts at concentrations that show the highest efficacy could be explored [34]. However, it is crucial to strike a balance between increasing concentrations for enhanced antimicrobial activity and ensuring safety for consumption or application [35]. Further research is needed to establish the optimal concentration range that maximizes antimicrobial effectiveness while maintaining safety standards.

Salvia officinalis is known to contain various bioactive compounds, including phenolic acids, flavonoids, and essential oils, which have been reported to possess antimicrobial properties [36]. These compounds work synergistically to exert antimicrobial effects through several potential mechanisms. Similarly, [37] have highlighted in previous study the antimicrobial effectiveness of phytochemicals on an array of microorganisms. Phenolic acids, abundant in sage, are renowned for their antioxidant and antimicrobial activities. These compounds play a crucial role in the plant's defense mechanisms and have been studied for their potential therapeutic effects. In the context of antimicrobial properties, phenolic acids are known to interfere with the growth and survival of microorganisms, including bacteria [38]. Their ability to disrupt cellular processes and inhibit the synthesis of essential microbial components contributes to their antimicrobial efficacy [39]. While acting as antioxidants, phenolic compounds such as Rosmarinus acid can also generate reactive oxygen species (ROS) within bacterial cells. Excessive ROS production can overwhelm the bacterial defense mechanisms, leading to oxidative stress and damage to cellular components, including proteins and DNA [38]. Ultimately, essential oils in Salvia officinalis have been reported to inhibit the formation of bacterial biofilms. Essential oils, particularly terpenes, are known for their direct antimicrobial activity. They can interfere with bacterial cell walls, inhibit protein synthesis, and disrupt other essential cellular processes [38]. This direct action contributes to the observed zones of inhibition in agar diffusion assays. Flavonoids, another class of bioactive compounds found in *Salvia officinalis*, also exhibit antimicrobial properties. Flavonoids are known for their diverse biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects [40]. Studies have suggested that flavonoids can disrupt bacterial cell membranes, interfere with microbial enzymes, and modulate microbial gene expression, ultimately inhibiting the growth and survival of bacteria [41]. The presence of flavonoids in sage contributes to its potential as an antimicrobial agent against various pathogens, including *Proteus mirabilis* [42}. Essential oils extracted from *Salvia*

officinalis are a concentrated source of volatile compounds that contribute to the plant's characteristic aroma and medicinal properties [43]. These essential oils have been extensively studied for their antimicrobial potential. The volatile components, such as monoterpenes and sesquiterpenes, can exhibit antimicrobial effects by disrupting microbial membranes, interfering with cellular processes, and inducing oxidative stress in microorganisms [44]. Also, essential oils in Salvia officinalis, particularly rich in monoterpenes like thujone and camphor, have been reported to disrupt bacterial cell membranes [44]. This disruption can lead to increased permeability, leakage of cellular contents, and ultimately, cell death. The lipophilic nature of essential oils allows them to interact with the lipid bilayer of bacterial cell membranes, compromising their integrity [44]. *Salvia officinalis* essential oil has demonstrated inhibitory effects against a range of bacteria such as *Streptococcus*, making it a valuable resource in the exploration of natural antimicrobial agents [45]. The collective action of these bioactive compounds in *Salvia officinalis* presents a multifaceted approach to combating microbial pathogens, including *Proteus mirabilis*. Some bioactive compounds in *Salvia officinalis* may interfere with bacterial quorum sensing, a communication system that regulates gene expression in response to cell population density [46]. Disrupting quorum sensing can hinder the coordination of bacterial activities, including virulence factor production and biofilm formation, contributing to the inhibition of pathogenicity. *Salvia officinalis* extracts may exert immunomodulatory effects, influencing the host's immune response. The extracts can contribute to the overall defense against bacterial infections by stimulating the activity of immune cells or modulating the release of inflammatory mediators [47]. The synergistic effects of phenolic acids, flavonoids, and essential oils contribute to the plant's overall antimicrobial efficacy [46]. While the specific mechanisms of action may vary for each compound, their combined presence in sage enhances its potential as a natural and holistic alternative for addressing antibiotic-resistant infections [47].

The comparison with the negative and positive controls serves as a critical aspect of validating the reliability and significance of the results obtained from the antimicrobial effects of *Salvia officinalis* extracts on *Proteus mirabilis* isolates. Negative control, represented by ethanol without *Salvia officinalis* extract, provides a baseline for assessing any intrinsic antimicrobial properties of the solvent and ensures that any observed effects are indeed attributable to the *Salvia officinalis* extracts. The minimal inhibition zone (≤3mm) in the negative control confirms that ethanol alone does not possess significant antimicrobial activity against the *Proteus mirabilis* isolates. This reinforces the notion that the observed inhibitory effects in the experimental groups are indeed due to the presence of *Salvia officinalis* extracts and not influenced by the solvent. The positive control, utilizing Ciprofloxacin as an established antibiotic, serves as a benchmark for the susceptibility of the *Proteus mirabilis* isolates to antimicrobial agents. The substantial inhibition zone (≥29mm) in the positive control validates the experimental setup, confirming that the *Proteus mirabilis* isolates used in the study are susceptible to inhibition by a well-known and widely used antibiotic. This control provides a point of reference, indicating that the *Proteus mirabilis* isolates are viable and responsive to antimicrobial agents, setting the stage for evaluating the effectiveness of *Salvia officinalis* extracts. In light of these controls, the results from *Salvia officinalis* extracts become more meaningful. The clear and concentration-dependent zones of inhibition observed in the experimental groups, in comparison to the controls, suggest that *Salvia officinalis* extracts indeed possess antimicrobial activity against *Proteus mirabilis* isolates. The comparison lends credibility to the experimental design, ensuring that any observed effects are not artifacts but genuine indications of the antimicrobial potential of *Salvia officinalis*.

The comparison between fresh and dried leaves of *Salvia officinalis* in terms of their antimicrobial efficacy against *Proteus mirabilis* isolates provides valuable insights into the impact of processing on the bioactivity of the plant. The observation that dried leaves generally exhibited larger zones of inhibition compared to fresh leaves at each concentration suggests notable differences in antimicrobial potency between the two forms. This occurrence has been observed by [48] which documented a greater zone of inhibition for dried leaf of *Vernonia amygdalina* on *Escherichia coli* and *Proteus species* isolated from urine samples compared to the fresh leaves of *Vernonia amygdalina.* One of the primary factors contributing to the variation in antimicrobial efficacy is likely the differences in bioactive compound concentrations between fresh and dried leaves [49]. During the drying process, certain volatile compounds, such as essential oils, may become more concentrated due to the removal of water content [50]. *Salvia officinalis* is known to contain various bioactive compounds, including flavonoids, phenolic acids, and essential oils, which contribute to its antimicrobial properties [31]. The higher concentration of these compounds in dried leaves could explain the observed increase in antimicrobial

efficacy [51]. Fresh leaves contain a significant amount of water, and this high-water content might dilute the concentration of bioactive compounds [52]. The drying process, by removing water, could lead to a more concentrated extract, enhancing its antimicrobial effects. Water content can also affect the release and availability of bioactive compounds, influencing their interactions with bacterial cells [48]. The drying process may also contribute to the stability of certain bioactive compounds. Fresh leaves might contain compounds that are more prone to degradation or evaporation, whereas the drying process could preserve these compounds more effectively [52]. This increased stability could result in a more sustained release of antimicrobial agents from the dried leaves, contributing to a prolonged inhibitory effect. Also, the drying process itself may induce chemical changes in the composition of the leaves. For instance, enzymatic reactions or oxidative processes may occur during drying, leading to the formation of new bioactive compounds or alterations in the structure of existing ones [53]. These changes could influence the overall antimicrobial activity of the dried leaves. The method used to extract bioactive compounds from fresh and dried leaves may also play a role [54]. Different compounds may have varying solubilities in the extraction solvent, and the efficiency of extraction can vary between fresh and dried plant material. In this current study, ethanol was used. Ethanol has been documented by 34 to be an effective menstruum for both dried and fresh leaf [48]. Water has also proved to be effective menstruum in the study by [31].

The isolation of *Proteus mirabilis* from two out of the 20 urine samples collected raises interesting observations, particularly the gender-based difference in positivity. All positive isolates were from female volunteers, while no positive isolates were observed among males. This gender-specific disparity in prevalence rates aligns with the well-documented higher susceptibility of females to urinary tract infections, reflecting the anatomical and physiological differences that make women more prone to such infections. It's worth noting that females generally have a higher susceptibility to urinary tract infections (UTIs) due to anatomical differences, such as a shorter urethra, which facilitates easier entry of bacteria into the urinary tract. This is not dissimilar with the findings of [55] which isolated *Proteus mirabilis* from 3 women. *Proteus mirabilis* is a common causative agent of UTIs, and its presence in the urine samples of female volunteers aligns with the higher incidence of UTIs in women. Possible contributing factors to the observed gender disparity could include hormonal influences, sexual activity, and individual variations in hygiene practices [56]. Hormonal fluctuations, particularly during the menstrual cycle, can affect the microbial environment in the urinary tract, potentially influencing the prevalence of bacteria like *Proteus mirabilis* [57]. Additionally, sexual activity can introduce bacteria into the urinary tract, contributing to the gender-specific patterns observed [58]. Interestingly, findings of this study suggest that age-related factors may play a role in influencing susceptibility to urinary tract infections (UTIs) among hospitalized patients. The only two isolates from this current research were recorded among females belonging to age groups 18–28 and 29-39, indicating a potential age-related dominance in UTI vulnerability. This outcome has been argued by previous studies [59] to be attributed to physiological changes associated with aging, including hormonal fluctuations, menopause, or alterations in urinary tract structure and function. Additionally, lifestyle factors such as sexual activity or childbirth may contribute to the heightened susceptibility to UTIs in this age group [59]. While these findings provide valuable insights into the microbial composition of the collected urine samples, it's essential to interpret them with caution. The observed prevalence may be influenced by the sample size and the specific characteristics of the study population. Further investigations, including a larger and more diverse sample pool, would be beneficial to generalize and validate these gender-specific trends in *Proteus mirabilis* prevalence. Additionally, exploring other factors such as, underlying health conditions, and hygiene practices among the participants could provide a more comprehensive understanding of the observed differences in bacterial isolation.

Importantly, the molecular characterization of the *UreR* gene of *Proteus mirabilis* culturally identified from urine samples holds significant importance due to its role in urease production, which is a pivotal factor in the pathogenesis and survival. The *UreR* gene stands out as a vital marker for *Proteus mirabilis* [26]. Tests that target this gene, like PCR amplification assays, offer precise and sensitive methods for detecting and confirming the presence of *Proteus mirabilis* in urine samples [60]. The urease activity regulated by the *UreR* gene generates an alkaline environment, a characteristic often linked with *Proteus mirabilis* infections [61]. While pH tests and assays checking for urease activity serve as indicators, molecular techniques focusing on the *UreR* gene provide highly specific and reliable identification [60]. The urease production facilitated by the *UreR* gene plays a significant role in the pathogenicity of *Proteus mirabilis* [62]. This enzyme's activity not only promotes urinary stone formation but also creates an immune-evading

environment, contributing to the bacterium's persistence within the urinary tract [62]. Moreover, the alkaline conditions resulting from urease activity impact antimicrobial susceptibility [63]. The elevated pH impedes the effectiveness of certain antibiotics, making it harder for them to penetrate bacterial cells and exert their antimicrobial effects [64]. Additionally, bacterial encrustation within urinary stones offers a sheltered environment where the bacteria may evade antibiotic treatments [65]. Understanding how the *UreR* gene influences urease activity sheds light on the mechanisms behind *Proteus mirabilis*' virulence and its ability to resist antimicrobial treatments. This comprehension is crucial in guiding effective diagnostic approaches and developing targeted therapeutic strategies to combat Proteus-related urinary tract infections while considering the pathogen's propensity for antimicrobial resistance.

4. Conclusion

The demonstrated antimicrobial efficacy of ethanolic extracts from *Salvia officinalis* against *Proteus mirabilis* isolates marks a significant stride in the quest for alternative treatments for urinary tract infections (UTIs). These findings shed light on the potential of natural compounds, such as those found in *Salvia officinalis*, to offer effective therapeutic options, especially in the face of rising antimicrobial resistance. The research demonstrated varying zones of inhibition, with dried extracts showing greater inhibitory effects across different concentrations This discrepancy was attributed to moisture content favoring microbial growth in fresh leaves.

Moreover, the observed gender-based differences in the prevalence of *Proteus mirabilis* among female participants accentuate the complex interplay of anatomical, physiological, and possibly hormonal factors contributing to UTI susceptibility. This highlights the importance of considering gender-specific approaches in UTI management and research.

The molecular characterization of the *UreR* gene of *Proteus mirabilis* offers invaluable insights into the pathogenesis and antimicrobial resistance mechanisms of this opportunistic pathogen. It provides a foundation for the development of targeted diagnostic and therapeutic strategies, potentially improving patient outcomes and reducing the burden of UTIs.

However, while these findings hold promise, it is essential to approach them with caution and recognize the need for further research. Rigorous clinical trials and in-depth studies are warranted to fully elucidate the mechanisms of action, safety profile, and optimal dosing regimens of *Salvia officinalis* in clinical settings. Additionally, exploring potential synergistic effects of *Salvia officinalis* with conventional antibiotics may enhance treatment efficacy and combat antimicrobial resistance.

In light of the increasing prevalence of antibiotic-resistant infections, there is a pressing need to explore alternative treatment modalities. *Salvia officinalis* stands as a promising candidate, but its practical application hinges on comprehensive research and evidence-based practice. Ultimately, bridging the gap between laboratory discoveries and clinical implementation enables us to harness the therapeutic potential of *Salvia officinalis* effectively, addressing the global challenge of UTIs and antimicrobial resistance.

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