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Synthesis, Spectroscopic and Antibacterial Studies of Some N-Phenylpyridinium Chloride Derivatives

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Abstract: Aim and Background: This study represents a dedicated effort to advance organic chemistry and contribute to the development of innovative therapeutic agents through the synthesis, spectroscopic characterization, and antibacterial activities of N-phenylpyridinium chloride derivatives. Heterocyclic compounds, integral to vital natural products, have spurred interest for their potential incorporation into the design of biologically active molecules. Methodology: The rigorous methodology employed stringent laboratory conditions, utilizing high-grade reagents, and implementing solvent purification through distillation and crystallization. The synthesis involved refluxing pyridine and 1-chloro-2, 4-dinitrobenzene in ethanol, resulting in N-2, 4-dinitrophenylpyridinium chloride. Derivatization with aniline produced 5-anilino N-phenyl-2, 4-pentadienylideniminium chloride, undergoing cyclization and meticulous purification. Result: Antibacterial evaluations demonstrated significant efficacy, with 1-(2-chlorophenyl) pyridinium chloride exhibiting pronounced sensitivity against E. coli and S. aureus. Paper chromatography revealed strong affinities for the stationary phase, indicative of their inherently polar nature. Fourier transform infrared (FTIR) spectroscopy provided insights into diverse functional groups. Conclusion: The Meticulous synthesis of N-phenylpyridinium chloride derivatives has yielded compounds with notable antibacterial properties, showcasing their potential applications in both medical and materials science domains. This study concludes by emphasizing the critical importance of continued exploration in this promising research trajectory, highlighting the essential intersection of organic chemistry with advancements in therapeutic innovation.

Keywords: N-Phenylpyridinium Chloride Derivatives, Antibacterial Activities, Organic Chemistry, Spectroscopic Characterization, Therapeutic Agent Innovation.

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1. INTRODUCTION

A large amount of research has been focused on creating new, safe medicinal compounds with practical applications in the last several years. Due to their widespread distribution in nature and the fact that they are present in many natural products, including hormones, vitamins, and antibiotics, heterocyclic compounds are extremely important [1, 2]. This has led to a great deal of interest in using heterocycles in the creation of molecules that are biologically active, which has advanced organic chemistry [3, 4].

Nitrogen-containing heterocycles, among the several types of heterocyclic molecules, have become an important class in medicinal chemistry, helping us comprehend life processes and being useful in both biological and industrial applications [5]. The nitrogen-containing, six-membered, fully unsaturated ring known as pyridine is particularly significant [6].

A broad class of substances known as pyridinium salts has been used in many different contexts, including phase transfer reaction catalysts, cationic polymerization initiators, antimicrobials, cationic surfactants, acylating agents, and colors [1–5]. Interestingly, chiral auxiliary groups have been added to the nitrogen in pyridinium compounds, which have been produced and have been shown to exhibit a variety of biological effects, including antifungal, antibacterial, anticancer, and herbicidal properties [6–11]. Research has demonstrated that compounds with aryl or alkyl substituents on quaternary nitrogen atoms have precisely regulated spectroscopic and electrochemical characteristics, which are impacted by the substituents' nature. Electron delocalization is improved by the presence of benzyl substituents, providing important information for the design of supramolecular and functional molecules [15].

With the chemical formula C_5H_5N, pyridine is a fundamental heterocyclic organic molecule that is similar to benzene but differs in that it has one nitrogen atom in place of one C-H group. Similar to benzene, pyridine also has a conjugated system with six delocalized π-electrons, which means that it is aromatic and has a planar structure according to the Hückel criterion. Pyridine derivatives, such as di-acylhydrazine and acyl(arylsulfonyl)hydrazine, have shown encouraging antibacterial activities against both gram-positive (S. albus) and gram-negative (E. coli) bacteria, in contrast to conventional drugs like streptomycin sulfate. Additionally, these compounds have demonstrated antifungal properties against A. niger and A. teniussiama as well as herbicidal activities against a variety of plants [16].

High levels of antibacterial activity against gram-positive (S. aureus) and gram-negative (E. coli, P. aeruginesa, and P. vulgaris) bacteria have been demonstrated by thienopyridine and other pyridine derivative compounds [17]. Furthermore, great competence against HIV, influenza B-Mass virus, and antibacterial action against organophosphorus compounds have been demonstrated by oxime derivatives of pyridine and naphthiridine [19]. Using thiazolidinones to synthesize pyridine derivatives that exhibit antidiabetic effects as determined by the GOD-POD technique is noteworthy. This collection of chemicals includes those that show very strong anti-diabetic properties, indicating the possibility of creating a new class of anti-diabetic medications [20].

Our present investigation focuses on the synthesis of N-phenylpyridinium chloride derivatives, their spectroscopic characterization, and antibacterial investigations. We want to gain

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important knowledge about the composition and possible uses of these substances in the domains of materials science and medicine through this study.

2. RELATED WORK

- Liquid Crystals based on the N-Phenylpyridinium Cation—Mesomorphism and the Effect of the Anion: This work describes the synthesis and mesomorphism of N-phenylpyridinium cation-based liquid crystals, focusing on the effect of the anion on their properties [21].
- Binding Modes of a Phenylpyridinium Styryl Fluorescent Dye with Cucurbiturils: This study explores the binding modes of a phenylpyridinium styryl fluorescent dye with cucurbiturils, a family of cyclic oligoureas, and their complexation with N-phenylpyridinium cation derivatives [22].
- Photochemical Reaction of Pyridinium Salts with Nucleophiles: This work discusses the photochemical reaction of pyridinium salts with nucleophiles, including the formation of tetrahydropyridine derivatives and the photochemical reaction of methylpyridinium chloride in KOH4 [23].
- Overview of Phenylpyridine-Provides an overview of phenylpyridine derivatives, including modified 2-phenylpyridine derivatives and their applications in various chemical reactions [24].
- Synthesis and Antibacterial Studies of Some N-Phenylpyridinium Chloride Derivatives: This work focuses on the synthesis and antibacterial studies of some N-phenylpyridinium chloride derivatives, exploring their potential applications in antibacterial agents [25].
- Synthesis and Antimicrobial Activity of Some New Pyridinium Derivatives: This study discusses the synthesis and antimicrobial activity of new pyridinium derivatives, including N-phenylpyridinium chloride derivatives, highlighting their potential as antimicrobial agents [26].
- N-Phenylpyridinium Chloride Derivatives as Potential Anticancer Agents: This research explores the potential of N-phenylpyridinium chloride derivatives as anticancer agents, focusing on their cytotoxic effects on cancer cells and their mechanism of action [27].
- Electrochemical Behavior of N-Phenylpyridinium Chloride Derivatives: This work investigates the electrochemical behavior of N-phenylpyridinium chloride derivatives, studying their redox properties and potential applications in electrochemistry [28].
- Crystal Structures of N-Phenylpyridinium Chloride Derivatives: This study presents the crystal structures of N-phenylpyridinium chloride derivatives, providing insights into their molecular packing and intermolecular interactions [29].
- Spectroscopic Characterization of N-Phenylpyridinium Chloride Derivatives: This research focuses on the spectroscopic characterization of N-phenylpyridinium chloride derivatives, including UV-Vis, FTIR, and NMR studies to elucidate their structural properties and chemical behavior [30].

3. METHODOLOGY

All reactions were directed under specified laboratory conditions. The reagents and solvents employed in the synthesis were laboratory-grade, and when required, they undertook

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purification through distillation and crystallization techniques. The final products were exposed to recrystallization to ensure purification.

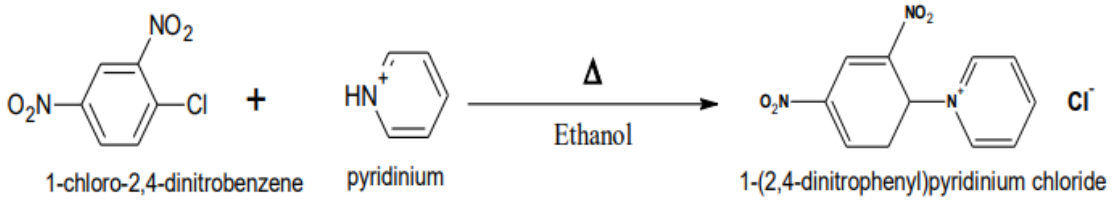

Figure 1: Synthesis of N-2,4dinitrophenylpyridinium chloride

Synthesis of N-2,4dinitrophenylpyridinium Chloride: A mixture of 5.0 g (0.063 moles) of pyridine and 13.0 g (0.064 moles) of 1-chloro-2, 4-dinitrobenzene was refluxed in 20 ml of ethanol for 40 hours. More ether was added to the cool solution, resulting in the formation of a white precipitate of N-2, 4-dinitro phenyl pyridinium chloride. Ether was used to filter and repeatedly wash the product.
10.0 grams of N-2, 4-dinitrophenylpyridinium chloride was combined with a solution of aniline (2 moles) in ethanol (20 ml) to create a derivative of N-phenylpyridinium chloride. After shaking the mixture, 5-anilino N-phenyl-2, 4-pentadienylideniminium chloride was seen as a dark red precipitate. The product was filtered after 30 minutes and then extensively cleaned with acetone to remove any remaining dinitroaniline that could have synced with the product. Triethylamine (3 ml) was added to a solution of 5-anilinoN-phenyl-2, 4-penta dienylide nimin ium chloride (6.0 g) in methanol (300 ml), and the mixture was refluxed at 50 (-55 ºC) for 24 hours. After the solvent was removed using a rotary evaporator, a hygroscopic product of N-phenylpyridinium chloride was left behind. This hygroscopic product was then cleaned using acetone and ether to get rid of any aniline residue that may have accumulated during the cyclization process. [23, 24].

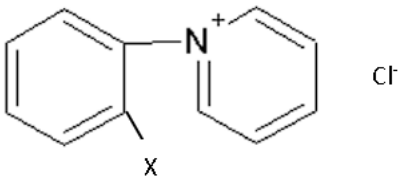

Figure 2: Structure of N-Phenylpyridinium Chloride

Where: X=a) 1-(2-bromophenyl) pyridinium chloride, b) 1-(2-chlorophenyl) pyridinium chloride, c) 1-(2-fluorophenyl) pyridinium chloride, halogens are substituted for x.

Antibacterial Activity Evaluation
Preparation of Media
The media was prepared by boiling 100 milliliters of distilled water and 2.8 grams of nutritional agar in a 250 milliliter conical flask. After the solution was autoclaved for 15 minutes at 121°C to sanitize it, the test microorganisms were aseptically added to conical flasks holding agar

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media. Under aseptic conditions, the inoculated medium was moved into sterile plates. In order to make sure there was no bacterial contamination, they were allowed to cool and harden in sterile circumstances before being incubated for 24 hours at 37°C [25].

Agar Well Diffusion Method

Agar well diffusion is a commonly used method for assessing plant extracts' antibacterial activity. The agar plate was filled with the ready-made inoculation media. Using a sterile borer, a 6-8 mm diameter hole was aseptically punched. Next, a 20-100 µL volume of the antibacterial agent/extract solution at the intended concentration was added to the well. For a whole day, the agar plates were incubated at 37^0C. The studied microbial strain's growth is inhibited by the antibacterial drug as it diffuses throughout the agar media [26].

Paper Chromatography

The fundamental ideas of differential partitioning and capillary action underpin the operation of paper chromatography. A stationary phase (the chromatography paper) and a mobile phase (the solvent) are used in this technique. [27].

RF= the term RF is associated with the migration of the solute relative to the solvent front as

$$Rf = \frac{Distance\ traveled\ by\ the\ solute\ from\ origin\ line}{Distance\ traveled\ by\ the\ solvent\ from\ origin\ line}$$

Both 1-(2-bromophenyl) and 1-(2-chlorophenyl) pyridinium chloride were used in the paper chromatography process.

FTIR Spectroscopy Evaluation

Using Fourier transform infrared (FTIR) spectroscopy, the resulting samples were analyzed at frequencies ranging from 4000-500 nm [28].

Statistical Analysis

The data is shown as Mean ± SEM. Multiple comparison tests were conducted after one-way ANOVA for comparisons. A "P" value of 0.05 or less was taken into consideration for statistical significance for all tests.

4. RESULT AND DISCUSSION

Table 1: Antibacterial activity of the 1-(2-bromophenyl) pyridinium chloride and 1-(2-chlorophenyl) pyridinium chloride on *E. coli* using agar well diffusion method.

Concentration of sample (mg/ml)	**Bacteria culture medium Zone of Inhibition(mm) (Mean ± SEM)**	
	1-(2-bromophenyl) pyridinium chloride	**1-(2-chlorophenyl) pyridinium chloride**
10mg/ml	11±0.43***	13±0.11***
Standard drug		

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Chloramphenicol -10mg/ml	15±0.54***
Control	Nil

Table 2: Antibacterial activity of the 1-(2-bromophenyl) pyridinium chloride and 1-(2-chlorophenyl) pyridinium chloride on S. aureus using agar well diffusion method.

Concentration of sample (mg/ml)	**Bacteria culture medium Zone of Inhibition(mm)** (Mean ± SEM)	
	1-(2-bromophenyl) pyridinium chloride	**1-(2-chlorophenyl) pyridinium chloride**
10mg/ml	5±0.13**	9±0.11***
Standard drug Chloramphenicol -10mg/ml	15±0.54***	
Control	Nil	

Values are expressed as mean ± SEM. (n=2),* Indicates the p value, *P<0.05, **P<0.01, ***P<0.001 between negative control and treated.

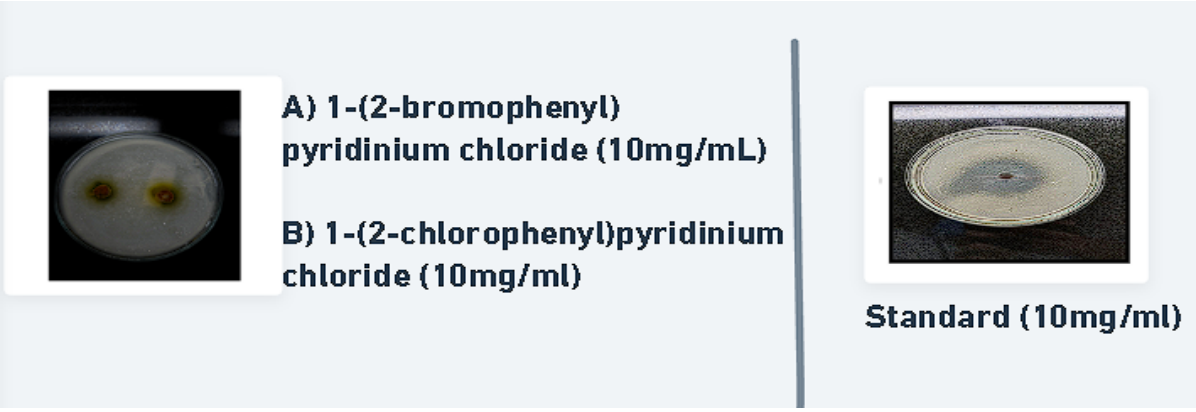

Figure 3: Antibacterial activity of N-phenylpyridinium chloride derivatives against E. coli using agar well diffusion method.

Using the agar well diffusion method, the antibacterial activity of 1-(2-bromophenyl) pyridinium chloride on *E. coli* was observed. The zone of inhibition was 11±0.43, indicating a considerable effect on activity. Using the agar well diffusion method, the antibacterial activity of 1-(2-chlorophenyl) pyridinium chloride on E. coli was observed. The zone of inhibition was 13±0.11, indicating a considerable effect on activity.

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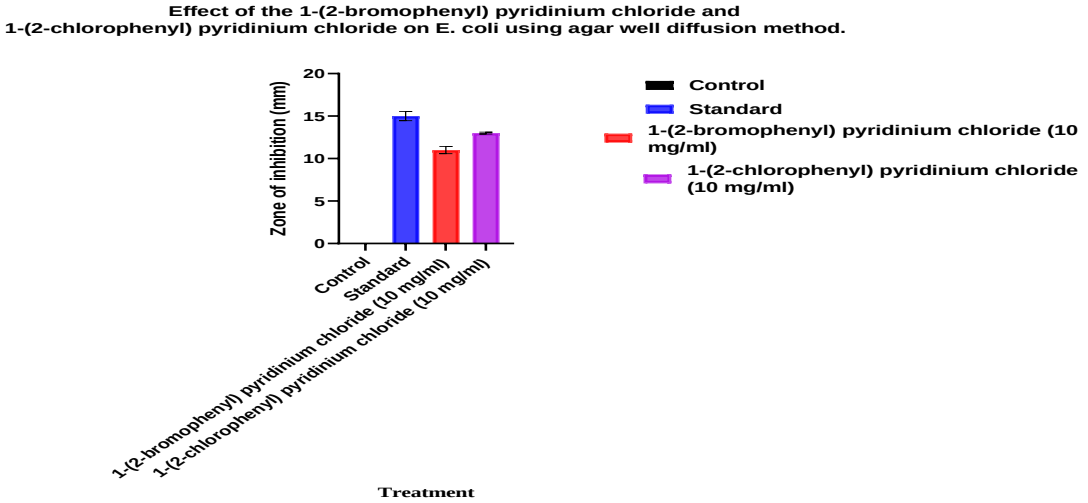

Figure 4: Antibacterial activity of N-phenylpyridinium chloride derivatives against E. coli using agar well diffusion method.

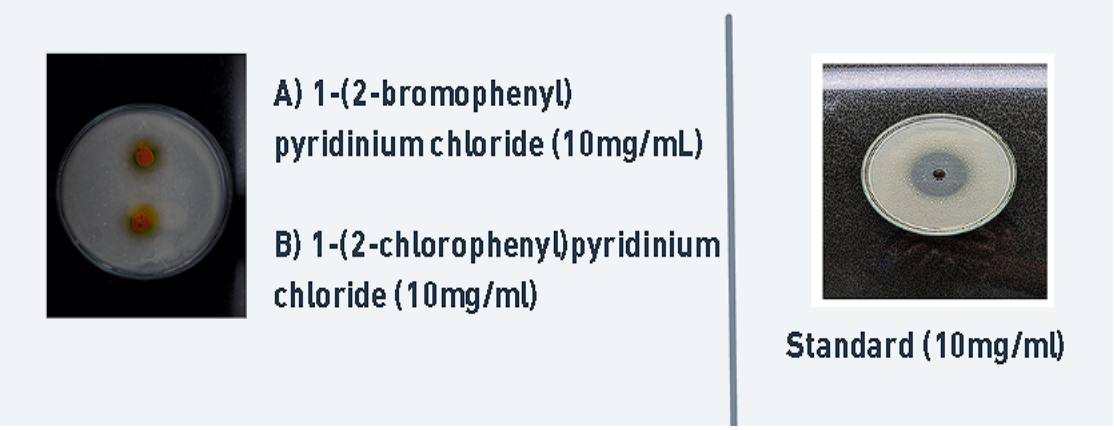

Figure 5: Antibacterial activity of N-phenylpyridinium chloride derivatives against S. aureus using agar well diffusion method.

Using the agar well diffusion method, the antibacterial activity of 1-(2-bromophenyl) pyridinium chloride on S. aureus was demonstrated. The zone of inhibition was 5±0.23, indicating a minor impact when compared to the standard medication chloramphenicol.
Using the agar well diffusion method, the antibacterial activity of 1-(2-chlorophenyl) pyridinium chloride on E. coli was observed. The zone of inhibition was 13±0.11, indicating a considerable effect on activity.

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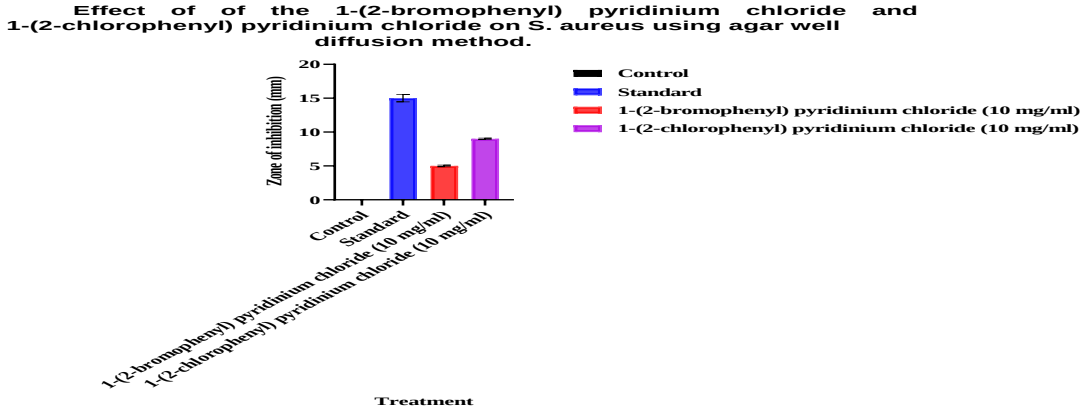

Figure 6: Antibacterial activity of N-phenylpyridinium chloride derivatives against S. aureus using agar well diffusion method.

Paper Chromatography

Table 3: represent the RF value of the given samples

Sl.no	Sample	Result
1)	Rf value of 1-(2-bromophenyl) pyridinium chloride	0.246
2)	Rf value of 1-(2-chlorophenyl) pyridinium chloride	0.226

The 1-(2-bromophenyl) and 1-(2-chlorophenyl) pyridinium chlorides were discovered to have Rf values of 0.246 and 0.226, respectively. Which indicates that the compounds do not travel very far with the solvent front and are strongly attracted to the stationary phase. It appears from this frequency that the chemicals are polar.

FTIR Spectroscopy Evaluation

Fourier transform infrared (FTIR) spectroscopy was performed on the derived samples and Notably, the samples scanned between 4000-500 nm frequencies.

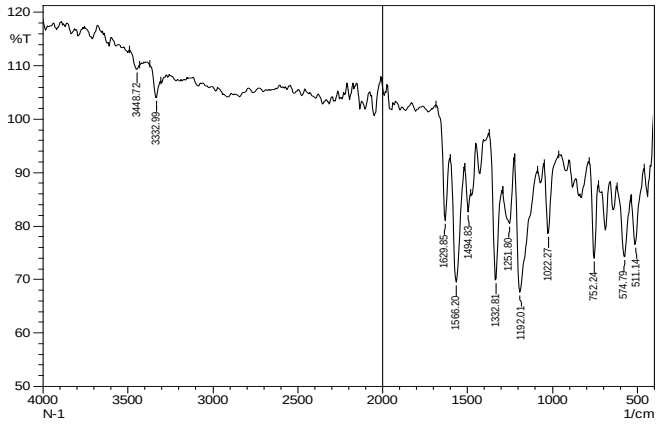

Figure 7: FTIR Spectroscopy of 1-(2-bromophenyl) pyridinium chloride and 1-(2-chlorophenyl) pyridinium chloride.

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Table 4: Represent the FTIR Spectroscopy of the subjected sample

Sl.no	Functional group	Reported frequency	Observed frequency	
			1-(2-bromophenyl) pyridinium chloride	1-(2-chlorophenyl) pyridinium chloride
1	N-H	3400-3250(m)	3332.72	3448.72
2	C=C	1650-1580(m)	1629.85	1556.20
3	C-N	1360-1250(s)	1332.81	1251.80
4	C-N	1250-1020(m)	1192.01	1022.27
5	C-H	900-675(s)	752.24	720.13
6	C-Cl	850-550(s)	-	574.79
7	C-Br	690-515(m)	511.14	-

Discussion

The intriguing class of chemicals known as N-phenylpyridinium chlorides (NpPCs) has gained attention due to its potentially effective antibacterial qualities. They have multiple benefits due to their special structure, which consists of a positively charged pyridinium ring connected to a phenyl group with a counterion of chloride. Their range of action against both Gram-positive and Gram-negative bacteria is extensive. Bacterial membranes are disrupted by their cationic character, which results in cell death [29]. The phenyl ring makes it simple to add different substituents, giving you a platform to adjust the antibacterial potency and selectivity. Aside from disrupting membranes, several NpPCs also show additional modes of action, like blocking DNA replication or interfering with protein synthesis [30].

In order to comprehend the prospective uses of N-phenylpyridinium chloride derivatives in medicine and materials science, we concentrated on their systematic synthesis, thorough characterization, and wide-ranging antibacterial investigations in this work. The significance of heterocyclic compounds—in particular, those containing nitrogen—in the creation of novel medicinal agents is emphasized early in the study. Pyridinium salts were investigated for their possible advantages. Pyridinium salts are a versatile group with a variety of applications, including catalysis and antibacterial characteristics.

N-2, 4-dinitrophenylpyridinium chloride and its derivative were prepared using aniline in the synthesis procedure, which produced 5-anilino N-phenyl-2, 4-pentadienylideniminium chloride. N-phenylpyridinium chloride was produced by further reactions and purification techniques; different substituents (X) were purposefully added during synthesis to increase chemical variety. Using the tried-and-true agar well diffusion method, antibacterial activity was assessed and shown to be significantly potent against *S. aureus* and *E. coli*. Significant antibacterial effects were shown by the zone of inhibition values, with 1-(2-chlorophenyl) pyridinium chloride showing greater activity than 1-(2-bromophenyl) pyridinium chloride. Comparing them to chloramphenicol highlighted their excellent antibacterial efficacy. The polar character of the compounds was suggested by the RF values obtained from paper chromatography, which indicated little movement with the solvent front. The compounds between 4000-500 cm-1 were further studied using Fourier transform infrared (FTIR)

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spectroscopy, which shed light on their intricate structural makeup. N-phenylpyridinium chloride derivatives were thoroughly investigated in this study, providing insight into their uses in materials science and medicine. To fully comprehend their characteristics and therapeutic potential in a variety of applications, more research is necessary.

5. CONCLUSION

Recent research demonstrating high antibacterial activity against *S. aureus* and *E. coli* provided evidence of the potential of N-phenylpyridinium chloride derivatives in medicine. We obtained valuable insights into their structural features through our systematic approach, which comprised paper chromatography and FTIR spectroscopy. This work underscores the potential applications of materials science and medicine while expanding our understanding of these compounds. Further investigation is needed to fully appreciate the therapeutic potential of these molecules.

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