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Fabrication and Characterization of Efficient Resin of Schiff base as Active Antimicrobial Agent

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ABSTRACT

In this study, formaldehyde (HCHO) and bis(2-Hydroxy-1-napthaldehyde)-o-phenylenediamine (BHNPhen) are heated to 120–130 °C for one to two hours in order to develop an effective antibacterial resin. Polymethylene bis(2-hydroxy-1-napthaldehyde)-o-phenylenediamine (PMBHNPhen), the resultant Schiff base, showed remarkable antibacterial qualities. Compounds consisting of a primary amine and a carbonyl molecule, which produce a chemical structure with an azomethine group (-HC=N-p), are referred to as "Schiff bases" by Hugo Schiff. The combination of Schiff bases and metal ions can lead to improvements in coordination and chemistry. The synthesized material was characterized by means of CHNS, FTIR, UV-visible spectroscopy, and SEM. analyses. The C.H.N. analytical results showed that the estimated values agreed well with the results, and the FTIR spectrum showed absorption bands at 1615cm^{-1} and 1620cm^{-1}. A UV-visible spectroscopy investigation revealed polymerization-related changes in the absorption spectra. Significant antibacterial action was demonstrated by the synthesized Schiff base and resin against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. This work highlights the potential uses of Schiff base resin in biomedical interventions and advances the development of antibacterial materials.

1. Introduction

A Schiff base is a subclass of imines, which are compounds made up of carbon-nitrogen double bonds and can either be para-aldehydes or ketamine, depending on their structural makeup. In 1864, Hugo Schiff, a well-known scientist, outlined condensation reactions of carbonyl compounds with primary amines forming Schiff bases [1]. The >CH=N is a Nitrogen associated with an aldehyde or a ketene. Ortho, para, and meta phenolic Schiff bases could undergo condensation *via* formaldehyde, resulting in successful yields of highly reactive resins. The resins can be used as adhesives in the hydrocarbon production of various plants. Several Schiff-based resins have thermal strength like polyamides [2-3]. These compounds are typically found together as an insoluble group that exhibits a wide range of biological activity. Schiff bases and resins are essential in many biological applications, mainly when synthesized with different functional groups. Reputably adaptable, Schiff bases can form complexes with common metals and demonstrate efficacy in a range of biological activities, including antioxidant, antiviral, anticancer, antibacterial, antifungal, anti-inflammatory, and anti-malarial activities. Antimicrobial resistance is driving an increase in the prevalence of bacterial illnesses, which highlights the need for efficient remedies. Schiff bases provide

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promise in resolving this issue, given their documented applicability in various disciplines. Beyond antimicrobials, their diverse responsibilities include antibacterial, antifungal, and other effects like antioxidant, antitumor, anticancer, and herbicidal properties. So, the main focus of the current research is to synthesize Schiff-base and Schiff-based resins with different functional groups [4].

The goal of this project is to further the development of materials with improved biological activity, with a focus on their potential application as effective antimicrobials against common pathogens such as *Staphylococcus aureus* and *Escherichia coli*. The thorough evaluation of these materials using methods including SEM analysis, FT-IR, UV-visible spectroscopy, and CHNS emphasizes how important they are to the advancement of antimicrobial research. The biological systems' transamination and racemization reaction explained the mechanism of the Schiff base's imine group (–N=CH). Therefore, these compounds are leading in developing therapeutic agents, the most essential sources of traditional medicine worldwide. Although the Schiff base of the aromatic aldehyde includes effective compounds and strength, it is relatively imbalanced and readily polymerizable. An agitated effect that typically happens in acid or base catalysis before leading to heat is the structure of the Schiff base as an aldehyde or ketene [5]. A water corrosive or base can hydrolyze certain Schiff bases into aldehydes, ketenes, or amines [6]. The stability is compromised on one side, and no carboxamide is formed. As a result, synthesizing several Schiff bases is well permitted at type acidic pH [7]. In general, Schiff bases make good chelating agents because they have functional groups like -OH or -SH to form a metal with a five- or six-member ring of ions and because they are relatively accepted formulations, synthetically produced, and complete products of the C=N group, especially around the methylamine group. Lone pairs of electrons in the imine nitrogen atom's sp^2 hybrid orbital have been found to have significant chemical and biological relevance, according to specific research [8]. Additional research in this area is highly desirable due to the flexibility of the Schiff base and the natural, analytical, and industrial applications of its complexes [9].

Bis(2-Hydroxy-1-napthaldehyde)-o-phenylenedimine (BHNPhen) and polymethylene bis(2-Hydroxy-1-napthaldehyde)-o-phenylenedimine (PMBHNPhen) are Schiff bases that are synthesized in the present investigation. The manufactured material has been satisfactorily characterized using various instrumental techniques, including CHNS, FT-IR, UV-visible spectroscopy, and SEM. The produced material has been well described using multiple instrumental methods, including CHNS, FT-IR, UV-visible spectroscopy, and SEM examination. The differences in absorption spectra caused by the polymerization were revealed by spectroscopic investigation in the ultra-violet visible. Schiff base most effectively and efficiently inhibited Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, with resin exhibiting remarkable results.

2. Materials and Methods

The chemicals and reagents used in this investigation are 2- hydroxy-1-napthaldehyde and o-phylenediamine. Solvents such as ethanol, methanol, chloroform, dimethyl sulfoxide, Agar, glucose, peptone, and meat extract were used. Each chemical and reagent used in this work was of pure grade of analytical value [10]. *Escherichia coli (Atcc-25922)* and *Staphylococcus aureus (Atcc-6538)* were used in the experiment.

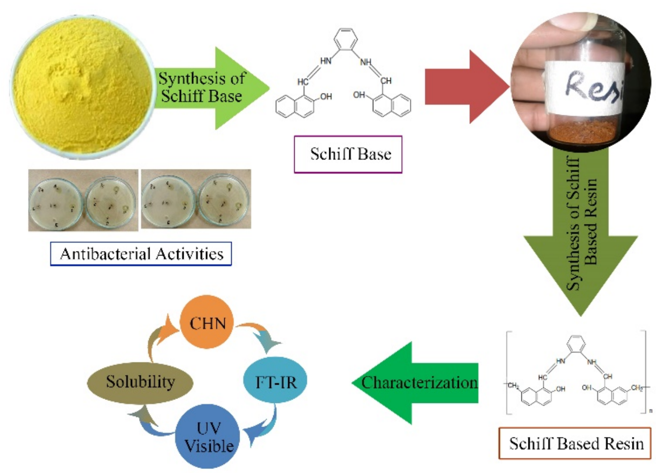

Fig. 1. Graphical abstract of the synthesis of Schiff base and Schiff based resin.

2.1 Bis(2-Hydroxy-1-napthaldehyde)-o-phenylenediamine (BHNPhen) Schiff Base Synthesis

40 ml of ethanol was used in a synthesis procedure to dissolve 0.18 grams of o-phenylenediamine and the aldehyde 2-Hydroxy-1-napthaldehyde. The resultant mixture was refluxed at 60 ^oC for two hours while being exposed to 2 cc of glacial acetic acid. The resulting mixture was then added to 250 cc of ice-cold distilled water, causing precipitation to occur over two to three hours and producing bright yellow sediments. Filtration, diethyl ether washing, vacuum desiccator drying for a long time, ethanol recrystallization, and thin-layer chromatography (TLC) purification were among the

processes these precipitates went through [11]. After the recrystallization procedure, the residue was cleaned with n-hexane and ethyl acetate as part of additional purification processes. It was then left to dry overnight in a vacuum desiccator. TLC was used to confirm the purity of the Schiff base, and found that its melting point was 85 °C. The BHNPhen reaction scheme is shown in Fig. 2.

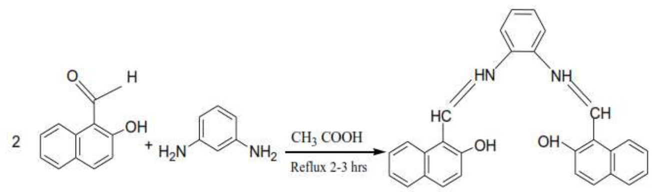

Fig. 2. Chemical scheme for Synthesis of Schiff base (BHNPhen) [12].

3. Polymerization of Polymethylene Bis (2-hydroxy-1-napthaldehyde)-o-phenylenediimine (PMBHNPhen), a resin based on Schiff

25 ml of distilled water was used to dissolve 0.5g of bis 2-hydroxy-1-napthaldehyde o-phenylenediamine, and roughly 7-8 drops of sodium hydroxide (2 ml) were added. The mixture was heated for 5 minutes at 50–60 °C while a further 37% formaldehyde was added at a molar ratio of 1:3. The mixture was refluxed for two hours at 120 to 130 °C in an oil bath. Diethyl ether and water were used to filter and wash the resin. Fine precipitates were recovered at 70-80 °C for 1-2 hours, and the melting point was reported at 140 °C [13]. **Figure 3** presents the reaction scheme for synthesized PMBHNPhen.

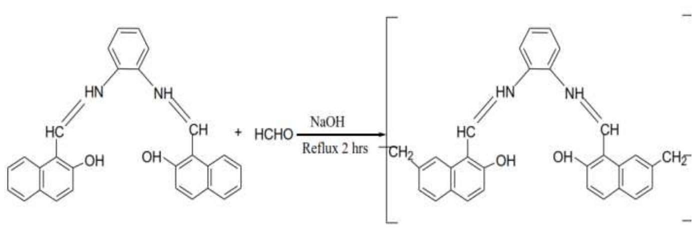

Fig. 3. Chemical Scheme for synthesis of Schiff based resin (PMBHNPhen) [13].

C.H.N Elemental microanalysis of BHNPhen and PMBHNPhen

Newly synthesized compounds were verified by CHN analysis. The experimental values are correlated to the found values [14]. CHN elemental microanalysis results approve the values presented in (Table 2).

FT-IR spectroscopy of BHNPhen and PMBHNPhen

Compounds' composition and geometrical features could be determined thanks to FT-IR research [15]. The outcomes are described by infrared spectroscopy and are presented in BHNPhen and PMBHNPhen. The FT-IR spectra of the Schiff base and Schiff-based resin are displayed in Figs. 4(a) and 4(b). Several high- and low-intensity vibrational modes were identified in the Schiff base's infrared spectra to help distinguish between the various groups, bonds, and fragments.. The (BHNPhen) azomethine peak is marked at 1615 cm^{-1}. In contrast, the resin (PMBHNPhen) showed a highly intense peak of 1621 cm^{-1} and a difference of 6 cm^{-1} toward greater frequency [16].

Ultra-Violet Spectroscopy of BHNPhen and PMBHNPhen

Ultra-violet visible spectra of newly synthesized BHNPhen and PMBHNPhen are shown in Figure 5(a) and Figure 5 (b). This study provides Ultraviolet-Visible spectra of BHNPhen and PMBHNPhen. The authors analyze the variation in the expected spectrum of the BHNPhen and PMBHNPhen [17].

The BHNPhen and PMBHNPhen spectra, which explain the spectrum groups in the Schiff Base 300-450 nm UV, showed that the transitions to π-π* caused the first absorption band at 361.34 nm (6000 $Lmol^{-1}cm^{-1}$) and the second band of absorption at 424.81 nm (4700 $Lmol^{-1}cm^{-1}$) in the Schiff Base. PMBHNPhen identified three distinct absorption bands: the first at 321.91 nm (1% 144 Lg^{-1} cm^{-1}), the second at 321.91 nm (1% 80 Lg^{-1} cm^{-1}), and the third at 423.19 nm (1% 80 Lg^{-1} cm^{-1}) [18]. The bands may be dispersed to the azomethine naphthyl ring's π-π∗ transition. Since the molecular weight of PMBHNPhen is undefined, the absorption rate of 1% is determined.

Scanning electron microscopy of BHNPhen and PMBHNPhen

Schiff base and Schiff-based resin surface morphology has been investigated using scanning electron microscopy. The Fig. 6 (a) shows that the SEM image of the Schiff base at magnification x55 indicated granular and irregular surfaces at lower magnification [19].

Antimicrobial activity of Schiff base (BHNPhen) and Schiff based resin (PMBHNPhen)

Staphylococcus aureus (G+ve) and *Escherichia coli* (G-ve) inhibitory zones were used in millimeter measurements to assess the antibacterial activity. For each component, 1 mg of each drug was separately dissolved in 1 ml of DMSO to create a solution of 100 µg [21].

Table-1. Shows the solvents in which Schiff-based and Schiff-based resin are soluble									
SL	**Compounds**	**H_2O**	**CH_3OH**	**C_2H_5OH**	**$CHCl_3$**	**Acetone**	**Hexane**	**Ether**	**DMSO**
1	BHNPhen	Insoluble	Partially soluble	Partially soluble	Soluble	Partially soluble	Insoluble	Insoluble	Soluble
2	MPBHNPhen	Insoluble	Partially soluble	Partially soluble	Soluble	Partially soluble	Insoluble	Insoluble	Soluble

0.05g of various compounds in 10 ml of liquids

Table 2. Schiff base and Schiff based resin CHN analytical values.								
Mixture	**Chemical formula**	**Melting point**	**Calculated %**			**Found %**		
			C	**H**	**N**	**C**	**H**	**N**
Schiff base BHNPhen	$2C_{11}H_8O_2+C_6H_8N$*	85°C	79.61	5.14	7.14	78.23	5.11	7.13
Schiff-based resin PMBHNPhen	$C_{27}H_{22}N_2O_2$	140 °C	79.50	5.21	7.11	78.90	5.10	6.90

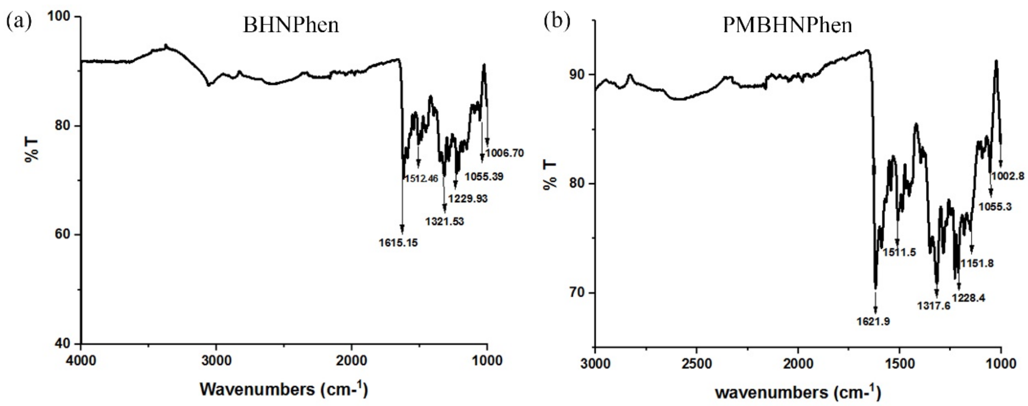

Fig. 4. (a) Infrared spectrum of Schiff base (BHNPhen), and (b) Infrared spectrum of Schiff based resin (PMBHNPhen).

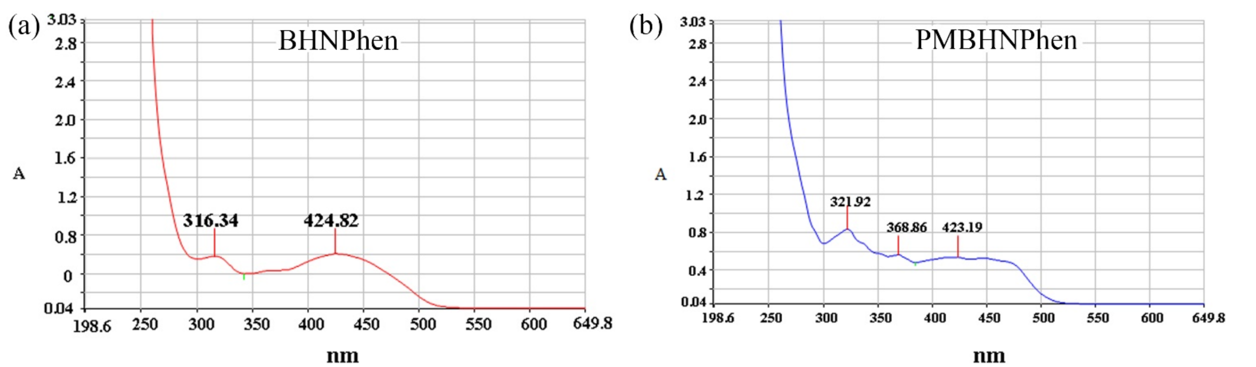

Fig. 5. (a) UV-Visible spectra of Schiff base (BHNPhen), and (b) UV-Visible spectrum of Schiff based resin (PMBHNPhen)

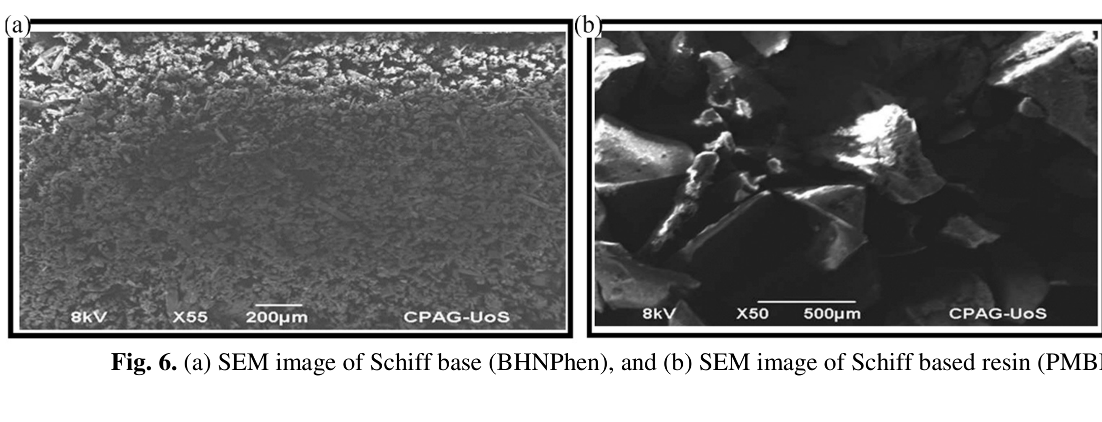

Fig. 6. (a) SEM image of Schiff base (BHNPhen), and (b) SEM image of Schiff based resin (PMBHNPhen)

Early researchers came to the idea that when bioactive compounds are coordinated, they become active compounds with lesser biological activity or more active compounds, supported by the antimicrobial activity results. Both substances demonstrated increased activity; however, Schiff-based resin performed better than Schiff-based. The medium used was Muller Hinton agar. [22]. The effects of Schiff base and resin's antibacterial action on two pathogens, Gram-positive *S. aureus* and Gram-negative *E. coli* are shown in Figs. 7(a) and 7(b). The highest zone of inhibition was observed against *S.aureus*, which is approximately (18mm) at a concentration of 10ug/ml, 22 mm at 30ug/ml, 23 mm at 50ug/ml, and 26mm at concentration 100ug/ml were observed in the Schiff based resins .while Schiff base showed 12mm at 30ug/ml, 13mm at 50ug/ml and 14mm at 100ug/ml, yet indicated no zone of inhibition at 10ug/ml against *S.aureus*. When tested individually at concentrations of 10, 30, 50, and 100 µg/mL against *E. coli*, Schiff-based resin exhibits a zone of 17 mm at a concentration of 10 µg/ml, 22 mm at a dosage of 30 µg/ml, 23 mm at a dose of 50 µg/ml, and 24 mm at a concentration of 100 µg/ml.

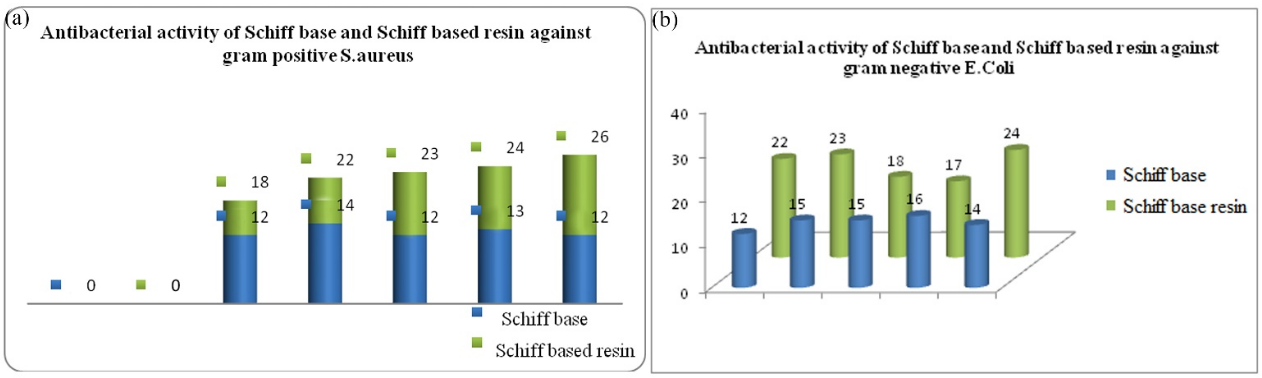

Fig. 7(a). Antibacterial activity of Schiff base and Schiff based resin against *S. aureus*, (b) Antibacterial activity of Schiff base and Schiff based resin against *E.Coli*

(Table-3) showed that (BHNPhen) and (PMBHNPhen) had a significant difference with a p.value of <5.562247, although Schiff-based resin has a significant difference of <0.00015. Standard medications (Ciprofloxacin and ketoconazole) are both (BHNPhen) and (PMBHNPhen); however, Schiff-based resin is more active than the parent Schiff-base and traditional drugs. The comparison between Schiff-based resin and Schiff base revealed that the antimicrobial tests improved the antibacterial potential of the substances, and the P value was <0.05 [23].

Table 3. Biological activity measured using Schiff base and Schiff-based resin at MIC 100µM

Organism	**Schiff Base**	**Schiff based resin**
E.Coli **(G-ve)**	14.41 ± 1.346456	20.81 ± 2.775668
S.aureus **(G+ve)**	12.61 ± 0.7901	22.61 ± 2.6643

Amazingly, the Schiff-based resin showed higher efficacy against *S. aureus* (MIC 100 µg / mL) than against *E. coli* (MIC 10 µg / mL). On the other hand, the DMSO control demonstrated antibacterial efficacy against gram-positive and gram-negative *S. aureus* and *E. coli*. According to the findings, the resin exhibited potent activity against *S. aureus* and *E. Coli* at 30 µg/mL. Schiff-based, in comparison, demonstrated extreme actions against *E. coli* and high activities against *S. aureus*. Thus, against *E. coli*, it was found that Schiff-base and Schiff-based resin were both increasingly productive. MIC is 10 µg/mL greater than that of *S. aureus*, which is 100 µg/mL. Under 2 mm (no action), 24-26 mm (relatively highly active), and 18 mm (very activity) (Table 4). [24]

Table 4. Inhibition zones (mm) for Schiff base and Schiff based resin's antibacterial properties

Microorganism Strains	**Concentration in µg/MIC**					**MIC Value (µg/mL)**
	100	**50**	**30**	**10**	**Control**	
Escherichia coli	24	23	18	12	-	10
Staphylococcus aureus	26	24	18	-	-	100

4. Conclusion

In summary, o-phenylenediamine and 2-Hydroxy-1-napthaldehyde were meticulously dissolved in ethanol to create the Schiff base (BHNPhen) and its polymer, polymethylene bis(2-Hydroxy-1-napthaldehyde)-o-phenylenediamine (PMBHNPhen), which were effectively produced in this study. A number of methods, including C.H.N., FT-IR, UV-visible spectroscopy, and SEM examination, were used to describe the compounds. The exact synthesis of the compounds was validated by the strong correlation observed between the computed and experimental C.H.N. values. PMBHNPhen's FT-IR spectra showed a strong azomethine absorption band at 1620 cm^{-1}, supporting polycondensation and confirming the structural consistency of the compound. The Schiff-based resin's UV-visible spectrum revealed a bathochromic shift that highlighted changes in absorption wavelengths. The synthesized compounds were shown by SEM examination to have an irregular granular spherical crystalline structure. Crucially, gram-positive *S. aureus* (Gram +ve) and gram-negative *E. coli* (Gram–ve) were both susceptible to the potent antibacterial activity of (BHNPhen) and (PMBHNPhen) in antimicrobial tests. The resin's increased antibacterial efficacy was linked to the polycondensation process. These results highlight the synthetic chemicals' potential uses as antibacterial agents. The thorough characterization and assessment of biological activities promotes the prospective application of Schiff base and resin materials in the biomedical and pharmaceutical sectors by furthering our understanding of them. This work represents a significant advancement in the creation of effective and versatile antibacterial materials.

Conflict of Interest Statement

The authors declare that they are engaged in no personal or financial conflicts that could have affected the research covered in this paper.

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The final version of this manuscript has been reviewed and approved by all of the authors.

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