INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Paper

Simple Techniques for The Synthesis and Evaluation of Schiff Bases

M. Kasthuri*, J. N. Suresh Kumar, Sk. Nazma, P. Priya Reetika, P. Sai Krishna, M. Sai Maneeswar, N. Siva Gopi

Department of Pharmaceutical Chemistry, Narasaraopeta Institute of Pharmaceutical Sciences Narasaraopet, Andhra Pradesh, 522601, India

ARTICLE INFO

Published: 06 Mar 2026

Keywords:
Schiff bases, Azomethines, Condensation reaction, Spectral analysis, Thin Layer Chromatography, Antimicrobial activity.

DOI:
10.5281/zenodo.18891522

ABSTRACT

Schiff bases are one of the most common imine derivatives that are prepared by the reaction of primary amines with aldehydes or ketones and contain the azomethine (C=N,) group. In this paper, simple and low-cost lab methods were used to synthesize, characterize, and test the Schiff bases derived from carbonyl compounds. The reactions were carried out under mild conditions using ethanol as a solvent and a few glacial acetic acids as a catalyst to form solid Schiff bases, which are purified by the process of recrystallization. The synthesized Schiff bases were analysed for their physical properties. Solubility and Melting point are the two properties that give an indication of the purity and stability of the Schiff bases. Structural characterization was done using spectral analysis, where the characteristic imine absorption bands appear and confirm the formation of Schiff base. Thin layer chromatography is used to check the progress of the reaction and purity. Chemical identification tests such as Schiff reagent test and 2, 4-dinitrophenylhydrazine test are conducted and gave negative results, which indicate the absence of unreacted carbonyl groups. Biological evaluation is done by antimicrobial tests to check whether the compound is potentially active or not

INTRODUCTION

Schiff bases are prepared by the condensation of primary amine and carbonyl compounds (aldehyde/ketone) discovered by a German chemist, Hugo Schiff. In the year 1864, he first synthesized Schiff's base through azeotropic distillation by using aldehyde or ketone and primary amine to get azomethine group (>C=N). They can be regarded as a sub-class of imines with the general structure R1R2C=NR3 (R3 ≠ H). Depending on their structure, they can be regarded as either secondary aldimines or secondary ketimines. Schiff bases are imines in which R3 is an alkyl or aryl group (not hydrogen). R1 and R2 may be hydrogen. Schiff bases have a wide range

***Corresponding Author:** M. Kasthuri
Address: *Department of Pharmaceutical Chemistry, Narasaraopeta Institute of Pharmaceutical Science, Narasaraopet, Andhra Predesh, 5200601, India*
Email : nazmashaik0802@gmail.com

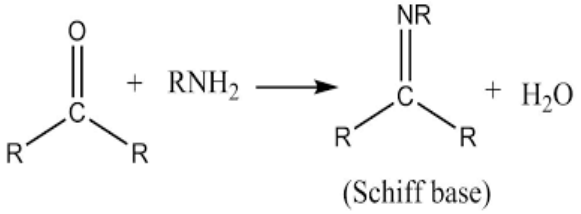
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

of biological activities such as antimicrobial, anticancer, and antiviral.

Schiff base are macrocyclic or macro-acyclic chelating agents that contain both nitrogen and oxygen donor atoms and are often multidentate and have chelating ability. Schiff bases and their metal coordination compounds have immense applications as a ligand in the co-ordination chemistry even after their discovery over a century. [1, 2, 3, 4]

Formation of the Schiff base

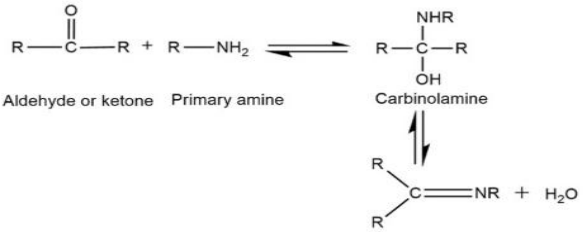
The condensation reactions of aldehyde and amines are carried out under different conditions and solvents like methanol or ethanol.



The formation of Schiff bases takes place at room temperatures or at refluxing conditions. If it happens, then the purification step can be done by crystallization. The formation of Schiff base from aldehyde or ketone is a reversible reaction and takes place under acidic or basic conditions.

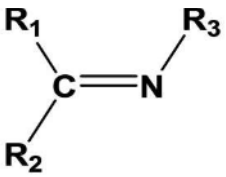
The reaction takes place through the formation of carbinolamine as an intermediate:

A Schiff base is a type of chemical compound where bonding to the alkyl or aryl group is through the Nitrogen atom and not through the Hydrogen atom. The other name for Schiff base is imine or azomethine because the carbonyl group is replaced by these groups. Schiff bases are the important mediators for the preparation of bioactive molecules. Schiff bases are significant not only in inorganic chemistry but also have vast applications in the organic, bio-organic, biochemical, and medicinal chemistry. [5, 6, 7, 8]



Chemistry of Schiff bases

A Schiff base is a compound that has a general chemical formula of R1N = CR2R3. The R1, R2, and R3 are either phenyl or alkyl groups, thus forming a stable imine compound. The Schiff bases are the condensation products of aldehydes and amines.



The Schiff bases can be formed either by acid or base catalysis or even in neutral solutions. The aryl Schiff bases have higher stability and can be formed easily compared to alkyl Schiff bases, which are less stable. The Schiff bases are considered to be subclasses of imines. They can be either secondary aldehydes or secondary ketimines based on their composition. The Schiff bases can be divided into two categories based on the number of C = N bonds formed, which are mono Schiff base and bis Schiff bases. [9, 10]

Physical Properties of Schiff Bases

The following are the general physical properties of Schiff bases. [11, 12, 13]

Table.1: Physical properties

S.no	Property	Description
1	Physical state	Solid, semi-solid, liquid
2	Colour	Yellow, Orange or Red
3	Odour	Odourless
4	Solubility	Soluble in organic solvents
5	Melting point	80-250^{0}C
6	Stability	Stable in dry conditions
7	Optical properties	Show UV-Visible absorption (250-400nm)

Applications of Schiff Basses

Schiff bases are a class of compounds that have an imine group (-C=N-) and are applied in various ways in chemistry, pharmacy, and industry.

1. Medicinal Applications

- Antibacterial agents – Applied to combat most of the bacterial infections.
- Antiviral agents – Applied to combat some specific viruses.
- Anti-inflammatory drugs – Applied to reduce inflammation.
- Antifungal agents – Applied to combat fungal diseases.
- Anticancer agents – Some Schiff bases are applied to combat cancers. [14, 15, 16]

2. Analytical Chemistry

- Applied as analytical agents to detect metal ions.
- Form metal complexes with copper, nickel, and cobalt.

Applied in qualitative and quantitative analysis. [17, 18, 19, 20,]

3. Coordination Chemistry

- Serves as a ligand and forms metal complexes that are stable.
- Applied to prepare transition metal complexes.
- Have applications in bioinorganic chemistry.

4. Industrial Applications

- Applied in the dye and pigment industry.
- Applied in the synthesis of polymers.
- Applied as corrosion inhibitors.

5. Catalysis

- Schiff base-metal complexes are applied as catalysts in organic reactions.

6. Agricultural Applications

- Applied in the preparation of pesticides and herbicides. [21, 22, 23, 24]

Objective of the study:

- To synthesize Schiff bases using aromatic amines and carbonyl compounds.
- To purify the synthesized compounds.
- To determine the physical properties.
- To evaluate the biological activity.

1. MATERIALS AND METHODS

1.1 Reagents and Solvents

O-phenylenediamine, Benzophenone, P-Dimethylaminobenzaldehyde, Aniline, Vanillin, Glacial acetic acid, Ethanol, Distilled water used for the synthesis of Schiff Bases.

1.2 Synthesis of Schiff Bases

Fig.1: Synthesis of Sample Compounds

Dissolve equimolar quantities reactants (aldehyde/ketone) in suitable solvent (ethanol).

↓

Add catalytic amount of glacial acetic acid to the above mixture.

↓

Stir continuously for about 2-3hrs (Magnetic Stirring or Manual Stirring) at room temperature.

↓

The solid precipitates out in the beaker during stirring.

↓

Filter the solid using filter paper and funnel.

↓

Dry the solid (Air drying).

↓

Powder the dried solid using mortar and pestle.

↓

Recrystallize the solid from ethanol

And store in an air-tight container. [11, 12]

PHYSICAL PROPERTIES

3.1 Solubility: Schiff bases are soluble in organic solvents like ethanol, methanol, chloroform, and so on. The solubility of Schiff bases varies depending on the substituents present on the imine functional group, especially polar and aromatic groups. The majority of Schiff bases are less soluble in water due to their nonpolar or weakly polar nature.

Table.1: Solubility of Synthesized Compounds

S.no	Drug	Solubility						
		Normal Water	Hot Water	Normal Ethanol	Hot Ethanol	Acetone	Chloroform	Benzene
1	S1	Insoluble	Insoluble	Insoluble	Soluble	Soluble	Soluble	Insoluble
2	S2	Insoluble	Insoluble	Insoluble	Soluble	Soluble	Soluble	Insoluble
3	S3	Insoluble	Insoluble	Insoluble	Soluble	Soluble	Soluble	Insoluble
4	S4	Insoluble	Insoluble	Insoluble	Soluble	Soluble	Soluble	Insoluble
5	S5	Insoluble	Insoluble	Insoluble	Soluble	Soluble	Soluble	Insoluble

3.2Melting Point Determination: Melting point determination is a simple and significant technique for identification of Schiff bases. The melting point of Schiff bases depends on the type of starting materials (aldehyde or ketone and the amine) used in the preparation.

Fig.2: Melting Point Apparatus

Table.2: Melting Point Determination

S.no	Drug	Melting Point (^{0}C)
1	S1	145-150 ˚c
2	S2	180˚c
3	S3	205-210 ˚c
4	S4	155-160 ˚c
5	S5	160-165 ˚c

4 CHARACTERIZATION AND EVALUATION

The Schiff bases can be characterized by the use of spectroscopic methods like IR, ^{1}H/^{13}C NMR, UV-Vis, and mass spectrometry to ensure the presence of the imine group (–C=N–). Evaluation of the Schiff bases will involve chemical tests (negative 2, 4-DNPH or Schiff reagent test) to ensure the absence of free carbonyl groups. Finally, the efficacy of the Schiff bases will be determined by the use of biological tests like antimicrobial, antifungal tests, among others.

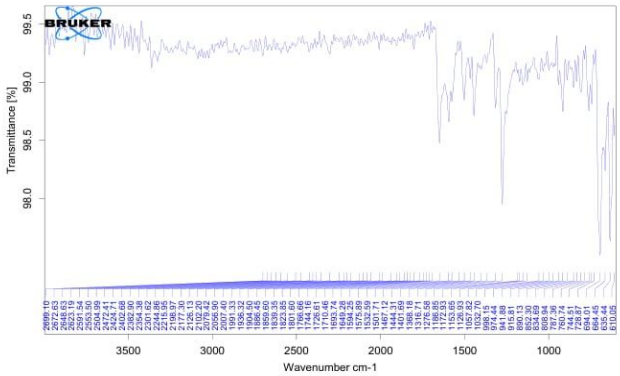
4.1 Spectral Analysis: Spectral analysis is the procedure of analysing a signal, waveform, or data set into its component frequencies to analyze its power spectrum and determine periodicities. Primarily founded on the Fourier theorem, it converts time-domain data into the frequency domain to detect, measure, and interpret frequency components. It is commonly employed in signal processing, engineering, physics, and medicine to detect oscillations or material composition.

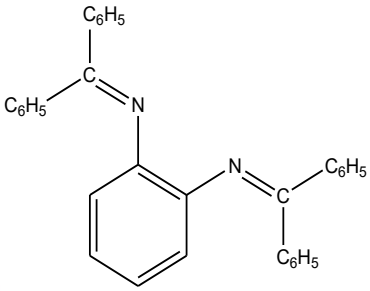
Infrared (IR) Spectroscopy: Infrared (IR) Spectroscopy is an analytical method employed to

identify and analyze chemical substances by measuring their interaction with infrared radiation. When IR radiation passes through a sample, molecules absorb specific frequencies that correspond to the vibrations of their chemical bonds. The IR spectrum, which is a graph of absorbance or transmittance at different wavelengths, can be analysed to identify functional groups and molecular structures.

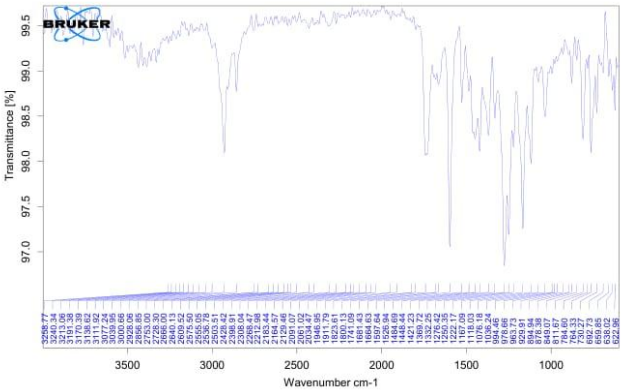
IR spectroscopy is a significant method for analysing Schiff bases by verifying functional group modifications. A distinctive absorption peak for the imine (–C=N–) group occurs around 1600-1650 cm^{-1}. [25, 26, 27]

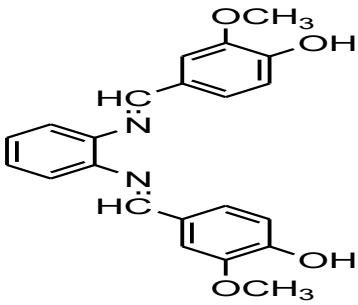
Compound-1(S1) :



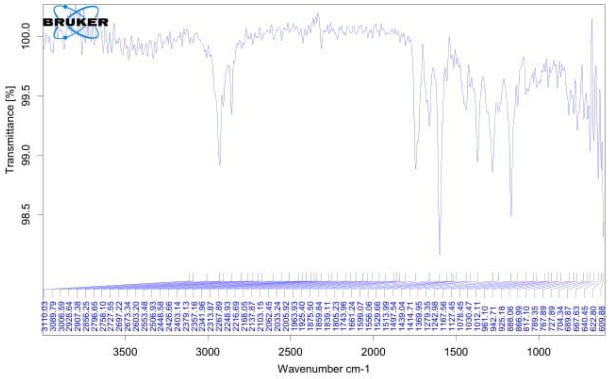


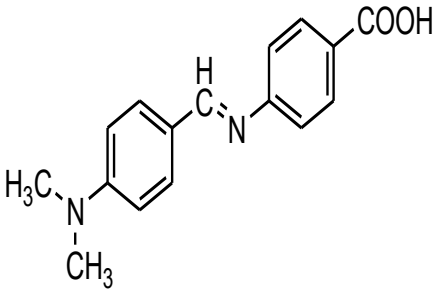
Compound-2(S2) :



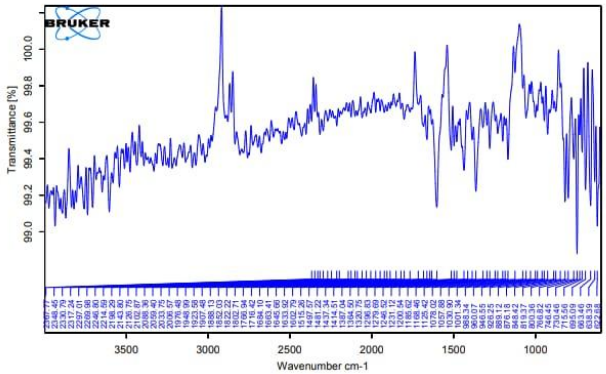


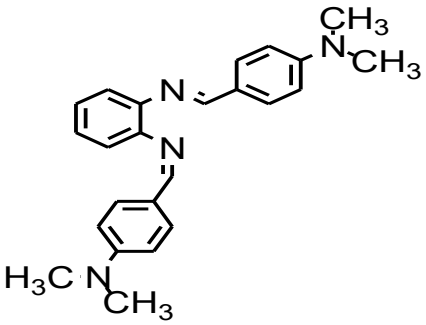
Compound-3(S3) :



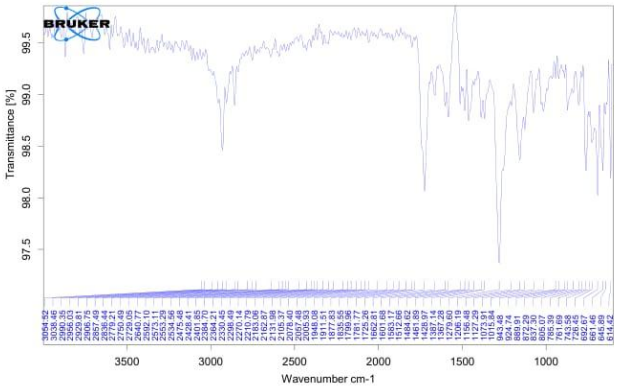


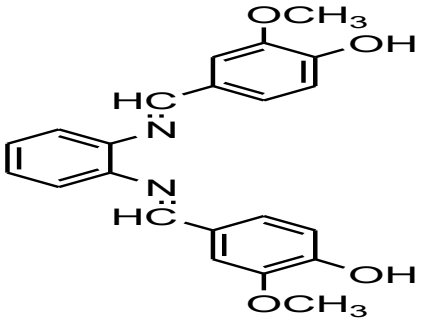
Compound-4(S4) :





Compound-5(S5) :





4.2 Thin Layer Chromatography (TLC): Thin Layer Chromatography (TLC) is a rapid and economical method of analysis used for the identification of various compounds on the basis of their RF values. Various compounds give different RF values, making it easy to identify the compound. The Stationary phase and mobile phase used are as follows:

Stationary phase: TLC grade Silica gel
Mobile phase: Hexane: Ethyl acetate (7:3) [28]

Table.3: Rf values of synthesized compounds

S.no	Compound	Rf value
1	S1	0.75
2	S2	0.54
3	S3	0.51
4	S4	0.56
5	S5	0.68

4.3 Identification Test:

Schiff reagent test: The Schiff reagent test is used for the identification of Schiff bases. This test is employed to check the presence of aldehydes. The Schiff reagent gives a pink or magenta colour in the presence of free aldehyde groups. Hence, the pure Schiff bases give a negative test as the aldehyde group is changed to an imine group (-C=N-). The positive test result may indicate the partial formation of Schiff base or hydrolysis to the aldehyde.

2, 4-dinitrophenylhydrazine (2, 4-DNPH) test: In the case of Schiff bases prepared from ketones, the 2, 4-dinitrophenylhydrazine (2, 4-DNPH) test is negative, which confirms the absence of the ketone group. The positive test result yields a yellow, orange, or red precipitate, which indicates the presence of carbonyl groups.

Table.4: Identification tests for Schiff Bases

S.no	Experiment	Observation	Inference
1	S1 + Solvent + 2,4-dinitrophenylhydrazine	Absence of yellow, orange, or red precipitate	Presence of Schiff Base
2	S2 + Solvent + Schiff's reagent	Absence of pink colour	
3	S3 + Solvent + Schiff's reagent		
4	S4 + Solvent + Schiff's reagent		
5	S5 + Solvent + Schiff's reagent		

4.4 Antimicrobial activity:

Biological evaluation for synthesized compounds by in vitro studies.

Method

Determination of antimicrobial activity by Agar well diffusion method.

Microorganism Used

Lactobacillus species isolated from curd (Gram-positive bacteria)
E. coli (Gram-negative bacteria)

Standard: Metronidazole

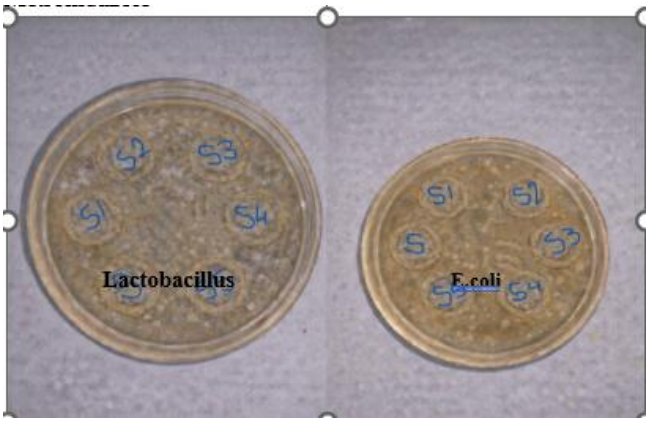


Fig.3: Antimicrobial activity

The medium was prepared, sterilized by autoclaving at 121°C for 15 minutes, and poured into sterile Petri plates for solidification. Then we have inoculated the bacteria and after incubation, wells are made using cork borer. 100microlitre solutions of the compounds and standard drug (Metronidazole) are poured into wells and again incubated in inversed position for about 28-32 hours. [29, 30, 31, 32, 33, 34, 35]

We have observed the zone of inhibition around the wells in which the compound is poured.

Table.5: Antimicrobial activity of Synthesized Compounds with Standard

Sample	Antimicrobial activity	
	Lactobacillus	E. Coli
S1	+	+
S2	+	+
S3	+	+
S4	+ +	+ +
S5	+ +	+ +
Standard (Metronidazole)	+ + +	+ + +

+ indicates presence of antimicrobial activity

RESULT

All five synthesized Schiff bases showed antibacterial activity against Lactobacillus isolated from curd and E. coli. Among them, S4 & S5 showed maximum zone of inhibition compared to other samples. The activity was compared with standard Metronidazole.

CONCLUSION

The current research has successfully shown simple and efficient methods for the synthesis, purification, characterization, and bioassay of five Schiff base derivatives obtained from aromatic amines and carbonyl compounds. The condensation reactions were performed under mild conditions using ethanol as the solvent and glacial acetic acid as the catalyst, giving stable solid products with good purity after recrystallization. Physical characterization, solubility tests, and melting point analysis revealed that the synthesized compounds have good stability and purity. Spectral analysis by IR spectroscopy confirmed the successful synthesis of the azomethine group (–C=N–), which was evident from the characteristic peak at 1600-1650 cm^{-1}. Thin Layer Chromatography analysis showed single spots with different RF values, indicating the purity of the compounds. Chemical identification tests like Schiff reagent and 2, 4-dinitrophenylhydrazine tests were negative, further confirming the absence of unreacted carbonyl groups and the successful synthesis of Schiff bases. The antimicrobial study carried out by the agar well diffusion method against Lactobacillus species isolated from curd showed that all the synthesized compounds have varying levels of antibacterial activity. Among the five samples (S1-S5), some derivatives have relatively higher zones of inhibition, indicating that structural variation has a significant effect on biological activity. Thus, this study clearly confirms that Schiff bases synthesized by simple laboratory methods have promising physicochemical and antimicrobial properties. These results indicate that the synthesized compounds can be used as potential lead molecules for further pharmacological studies and metal complexation to improve their biological activity.

REFERENCES

1. Schiff H. Mittheilungen aus dem Universitätslaboratorium in Pisa. Justus Liebigs Ann Chem.1864; 131:118–119.
2. Layer RW. The chemistry of imines. Chem Rev. 1963; 63(5):489–510.
3. Dhar DN, Taploo CL. Schiff bases and their applications. J Sci Ind Res. 1982; 41:501–506.
4. Patai S, editor. The Chemistry of the Carbon-Nitrogen Double Bond. London: Wiley; 1970.
5. Singh P, Goel RL, Singh BP. Schiff bases as ligands. J Indian Chem Soc. 1975; 52:958–960.
6. Solomons TWG, Fryhle CB. Organic Chemistry. 10th ed. New York: Wiley; 2011.
7. Morrison RT, Boyd RN. Organic Chemistry. 6th ed. New Delhi: Pearson; 1992.
8. Carey FA, Sundberg RJ. Advanced Organic Chemistry. 5th ed. New York: Springer; 2007.
9. March J. Advanced Organic Chemistry. 4th ed. New York: Wiley; 1992.
10. Atkins P, Jones L. Chemical Principles. 5th ed. New York: Freeman; 2010.
11. Vogel AI. Practical Organic Chemistry. 4th ed. London: Longman; 1978.
12. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR. Vogel's Textbook of Practical Organic Chemistry. 5th ed. London: Longman; 1989.

13. IUPAC, Compendium of chemical terminology (Gold Book), 2nd ed. Oxford: Blackwell; 1997.
14. Da Silva CM, da Silva DL, Modolo LV, Alves RB, de Resende MA, Martins CV, et al. Schiff bases: A short review of their antimicrobial activities. J Adv Res. 2011; 2(1):1–8.
15. Kajal A, Bala S, Kamboj S, Sharma N, Saini V. Schiff bases: A versatile pharmacophore. J Catal. 2013; 2013:1–14.
16. Singh K, Barwa MS, Tyagi P. Synthesis, characterization and biological studies of transition metal complexes of Schiff bases. Eur J Med Chem. 2006; 41:147–153.
17. El-Sayed NS. Synthesis and antimicrobial activity of some new Schiff bases. J Appl Sci Res. 2006; 2(12):1195–1200.
18. Raman N, Kulandaisamy A, Thangaraja C, Jeyasubramanian K. Synthesis and biological activity of Schiff base metal complexes. Trans Met Chem. 2004; 29:129–135.
19. Singh RV, Fahmi N, Biyala MK. Coordination behaviour and biological activity of Schiff base complexes. J Iran Chem Soc. 2005; 2:40–46.
20. Shukla SN, Gaur P. Schiff bases in medicinal chemistry. Int J Pharm Sci Res. 2011; 2:110–118.
21. Yadav R, Srivastava S. Biological importance of Schiff bases. Int J Pharm Sci Drug Res. 2012; 4:1–7.
22. Gupta KC, Sutar AK. Catalytic activities of Schiff base transition metal complexes. Coord Chem Rev. 2008; 252:1420–1450.
23. Kureshy RI, Khan NH, Abdi SHR, Patel ST. Schiff base metal complexes in catalysis. J Mol Catal A Chem. 2003; 206:29–40.
24. Dwyer FP, Mellor DP. Chelating Agents and Metal Chelates. New York: Academic Press; 1964.
25. Silverstein RM, Webster FX, Kiemle DJ. Spectrometric Identification of Organic Compounds. 7thed. New York: Wiley; 2005.
26. Kemp W. Organic Spectroscopy. 3rd ed. London: Macmillan; 1991.
27. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Belmont: Thomson Brooks/Cole; 2007.
28. Harborne JB. Phytochemical Methods. 3rd ed. London: Chapman & Hall; 1998.
29. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disk method. Am J Clin Pathol. 1966; 45:493–496.
30. CLSI. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Wayne, PA; 2020.
31. De Man JC, Rogosa M, Sharpe ME. A medium for the cultivation of lactobacilli. J Appl Bacteriol. 1960; 23:130–135.
32. Cheesbrough M. District Laboratory Practice in Tropical Countries. Cambridge: Cambridge University Press; 2006.
33. Prescott LM, Harley JP, Klein DA. Microbiology. 6th ed. Boston: McGraw-Hill; 2005.
34. Aneja KR. Experiments in Microbiology, Plant Pathology and Biotechnology. 4thed. New Delhi: New Age International; 2012.
35. Singh K, Barwa MS, Tyagi P. Synthesis and characterization of metal complexes with Schiff base ligands. Eur J Med Chem. 2006; 41:147–153.

HOW TO CITE: M. Kasthuri, J. N. Suresh Kumar, Sk. Nazma, P. Priya Reetika, P. Sai Krishna, M. Sai Maneeswar, N. Siva Gopi, Simple Techniques for The Synthesis and Evaluation of Schiff Bases., Int. J. of Pharm. Sci., 2026, Vol 4, Issue 3, 562-571. https://doi.org/10.5281/zenodo.18891522