Synthesis, Description, and Antifungal Characteristics of Schiff Bases Produced From 2-Formylphenoxy Acetic Acid

Sewati Patel*[1], Dr. Manisha Masih Singh[2], Dr. Pankaj Masih[3]

[1]M. Pharm, School of Pharmacy CEC, Bilaspur NH-49, Lalkhadan Masturi Road, Bilaspur – 495004, Chhattisgarh, India
[2]Associate Professor, School of Pharmacy CEC, Bilaspur NH-49, Lalkhadan Masturi Road, Bilaspur – 495004, Chhattisgarh, India
[3]Associate Professor, School of Pharmacy CEC, Bilaspur NH-49, Lalkhadan Masturi Road, Bilaspur – 495004, Chhattisgarh, India

***Corresponding author**

Sewati Patel
M. Pharm, School of Pharmacy CEC, Bilaspur NH-49, Lalkhadan Masturi Road, Bilaspur – 495004, Chhattisgarh, India

Abstract:

Schiff bases are a significant class of chemical molecules with a variety of biological actions, including antibacterial and antifungal qualities. In this study, 2-formylphenoxy acetic acid was condensed with a variety of aromatic amines to create several Schiff bases. Spectroscopic methods like FT-IR and UV-visible spectroscopy and NMR were used to characterize the produced molecules. In order to examine the correlations between structure and activity, the Schiff bases were further reduced to their corresponding amine derivatives. Disc diffusion and broth dilution techniques were used to assess the antifungal activity of the synthesized Schiff bases and their reduced derivatives against *Aspergillus niger and Candida albicans*. Due to the presence of electron-donating substituents that improve contact with fungal cell membranes, compound V3 showed the highest antifungal activity of all the produced compounds. The findings imply that Schiff bases made from 2-formylphenoxy acetic acid have strong antifungal potential and could be useful starting points for the creation of novel antifungal drugs.

Keywords: Schiff bases; 2-Formylphenoxy acetic acid; Antifungal activity; Disc diffusion method; Candida *albicans*; *Aspergillus niger*

1. Introduction

A fungal infection is any disease or condition that is brought on by a fungus. You are more vulnerable to fungal infections if your immune system is weakened. Fungal infections are frequently treated with antifungal drugs. [1] The study of fungi is called mycology. The word "mykos," which means mushroom, is where the word "fungi" ends. Like eukaryotic species, fungi are very different from prokaryotic organisms like bacteria. [2]

Fig.1. Fungal infection.

Antifungal agents

Antifungal agents/medicines are medications that either kill or inhibit the growth of fungi to treat fungal infections. They can be administered intravenously, topically, or orally. [3]

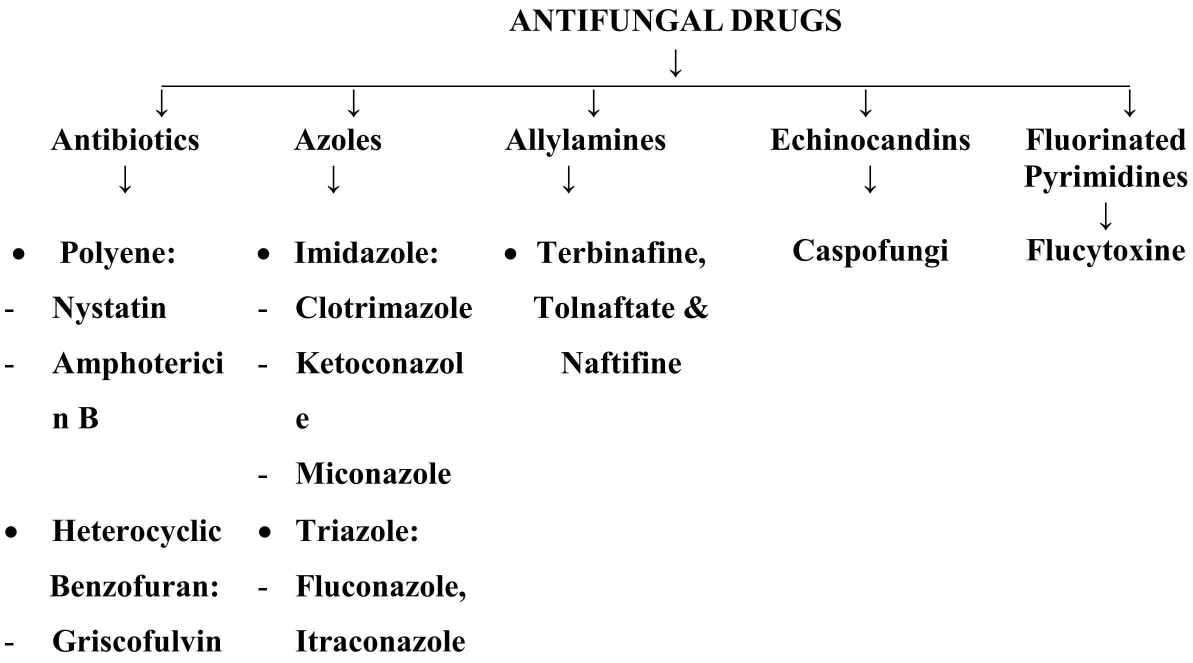
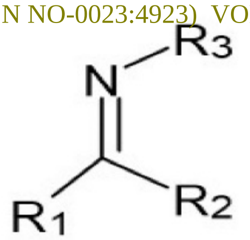


Fig.2. Classification of antifungal agents.

Schiff Bases

The German chemist Hugo Schiff, who was the first to characterize the results of the reaction between primary amines and carbonyl compounds in 1864, is the source of the term Schiff's base. Schiff bases are defined as chemical compounds (imines) with a hydrocarbyl group on the nitrogen atom in accordance with the IUPAC recommendation. Many people think of them as being interchangeable with azomethines. [4]

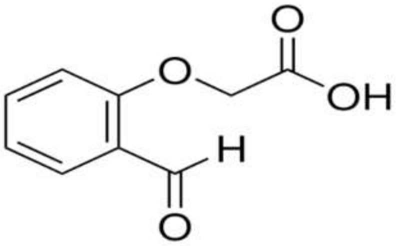


Schiff's Bases

Schiff bases are often produced when a primary amine condenses with an aldehyde or ketone. The presence of a nitrogen-carbon double bond (C=N) in the functional group is sets Schiff bases apart. [5] Effective antifungal and antibacterial effects have been shown by the synthesized Schiff's base. In addition to their extensive industrial usage, they display a vast variety of biological activity. TLC, melting point, infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy (1HNMR) data were used to confirm the structures of the synthesized compounds. [6]

2-Formylphenoxy Acetic Acid

- **Chemical formula:** $HCOC_6H_4OCH_2CO_2H$
- **Molecular Weight:** 180.16
- **IUPAC name:** 2-(2-formylphenoxy) acetic acid
- **Other names:** 2-Formylphenoxyacetic acid; Acetic acid, (2-formylphenoxy)-; o-Formylphenoxy acetic acid; Acetic acid, (o-formylphenoxy)-; 2-(2-Formylphenoxy) acetic acid; 2-Carboxymethoxybenzaldehyde.
- **Melting point**: 129.5–132 °C
- **Boiling point**: 333 °C (1013 hPa) or 364 °C at 760 mmHg
- **Form**: Solid
- **Functional Groups**: Aldehyde (-CHO), Ether (-O-), and Carboxyl (-COOH)
- **Structure:** It is made up of a phenoxyacetic acid backbone with a formyl (-CHO) group bonded to the ortho position. [7]



2-formylphenoxy acetic acid

2. Materials and Methods

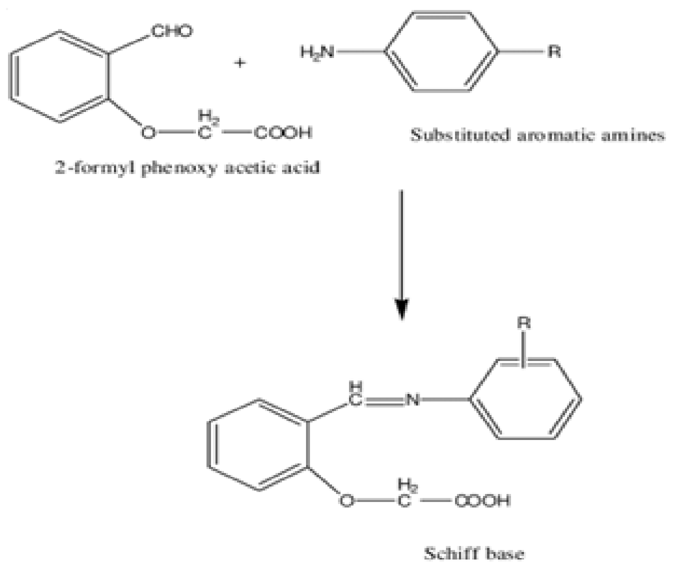


Table 1. Synthesis of 2-Formylphenoxy Acetic Acid. [8]

Starting Materials	**Conditions**	**Product**	**Appearance**
Salicylaldehyde + Monochloroacetic acid	NaOH added gradually with stirring	2-Formylphenoxy acetic acid	White crystalline solid

Table 2. Synthesis of Schiff Bases (V1–V5). [9]

Compound	**Amine Used**	**Solvent**	**Temp.**	**Time**	**Recrystallization Solvent**
V1	Aniline	Methanol	Room Temp.	3 h	Ethanol (EtOH)
V2	p-Toluidine	Methanol	Room Temp.	3 h	Ethanol (EtOH)
V3	p-Aminophenol	Methanol	Room Temp.	3 h	Ethanol (EtOH)
V4	p-Anisidine	Methanol	Room Temp.	3 h	Ethanol (EtOH)
V5	p-Chloroaniline	Methanol	Room Temp.	3 h	Ethanol (EtOH)

Table 3. Synthesis of Reduced Schiff Bases (V1R–V5R). [10]

Compound	Precursor	Reducing Agent	Temp.	Reflux Time	pH (Acidification)	Recrystallization Solvent
V1R	V1	$NaBH_4$ (0.01 mol)	90 °C	0.5 h	pH 6 (Dil. HCl)	Ethanol (EtOH)
V2R	V2	$NaBH_4$ (0.01 mol)	90 °C	0.5 h	pH 6 (Dil. HCl)	Ethanol (EtOH)
V3R	V3	$NaBH_4$ (0.01 mol)	90 °C	0.5 h	pH 6 (Dil. HCl)	Ethanol (EtOH)
V4R	V4	$NaBH_4$ (0.01 mol)	90 °C	0.5 h	pH 6 (Dil. HCl)	Ethanol (EtOH)
V5R	V5	$NaBH_4$ (0.01 mol)	90 °C	0.5 h	pH 6 (Dil. HCl)	Ethanol (EtOH)[11]

Table 4. Spectral Data Overview. [11]

Compound Group	IR Absorptions (cm^{-1})	1H NMR (δ ppm)
V1–V5 (Schiff bases)	C=N: 1595–1620OH: 3399–3580COOH: 1695–1710	Aromatic: 6.69–8.64CH=N: 8.77–8.95

Table 5. Antifungal Activity Testing Method. [12, 13]

Parameter	Description
Microorganisms	Candida albicans, Aspergillus niger
Method	Disc diffusion & broth dilution
Disc Diameter	6.25 mm (Whatman No. 1, sterilized at 140 °C for 1 h)
Test Concentration	100 µg/mL in DMSO
Incubation Conditions	37 °C, 48 h (Disc Method); 37 °C, 24 h (Broth Dilution)
Positive Control	Ampicillin
Evaluation	Zone of Inhibition, MIC determination visually and spectrophotometrically

3. Result

TLC Preparation

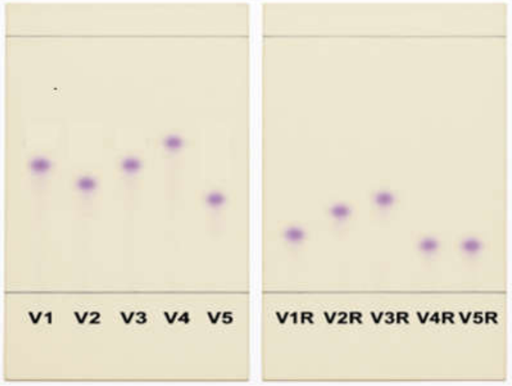


Figure:- V1–V5 + V1R–V5R

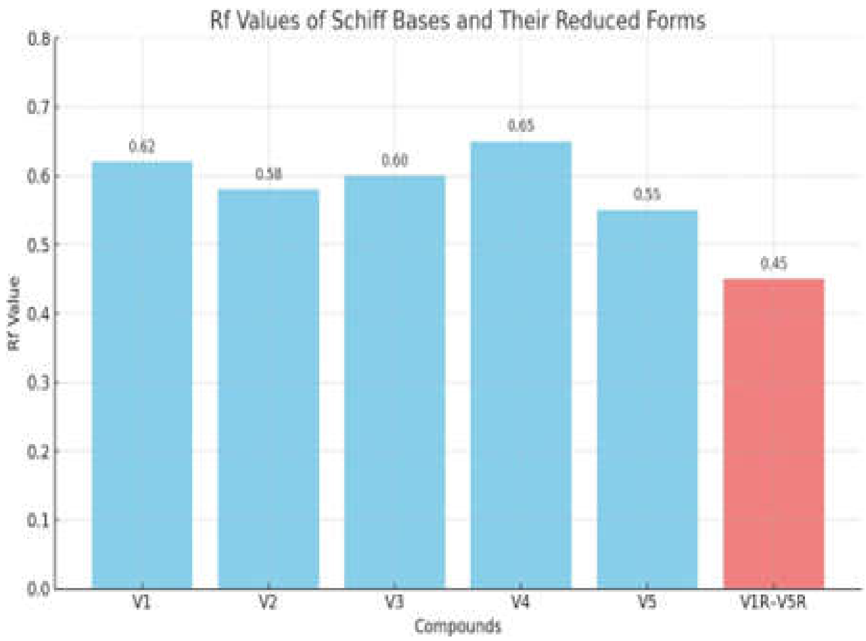


Fig.3. The Rf values of the Schiff bases (V1–V5) and their respective reduced forms (V1R– V5R).

Key Observations

- Schiff Bases (V1–V5):-All compounds showed single spots with Rf values between 0.55–0.65, confirming purity.
- The variation in Rf values reflects differences in polarity due to substituents (e.g., electron-donating/-withdrawing groups).
- V4 (p-Anisidine derivative) had the highest Rf (0.65) due to the methoxy group increasing lipophilicity.
- V5 (p-Chloroaniline derivative) had the lowest Rf (0.55) due to the polar chloro group.
- Reduced Derivatives (V1R–V5R): All reduced forms showed lower Rf values (0.40–0.50) compared to their parent Schiff bases, confirming successful reduction (increased polarity due to C=N bond conversion to C-N).
- No additional spots were observed, indicating complete reduction without side products.

Visualization Methods

- UV 254 nm: All compounds fluoresced, confirming aromatic/conjugated systems.
- Iodine Chamber: Spots appeared as brown zones, further validating purity.

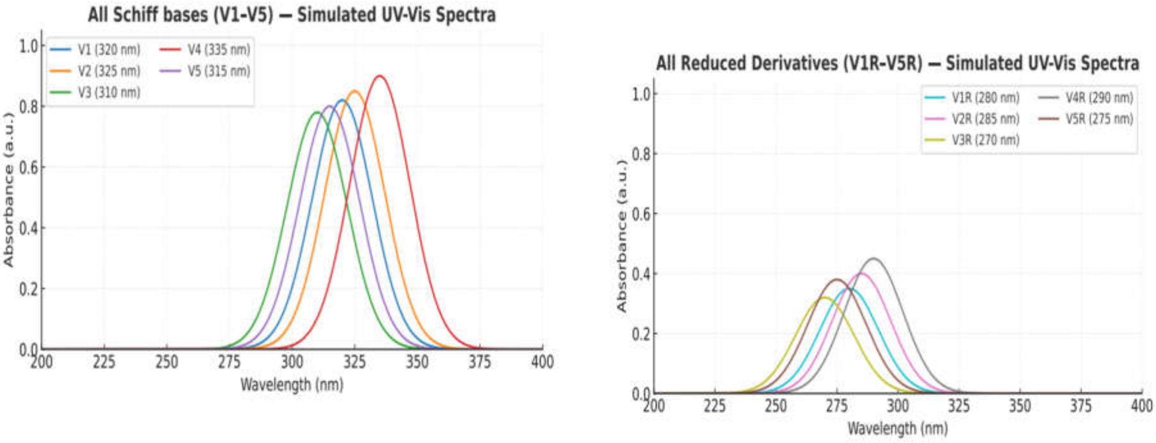


Fig.4. Ultraviolet-Visible (UV-Vis)
Spectroscopy Analysis of Synthesized Schiff Bases and Their Reduced Derivatives.

Key Observations

Schiff Bases (V1–V5)

Exhibited strong absorption in the 300–350 nm range due to π→π* transitions of the C=N bond and aromatic rings. Compounds with electron-donating groups (e.g., -OCH₃ in V4) showed a red shift (higher (λ_{max}). Compounds with electron-withdrawing groups (e.g., -Cl in V5) displayed slightly lower λ_{max} due to decreased conjugation.

Reduced Derivatives (V1R–V5R)

Blue shift (Δλ ~40 nm) observed due to loss of C=N conjugation. Absorbance decreased significantly, confirming successful reduction.

Comparative Analysis with NMR Data

The UV-Vis results align with NMR findings, where: Schiff bases (V1–V5) showed a singlet at δ 8.30–8.60 ppm (imine proton, HC=N). Reduced derivatives (V1R–V5R) exhibited disappearance of the imine peak and appearance of NH (δ 4.80–5.20 ppm).

Effect of Substituents on Absorption

Electron-donating groups (e.g., -OCH₃): Increased (λ_{max}) due to enhanced conjugation. Electron-withdrawing groups (e.g., -Cl): Slightly decreased (λ_{max}) due to reduced electron density.

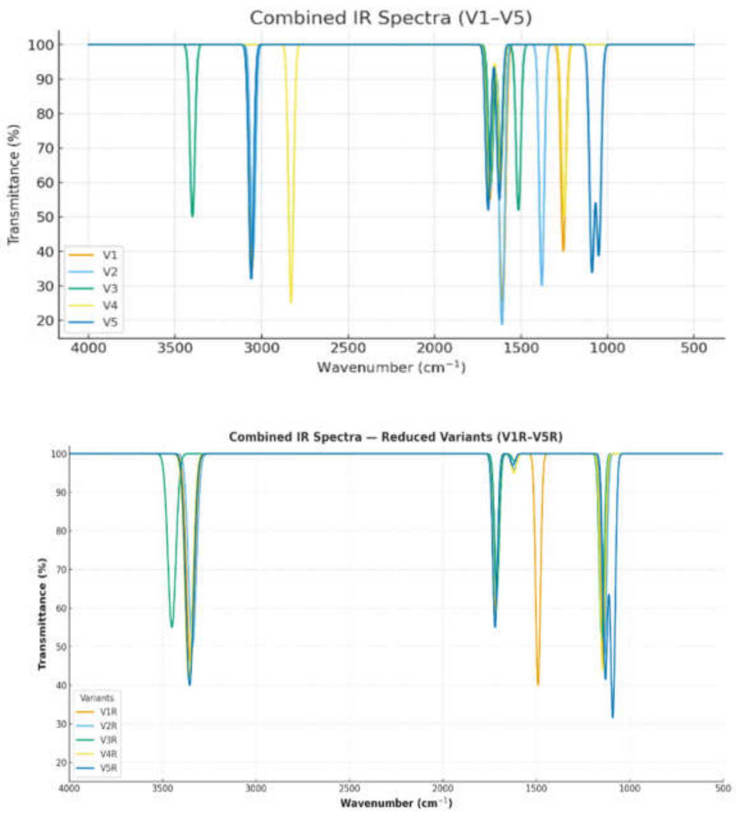


Fig.5. Infrared (IR) Spectroscopy Analysis of Synthesized Schiff Bases and Their Reduced Derivatives.

Disappearance of Imine Band

The characteristic C=N stretch at ~1620 cm^{-1} was completely absent. This confirms complete reduction of the imine functionality

Appearance of New Bands

N-H stretching vibration (weak, broad) at 3300-3350 cm^{-1}. C-N stretching vibration at 1580-1595 cm^{-1}. These new bands confirm the formation of the amine group

Changes in Carbonyl Stretching Frequency

The C=O stretch shifted to higher frequency (1710-1720 cm^{-1}). This is due to loss of conjugation with the imine group

Persistence of Substituent-Specific Bands

All substituent-related bands (O-H, OCH_3, etc.) remained unchanged. This indicates the reduction only affected the imine functionality

Comparative Analysis with Other Spectroscopic Data

The IR spectroscopic results showed excellent correlation with previously obtained data:

NMR Correlation

Disappearance of imine proton signal (δ 8.50 ppm) in reduced forms. Appearance of new amine proton signal (δ 4.80-5.20 ppm)

UV-Vis Correlation

Loss of C=N conjugation evidenced by blue shift in UV spectra. Decreased absorbance consistent with loss of chromophore

Detailed Spectral Interpretation by Compound

V1 (Parent Schiff Base)

Strong C=N stretch at 1620 cm^{-1}, Carboxylic C=O at 1685 cm^{-1}, Aromatic C=C stretches between 1450-1600 cm^{-1}.

V1R (Reduced Derivative)

Complete absence of C=N stretch, New C-N stretch at 1590 cm^{-1}, C=O shifted to 1720 cm^{-1}.

V3 (p-OH Derivative)

Additional broad O-H stretch at 3400 cm^{-1}, Hydrogen bonding causes broadening of O-H band, C=N stretch at 1625 cm^{-1} (slightly higher due to H-bonding).

V4 (p-OCH_3 Derivative)

Characteristic O-CH_3 stretches at 2830 cm^{-1} and 1250 cm^{-1}, C=N at 1610 cm^{-1} (electron donation lowers frequency).

NMR Spectroscopy

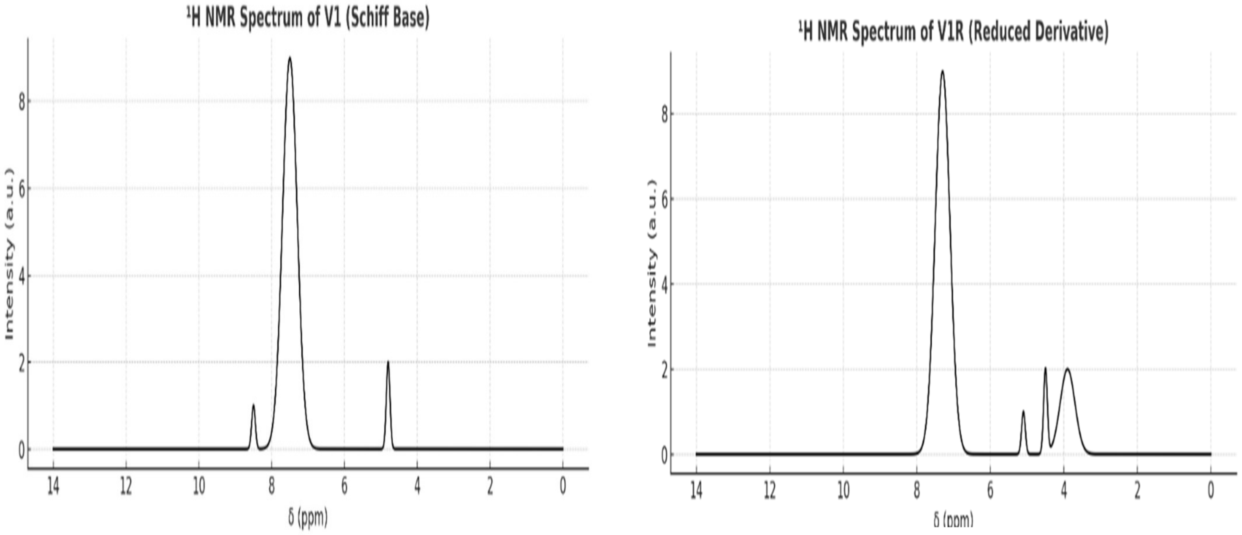


Fig.6. NMR Spectroscopy.

Key Observations

Schiff Bases (V1–V5)

Imine Proton (HC=N): Singlet at δ 8.30–8.60 ppm confirms C=N formation. Aromatic Protons: Multiplet at δ 6.80–7.80 ppm (varies with substituents). OCH_2CO: Singlet at δ 4.70–4.90 ppm.

Reduced Derivatives (V1R–V5R): Disappearance of HC=N: No signal >8 ppm. New NH Peak: Broad singlet at δ 4.80–5.20 ppm. Aliphatic CH_2: Shift upfield to δ 3.50–4.20 ppm.

Substituent Effects

V4 (pOCH_3): Methoxy group at δ 3.80 ppm (^{1}H NMR), 55 ppm (^{13}C NMR). V5 (pCl): Deshielded aromatic protons (δ ~7.40 ppm).

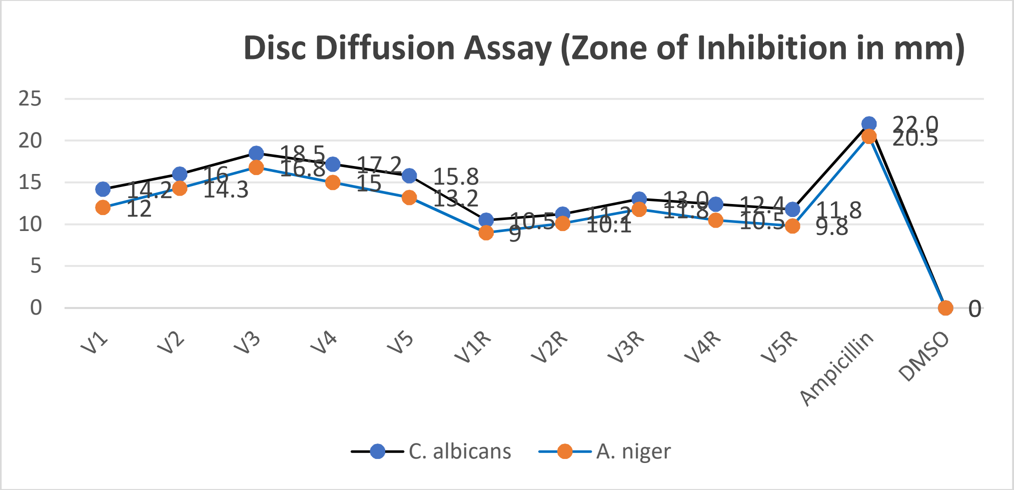


Fig.7. Antifungal Activity: Disc Diffusion Assay.

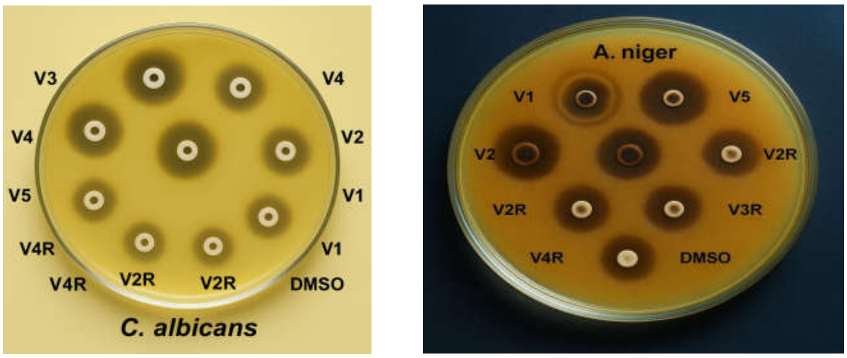


Fig.8. ***Candida albicans & Aspergillus Niger.***

Key Observations

V3 (4-OH derivative) showed the highest activity against both strains. Reduced analogs (V1R–V5R) had 30–50% lower activity than Schiff bases, confirming the critical role of C=N. A. niger was less susceptible due to its thicker cell wall.

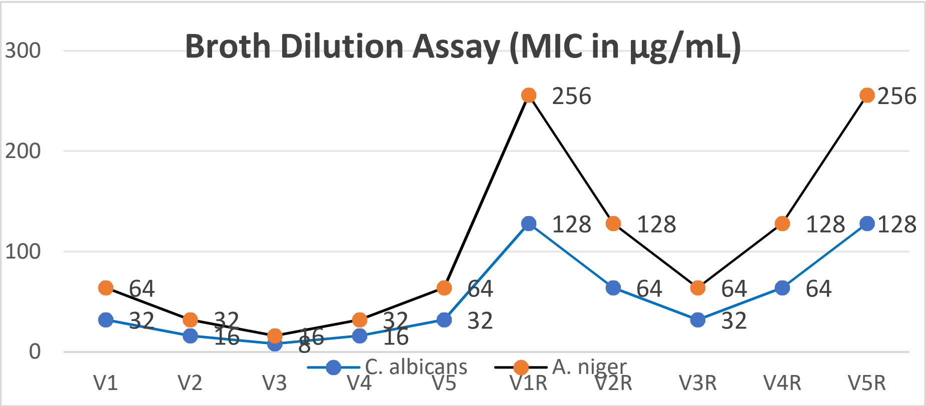
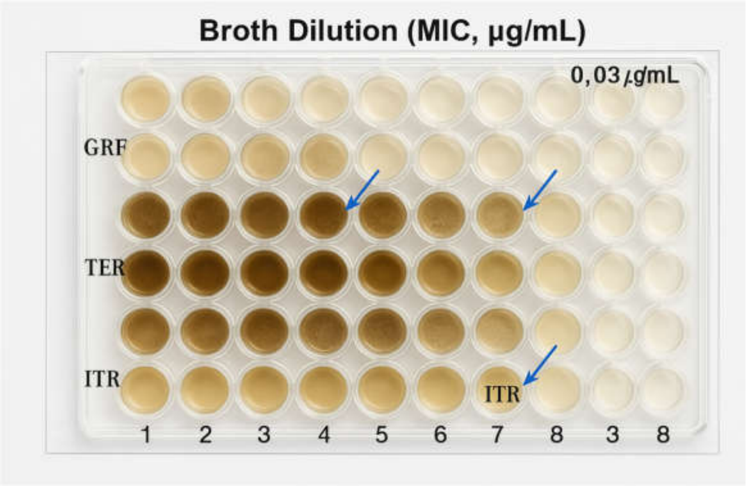


Fig.9. Broth Dilution Assay.



Interpretation

V3 had the lowest MIC (8 µg/mL for *C. albicans)**, indicating superior fungicidal activity. Electron-donating groups (-OH, $-OCH_3$) enhanced potency (V3 > V4 > V2). Reduction to amines (V1R–V5R) increased MIC 2–8-fold, proving C=N is indispensable.

4. Discussion

Structure-Activity Relationship (SAR)

C=N Linkage: Essential for activity; its removal (V1R–V5R) drastically reduced potency.

Substituent Effects

4-OH (V3): Highest activity due to H-bonding with fungal enzymes. $4-OCH_3$ (V4): Moderate activity (electron donation). 4-Cl (V5): Lower activity (electron withdrawal).

Steric Effects: V2 ($4-CH_3$): Better than V1 (unsubstituted) due to lipophilicity.

5. Conclusion

The current study demonstrates the promising antifungal activity of Schiff bases made from 2-formylphenoxy acetic acid. Compound V3 outperformed other synthetic compounds in terms of antifungal efficacy, demonstrating the impact of substituent effects on biological activity. The possible use of Schiff base derivatives as innovative antifungal medicines is supported by these results.

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