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# Synthesis, characterization and *in vitro* biological evaluation of the Schiff base derived from Benzidine and 1,3-Diphenyl-1,3-propanedione

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**Abstract**

Schiff bases are organic compounds formed by the reaction of the primary amine with carbonyl compounds (aldehydes or ketones). These are mainly bi- or tridentate ligands capable of forming very stable complexes with transitional metals. They are used as catalysts in oxygenation, hydrolysis, electro-reduction and decomposition reactions. Many Shiff bases show significant anti-tumor, antimicrobial and antifungal activity, which are the subject of research by many scientists in the world.
In this paper Schiff base from benzidine and 1,3-diphenyl-1,3-propanedione was synthesized. To characterize the product, FTIR spectroscopy and stereo-microscopy were used. In order to determine biological activity, antibacterial, antifungal and antioxidant activity of the product was tested.
The results showed that the interaction of benzidine and 1,3-diphenylpropanedione results in a Schiff base showing antibacterial, antifungal and antioxidant activity.

**Keywords:** Schiff base, benzidine, FTIR, UV/Vis, antimicrobial activity.

## 1. INTRODUCTION

Schiff bases (also known as imine or azomethine) are synthetically accessible and structurally diverse compounds, typically obtained by facile condensation between an aldehyde, or a ketone with primary amines (Hameed, al Rashida, Uroos, Abid Ali, & Khan 2017). Studies have shown that Schiff's bases have antimicrobial, antifungal, antioxidant, antitumor, antituberculous, catalytic and inhibitory activity (Bader 2010).

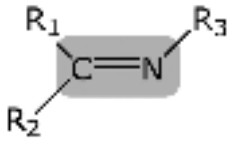


**Figure 1.** General structure of Schiff bases

The nitrogen atom of azomethine may be involved in the formation of a hydrogen bond with the active centers of cell constituents and interferes in normal cell processes (Kajal, Bala, Kamboj, Sharma, & Saini 2013; Vashi & Naik 2004; Venugopala & Jayashree 2003). Schiff bases are used in the photostabilization of poly(vinyl chloride) polymers against photodegradation by ultraviolet radiation (Hussain, Yousif, Ahmed, & Altaie 2014). Schiff base ligands are essential in the field of coordination chemistry, especially in the development of complexes of Schiff bases because these compounds are potentially capable of forming stable complexes with metal ions (Brodowska & Lodyga-Chruscinska 2014).

Benzidine (*p*-diaminodiphenyl) is a member of a large class of carcinogens referred to as aromatic amines. Benzidine and certain benzidine-based dyes can covalently react with DNA, and benzidine has been shown to induce chromosomal damage *in vivo* (Abel & DiGiovanni 2015).

1,3-diphenyl-1,3-propanedione (dibenzoylmethane or DMB) is a symmetrical ß-diketone. These compounds are interesting to scientists because of their ability to form

strong intra- and intermolecular hydrogen bonds (Kaitner & Meštrović 1993).

## 2. MATERIAL AND METHODS

All chemicals were of reagent grade, where possible, purchased from Aldrich and used without further purification.

### 2.1. Synthesis of Schiff base

Absolute ethanol was used as solvent. 0.01 mol of benzidine was dissolved in a 30 mL solvent. The solution was then transferred to a round-bottomed flask in which 0.005 mol of 1,3-diphenyl-2,3-propanedione was previously dissolved. The mixture was then refluxed to 70°C for 4 hours. After refluxing, the solution was cooled on ice where yellow crystals were isolated. The product was recrystallized in ethanol and stored in a desiccator until the analysis was carried out.

### 2.2. Spectral characterization

In order to determine the structure, products were recorded on Nicolet iS10 FT-IR spectrophotometer-Thermo Fisher Scientific. The ATR technique was used for sample analysis. Samples were recorded in the range of 4000-650 $cm^{-1}$.

Electronic spectra was recorded in the wavelength range of 200-400 nm on the Perkin Elmer $\lambda$25 device. A methanol solution of the Schiff base with the concentration of 0.02 mg/mL was used for recording.

### 2.3. Morphological characterization

To obtain better microscopic images, the crystals were treated with 100 $\mu$L of DMSO. The color, size and shape of the Schiff base were determined by microscopic analysis. Microscopic images were recorded on the binocular microscope, the Leica DM 2500P mark.

### 2.4. Antioxidant activity *in vitro*

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to the procedure described earlier (Benvenuti et al., 2004). The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$$\left[(A^{control} - A^{sample})/A^{control}\right] \times 100 \qquad (1)$$

where $A^{sample}$ is the absorbance of the solution containing the sample at 517 nm and Acontrol is the absorbance of the DPPH solution. The results are expressed as the IC50 value (mg/mL) or the concentration of the sample that caused 50% neutralization of DPPH radicals.

The determination of ferric reducing antioxidant power or ferric reducing ability (FRAP assay) was performed as described earlier (Jiménez-Aspee et al., 2014). To obtain the calibration curve, solutions of $FeSO_4 \times H_2O$ were prepared in the concentration range of 200-1000 $\mu$mol/L ($y = 0.001x + 0.0615$; $R^2 = 0.9907$). In each tube, 0.1 mL of Schiff base and 3 mL of FRAP reagent were added. The samples were incubated in an aqueous bath for 30 minutes at 37°C, and the absorbance was measured at 593 nm.

### 2.5. Antimicrobial activity *in vitro*

Antibacterial activity was investigated by diffusion method on reference bacterial strains *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*. Antifungal activity was tested on *Candida albicans*. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared (density $10^7 - 10^8$ CFU/mL, depending on soy). The strains were then placed on the surface of the nutrient substrate-Mueller-Hinton agar (MH), dispersed in sterile Petri dishes. Sub-

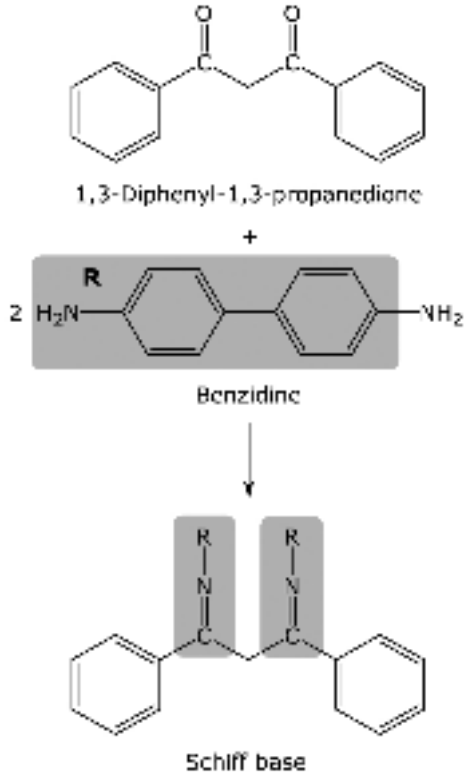


**Figure 2.** Reaction scheme and proposed structure of Schiff base

strate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 100 $\mu$L of Schiff base solutions were added at the concentration of 5 mg/mL. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at 37°C/24 h. After the incubation period, the size of the inhibitory zone was measured and the sensitivity of the microorganisms was expressed (Pirvu, Hlevca, Nicu, & Bubueanu 2014).

## 3. RESULTS AND DISCUSSION

### 3.1. Structure of Schiff base

The reaction scheme and the proposed structure of the Schiff base is shown in Figure 2. The melting point of the product is 105.1°C. Melting point of the product is lower in relation to benzidine (120°C) and higher than the melting point 1,3-diphenyl-1,3-propanedione (77°C). The product obtained is intensively yellow colour crystals, soluble in DMSO, methanol and ethanol.

### 3.2. Spectral characterization

Figures 3–5 show the FTIR spectra of the reactants and the Schiff base.

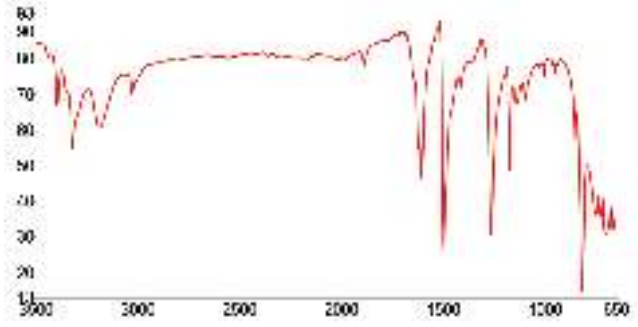

**Figure 3.** FTIR spectra of Benzidine

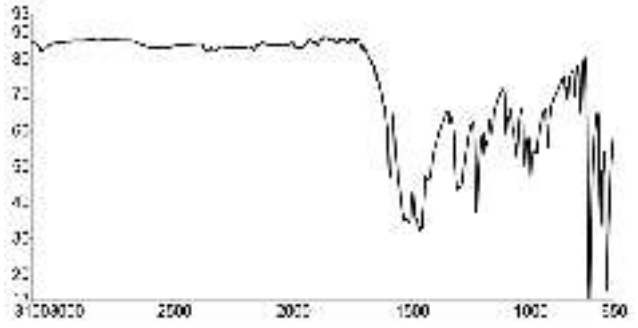

**Figure 4.** FTIR spectra of 1,3-Diphenyl-1,3-propanedione

The benzidine spectrum is characterized by band range of 3193-3401 cm$^{-1}$ corresponding to valence

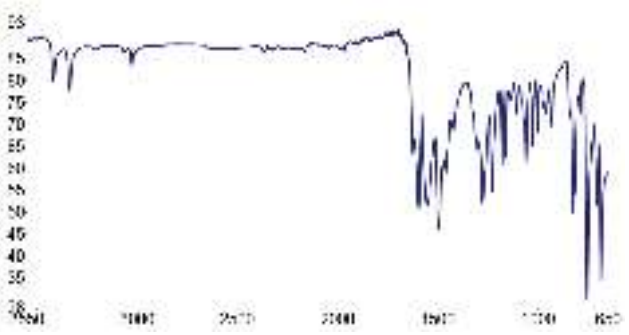

**Figure 5.** FTIR spectra of Schiff base

N–H vibrations [$\nu$(NH)]. N–H bending vibration was recorded at 1605 cm$^{-1}$, and the C–N stretch vibration at 1263 cm$^{-1}$. In the FTIR spectrum of 1,3-diphenylpropanedione, the intensive band at 1592 cm$^{-1}$ corresponds to the C=O bond. Compared to the interpreted spectra of the reactants, the Schiff base spectrum retains the appearance of the reactant spectra, with changes in intensity and a slight movement of the band to smaller or larger wavelengths. The new band in the product range at about 1550 cm$^{-1}$ corresponds to the C=N bond.

Electronic spectra of the Schiff base is shown in Figure 6. The spectrum is characterized by three bands (at 341, 290 and 221 nm). The absorption maximum was recorded at 341 nm, which corresponds to $\pi \rightarrow \pi^*$ transition.

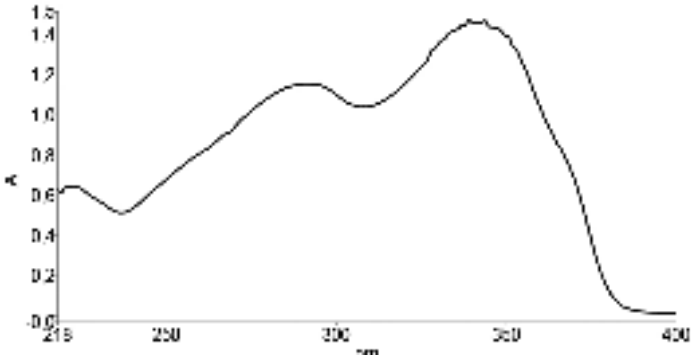

**Figure 6.** Electronic spectra of Schiff base

### 3.3. Morphological characterization

The morphology of the Schiff base crystal is shown in Figure 7. Crystals are radially-airlike with a characteristic "spinning" ending. They are incorrectly oriented and intertwined in the sample. The length of the air-needle crystals forms up to 3 mm.

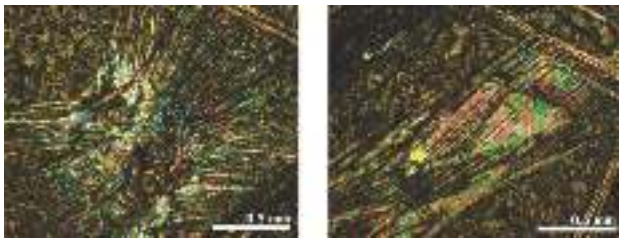

**Figure 7.** Morphology of the Schiff base crystal

### 3.4. Antioxidant activity *in vitro*

The results of the antioxidative capacity obtained by FRAP and DPPH method are shown in Table 1.

**Table 1.** Results of antioxidant activity *in vitro*

| Sample | FRAP value [$\mu$mol/L $Fe^{2+}$] | $IC_{50}$ value [mg/mL] |
|---|---|---|
| Schiff base | 737 | 0.067 |
| Vitamin C | 1425 | 0.031 |

Based on the constructed diagrams, an $IC_{50}$ value for a Schiff base of 0.067 mg/mL was calculated. The indicated value is significantly higher in relation to vitamin C whose $IC_{50}$ value is 0.031 mg/mL. The results of FRAP analysis confirm the results obtained by the DPPH method. For a Schiff base concentration of 0.1 mg/mL, the resulting FRAP value is 737 $\mu$mol/L. For vitamin C (at a concentration of 0.1 mg/mL), the reduction potential is 1425 $\mu$mol/L.

### 3.5. Antimicrobial activity *in vitro*

The results of antimicrobial activity of the synthesized Schiff base obtained by diffusion technique are shown in Table 2. The Schiff base has shown a weaker effect in the case of gram-positive bacteria *S. aureus* and *E. faecalis*. More effective action was found against *C. albicans*. The control agents showed greater activity in relation to the synthesized Schiff base.

**Table 2.** Results of antimicrobial activity obtained by diffusion technique

| Sample | Inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Schiff base | – | + | + | – | – | – | ++ |
| Control | +++ | +++ | +++ | +++ | +++ | +++ | ++ |

Legend:
**1** – *E. coli*; **2** – *S. aureus*; **3** – *E. faecalis*; **4** – *L. monocytogenes*; **5** – *B. subtilis*; **6** – *P. aeruginosa*; **7** – *Candida albicans*;
Control: **CPF** – Ciprofloxacin (conc. 1 mg/mL) and Nystatin

## 4. CONCLUSION

The reaction of benzidine and 1,3-Diphenyl-1,3-propanedione produces a Schiff base with a significant antioxidant capacity. Poor antimicrobial activity was demonstrated on bacterial strains of *S. aureus* and *E. faecalis*, as well as *C. albicans*.

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