



BENZYLIDENE ANILINE AND BENZIMIDAZOLIUM FLUOROCHROMATE - SPECTRAL AND MICROBIAL CHARACTERIZATION

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ABSTRACT

Benzylidene aniline is one of the important aromatic compounds for an additional stain which is used in various immuno assays. Benzimidazolium fluorochromate (BIFC) is one of the chromium (VI) compounds, which is used as a mild oxidising agent. This study aims to describe the synthesis and determination of antimicrobial activity of bezylidene aniline and BIFC. IR and UV spectral data were used to confirm the structure of compounds and antimicrobial activities were characterised by the nature of biological activities. Gram-positive and negative bacteria and also fungi were used for antimicrobial study by disc diffusion method.

Keywords: Benzylidene aniline, BIFC, Microbial study, Disc diffusion method, Bacteria, Fungi.

1. INTRODUCTION

Benzylidene aniline (X-CH=N-Y) consist of two phenyl ring such as benzaldehyde (X) and aniline (Y) moiety. Benzylidene aniline can be formed the azomethine group (-CH=N-) by the condensation of aniline and benzaldehyde under specific conditions [1, 2]. Benzylidene aniline like Schiff base having azomethine group (-CH=N-) play an important role in biological, analytical, industrial and pharmacological activity. It is used in polymer stabilizers and acts as intermediates in organic synthesis [3-6].

Benzimidazole is a heterocyclic compound, which is a benzene ring fused with 4, 5- position in imidazole ring. It is used in a wide range of pharmaceutical and biological applications [7]. Benzimidazole derivatives display numerous properties like anti-inflammatory, anti-cancer, anti-fungal and anti-oxidant [8]. But, the derivative of benzimidazolium fluorochromate is the chromium (VI) compounds used as a mild, efficient, stable and selective oxidizing agent in synthetic organic chemistry [9]. These two compounds are screened for their biological activities towards gram positive & negative bacteria and fungi.

2. EXPERIMENTAL

2.1. Material

AnalaR grade of reagents are used for the preparation of

benzylidene aniline and benzimidazolium fluorochromate. The physical constants of these two compounds are characterized by Thomas Hoover capillary melting point Instrument. All other chemicals were used as AnalaR grade and purity was checked with comparison of standard physical constants.

2.2. Preparation of benzylidene aniline

Benzylidene aniline was prepared by refluxing equimolar quantities of benzaldehyde and aniline in ethanol for about 2-3 h. The resulting mixture was cooled to room temperature and stream into ice-cooled water with constant stirring. The precipitate was filtered off, soaked with ethanol and then dried. The product was recrystallized with ethanol. The purity of benzylidene aniline was verified by determining its melting point (53°C) and comparing the literature value (53°C) [2].

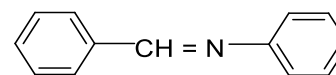


Fig. 1: Structure of Benzylidene Aniline

2.3. Preparation of BIFC (Benzimidazolium Fluorochromate)

Benzimidazolium fluorochromate (C₇H₄N₂H)CrO₃F has been prepared by mixing Benzimidazole, hydrofluoric

acid and CrO_3 (chromium trioxide) of the molar ratio 1: 1.3: 1 in frozen temperature. Benzimidazolium fluorochromate was acquired as yellow-orange crystals. BIFC is non-hygroscopic in nature and light unresponsive to storage. The yield of benzimidazolium fluorochromate was 86%. The compound melted at 195°C (literature value of melting point $194\text{-}196^\circ\text{C}$). The purity of benzimidazolium fluorochromate was verified by iodometric method [9].

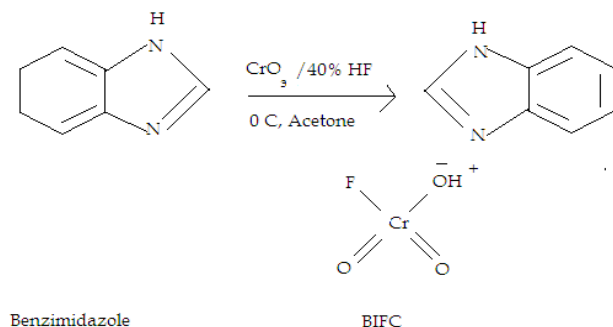


Fig. 2: Synthesis of Benzimidazolium fluorochromate

2.4. Characterization of prepared compounds

The structures of benzylidene aniline and benzimidazolium fluorochromate were confirmed by its elemental analysis. Further, it was confirmed by melting point because of reported compound. Benzylidene aniline and benzimidazolium fluorochromate have been characterized by electronic spectroscopy (Perkin Elmer, Model: Lambda 35, Range: 190 nm - 1100 nm) in the UV visible range and vibrational spectroscopy (Perkin Elmer, Model: Spectrum Two, Range: 4000 cm^{-1} - 400 cm^{-1}) in the IR range.

2.5. Antimicrobial screening

The prepared compounds were characterized with antimicrobial screening by Disc Diffusion method. Bacterial cultures such as, *Aspergillus Niger* (MTCC 1344), *Trichoderma Viride* (MTCC 5179), *Streptococcus Epidermidis* (MTCC 435), *Mycobacterium Tuberculosis* (MTCC 9506), *Escherichia Coli* (MTCC 118) and *Pseudomonas Aeruginosa* (MTCC 424) were obtained from Eumic Analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on Nutrient agar slants (Hi Media) at 4°C . Bacterial cultures were subcultured in liquid medium (Nutrient broth) at 37°C for 8h and further used for the test ($10^5\text{-}10^6\text{CFU/ml}$). These suspensions were prepared immediately before the test was carried out.

3. RESULTS AND DISCUSSION

3.1. Electronic Spectroscopy

The electronic spectra of benzylidene aniline and benzimidazolium fluorochromate in UV Visible region were obtained in DMSO using a spectrometer in the range of 200-800 nm shown in Fig. 3 and Fig. 4. The electronic spectra of benzylidene aniline show absorption bands at 211, 228 and 276 nm. These bands explain the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the present azomethines, chromophore group and aromatic ring. An extra absorption band was observed above 400 nm in the electronic spectra of the benzylidene aniline [3-4].

The electronic spectra of benzimidazolium fluorochromate show the absorption bands at 211, 215, 228 and 286 nm. These bands explain the indication of $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the present azomethines, chromophore group and aromatic ring. An additional absorption band was observed above 523 nm in the electronic spectra of the benzimidazolium fluorochromate charge transfer transition [4].

3.2. Vibrational Spectroscopy

The FTIR spectra of benzylidene aniline show the stretching vibration at 2976.24cm^{-1} , indicates the presence of aromatic C-H group. The peak obtained at 1630.38cm^{-1} , 1430.48cm^{-1} , 1259.54cm^{-1} and 1308.06cm^{-1} , indicate the presence of C=N group, aromatic C=C group, C-C group, aromatic C-N group respectively [3-4].

The FTIR spectra of benzimidazolium fluorochromate show the stretching vibration obtained at 3038.20cm^{-1} , indicates the presence of aromatic C=C-H group. The peak obtained at 1580.79cm^{-1} , 1450.48cm^{-1} , 1200.80cm^{-1} , 3439.92cm^{-1} and 951.30cm^{-1} , shows the presence of C-C=C, aromatic N-H, aromatic C-C=C, C-N and aromatic Cr=O group [9].

3.3. Evaluation of Biological Activity

Benzylidene aniline and benzimidazolium fluorochromate were tested for their microbial activity against three strains including two gram-positive species i.e. *Streptococcus Epidermidis* and *Mycobacterium Tuberculosis*, two gram-negative species i.e. *Escherichia Coli* and *Pseudomonas Aeruginosa* and two fungal strains i.e. *Aspergillus Niger* and *Trichoderma Viride* by utilizing the disc diffusion method for the various concentrations of 25, 50, 75 and 100 μL . The bacteria and fungi were subcultures in agar. The bacteria agar plates were incubated at 37°C 18-24 hrs. The fungi agar plates were incubated at 25°C 18-24 hrs [10-14].

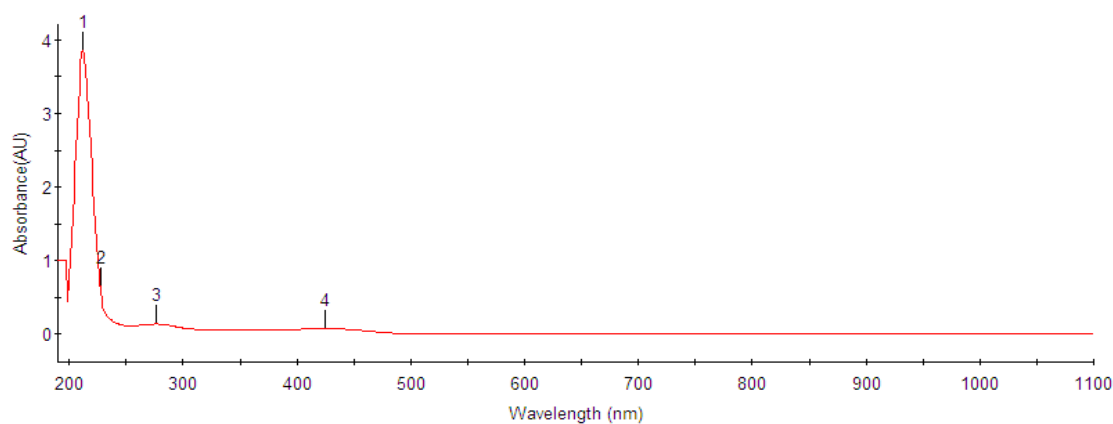


Fig. 3: Electronic Spectrum of Benzylidene Aniline

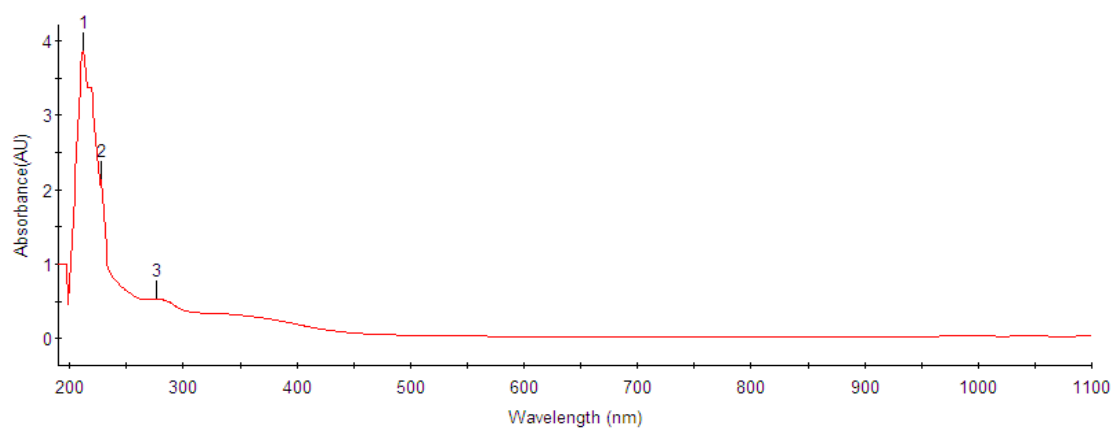


Fig. 4: Electronic Spectrum of Benzimidazolium Fluorochromate

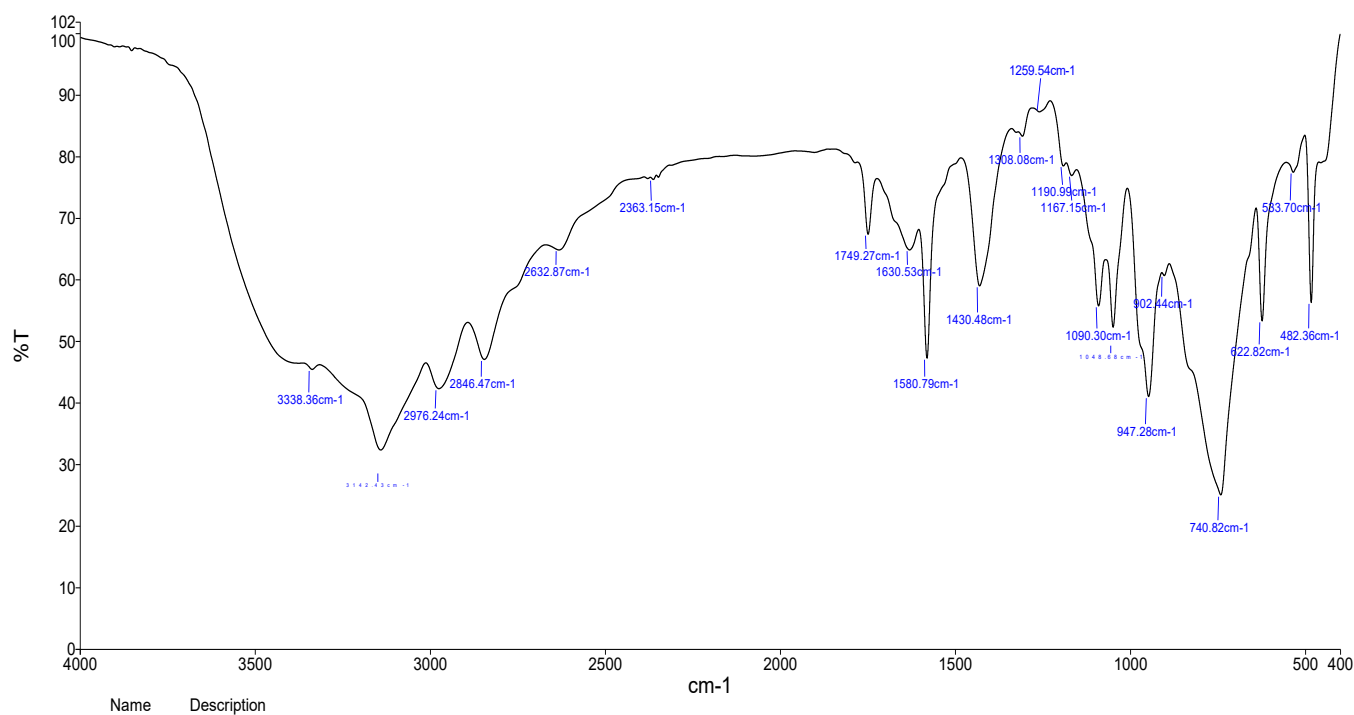


Fig. 5: FT-IR Spectrum of Benzylidene Aniline

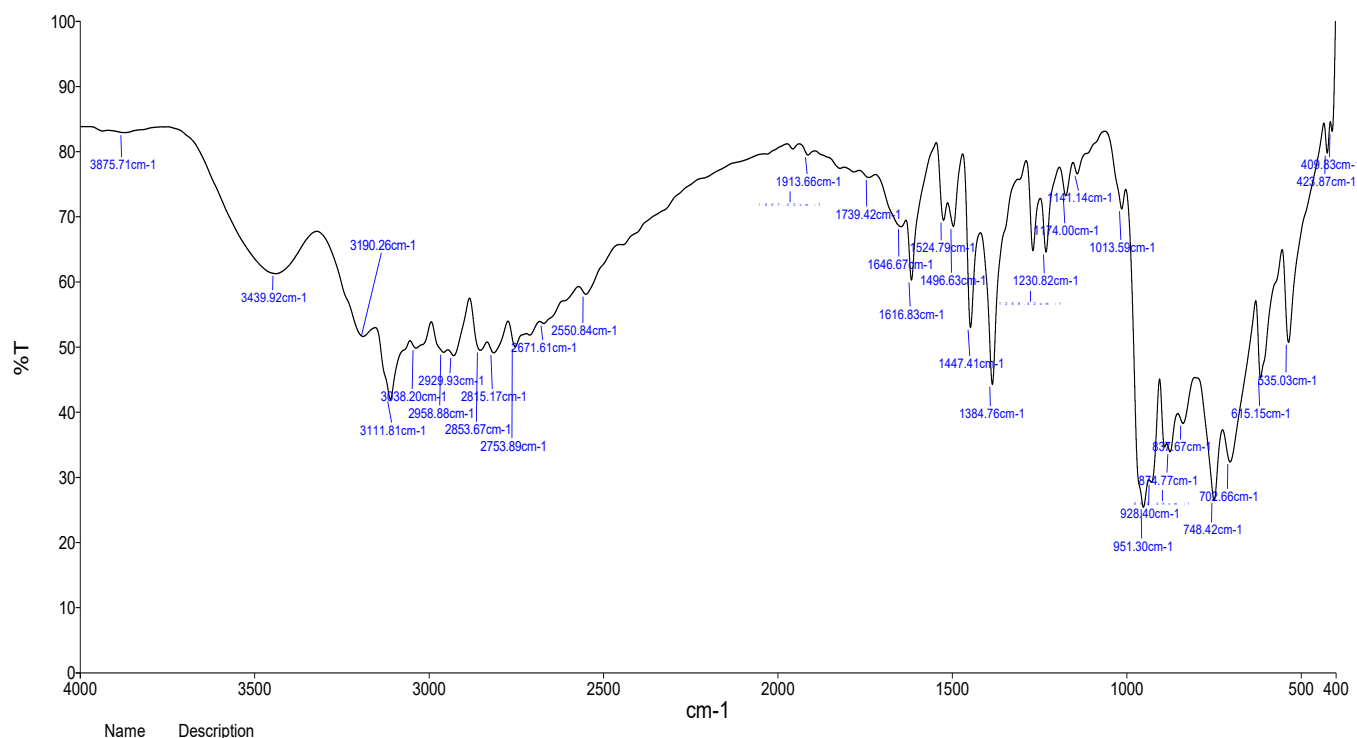


Fig. 6: FT-IR Spectrum of Benzimidazolium Fluorochromate

3.3.1. Anti Fungal and anti bacterial activity of benzylidene aniline

The biological screening result of the benzylidene aniline showed a varying degree of inhibition zone in tested microbes. The antimicrobial activity of the test samples increases with increase of their concentration (table 1). In this study, gentamycin was used as a standard antibiotic for comparison with the benzylidene aniline. Fungal strains such as *Aspergillus Niger* and *Trichoderma Viride* showed higher activity as compared to the standard antibiotic (Gentamycin) in 100 μ L. Gram-positive bacteria *Mycobacterium Tuberculosis* shows a higher activity in 75 μ L and 100 μ L and also gram-negative bacteria *Pseudomonas Aeruginosa* have higher activity in 100 μ L [10-14].

3.3.2. Anti Fungal and Anti Bacterial Activity of Benzimidazolium Fluorochromate

The biological screening result of the benzimidazolium fluorochromate showed a varying degree of inhibition zone in tested microbes. The antimicrobial activity of the test samples rises with rises in the concentration (table 2). In this study, gentamycin was used as a standard antibiotic for comparison with the benzimidazolium fluorochromate. Fungal strains such as *Aspergillus Niger* showed that a higher activity as compared to the standard antibiotic (Gentamycin) in 100 μ L. Gram-negative bacteria *Pseudomonas Aeruginosa* was only had higher activity in 100 μ L. In general, it is a very considerable activity as compared to the standard antibiotic (Gentamycin) [10-14].

Table 1: Anti Bacterial and Anti Fungal Activity of Benzylidene Aniline

Organisms	DMSO Extract Added in the Zone of Inhibition(mm/ml)				
	25 μ L	50 μ L	75 μ L	100 μ L	Standard Control
<i>Aspergillus Niger</i>	22	24	27	32	25
<i>Trichoderma Viride</i>	22	26	30	34	30
<i>Streptococcus Epidermidis</i>	20	23	26	30	30
<i>Mycobacterium Tuberculosis</i>	18	22	25	28	22
<i>Escherichia Coli</i>	18	20	23	26	26
<i>Pseudomonas Aeruginosa</i>	17	20	24	30	25

Table 2: Anti Bacterial and Anti Fungal Activity of Benzimidazolium Fluorochromate

Organisms	DMSO Extract Added in the Zone of Inhibition(mm/ml)				
	25 μ L	50 μ L	75 μ L	100 μ L	Control
<i>Aspergillus Niger</i>	10	12	14	26	23
<i>Trichoderma Viride</i>	12	14	16	19	30
<i>Streptococcus Epidermidis</i>	10	13	16	22	30
<i>Mycobacterium Tuberculosis</i>	10	13	16	20	20
<i>Escherichia Coli</i>	10	12	14	16	20
<i>Pseudomonas Aeruginosa</i>	12	14	17	20	25

4. CONCLUSION

We have synthesized and characterized benzyldene aniline and benzimidazolium fluorochromate using UV and IR spectroscopic analysis. Both the compounds were screened against two gram-positive, gram-negative bacteria and two fungal strains. The concentration of the above compounds increases with an increase in activity. Fungal strains such as *Aspergillus Niger* only have higher activity as compared to the standard antibiotic (Gentamycin) in 100 μ L. The gram-positive bacteria *Mycobacterium Tuberculosis* showed only nearly higher activity in 100 μ L. But, the gram-negative bacteria *Pseudomonas Aeruginosa* has a nearly higher activity in 100 μ L for benzyldene aniline as compared to Gentamycin.

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Conflict of interest

NIL

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