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# B-CYCLODEXTRIN ENCAPSULATION OF (E)-7-(DIETHYLAMINO)-3-((3-HYDROXYNAPHTHALEN-2-YL)IMINOMETHYL)-2H-CHROMEN-2-ONE SCHIFF BASE: SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND POTENT ANTIMICROBIAL PROPERTIES

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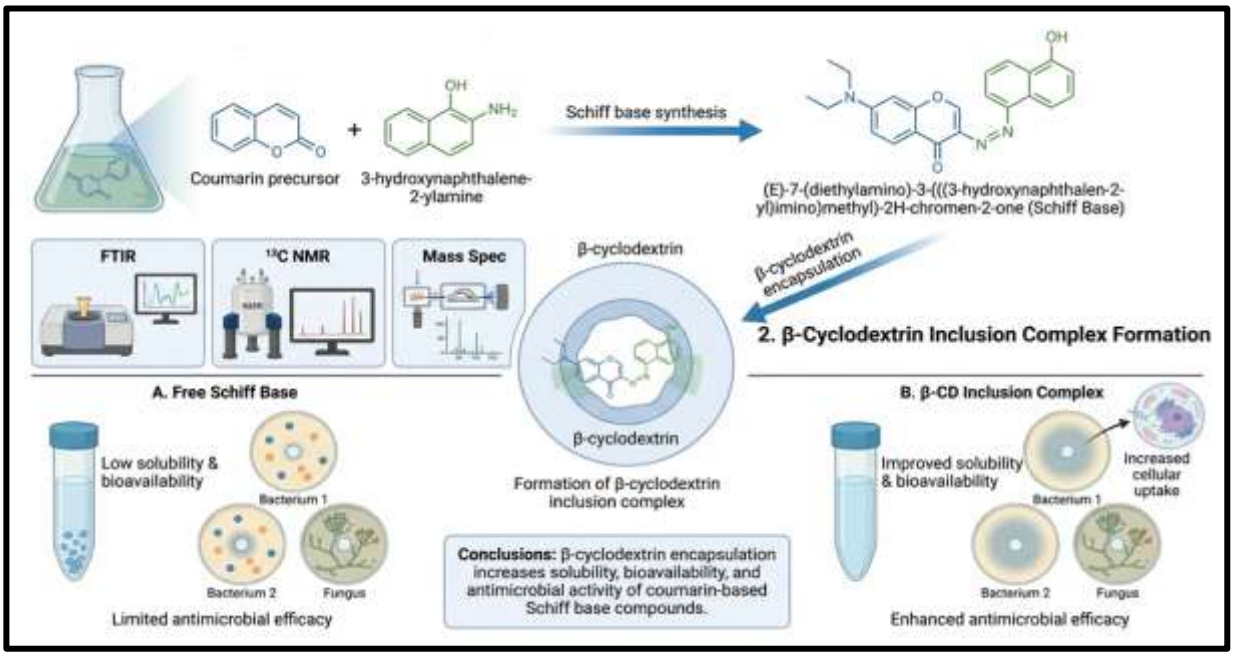



**ABSTRACT**
Coumarin-based Schiff bases have received much interest in medicinal and supramolecular chemistry due to their diverse nature, easy synthetic accessibility, and wide-ranging bioactivity. This article describes the synthesis, characterization, and preparation of the β-cyclodextrin inclusion complex of a new Schiff base based on coumarin structure, (E)-7-(diethylamino)-3-(((3-hydroxynaphthalen-2-yl)imino)methyl)-2H-chromen-2-one. Characterization of the Schiff base compound and its inclusion complex was performed by Fourier Transform Infrared Spectroscopy, Carbon-13 Nuclear Magnetic Resonance, and Mass Spectrometry. It can be clearly seen that inclusion complex showed better results in terms of solubility and bioavailability compared to Schiff base alone. In vitro testing of its antimicrobial efficacy showed higher inhibition of selected bacteria and fungi due to the improved dissolution properties of the compound along with increased cellular uptake after encapsulation in the inclusion complex. Thus, it is evident that β-cyclodextrin encapsulation can serve as an effective approach towards increasing pharmacological activity of coumarin-based Schiff bases as antimicrobial agents.

**KEYWORDS:** Schiff base, Coumarin derivative, β-Cyclodextrin inclusion complex, Host-guest chemistry, antimicrobial activity, Antifungal activity, Imine bond, Supramolecular chemistry.

## INTRODUCTION

Schiff's bases or azomethines/imines are chemically distinct and therapeutically important organic molecules synthesized as a result of the condensation reaction involving a primary amine and an active carbonyl group such as aldehydes or ketones [1]. The discovery of this family of chemicals is credited to Hugo Schiff in the year 1864, after which they have gained immense popularity within the domain of medicinal chemistry, coordination chemistry, and material sciences. The

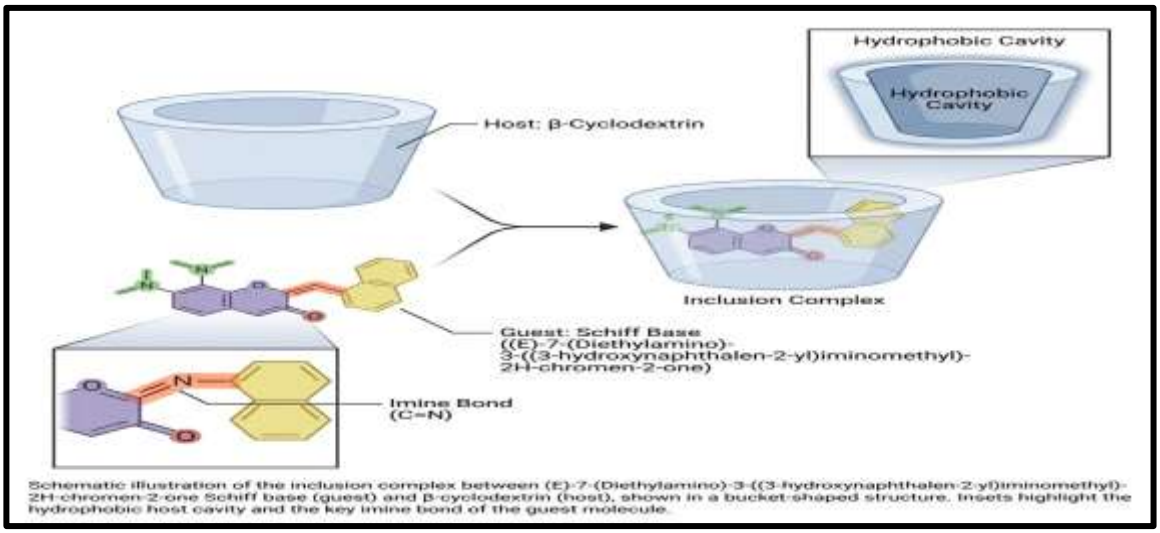
most important structural characteristic of Schiff bases is the presence of an azomethine bond (–C=N–)[1,2] [3,4]. This unique property not only gives them a distinctive electronic character but also acts as an important moiety that confers a wide range of biological activities such as antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory effects on them[5,6]. It is the electron-rich nature of the nitrogen atom in C=N that helps in coordinating with diverse transition metal cations to form metal complexes that have increased biological activity. Schiff bases' economic advantages are many owing to their easy synthesis, structural modification and lipophilicity [7,8] [1,9,10].

Among several carbonyl precursors used in Schiff base formation, the coumarin group has drawn significant attention due to its wide availability and low toxicity towards mammals along with diverse biological activity [11,12]. Coumarins constitute a heterocyclic organic compound of benzopyranone type that is widely available in nature [15]. These compounds are effective against microbes, fungi, cancers, inflammation, coagulation, and oxidation processes [16]. The coumarin core presents a unique rigid and planar core structure, which enables the easy process of DNA intercalation and enzyme site interaction, thus allowing the development of a wide range of biological activities [17]. Introduction of functional groups on the coumarin ring, especially by introducing electron-donating groups such as the diethylamino moiety on the C-7 position, greatly improves the electronic push-pull effect of the molecule by extending its π-conjugation and increasing its charge transfer capability, which subsequently improves the biological efficacy of the coumarin derivatives obtained [18,19]. The C-3 position on the coumarin structure is equally significant since the introduction of an azomethine group on this position develops a conjugation between the coumarin pharmacophore and the amine portion, leading to the formation of Schiff bases with better electronic delocalization and greater affinity to receptors [3].

The naphthol function is equally important in medicinal chemistry, where hydroxynaphthyl compounds have been extensively studied due to their high efficacy against microbes, oxidation, inflammation, and enzymes [20]. The presence of naphthalene as the backbone offers enhanced π-π stacking capabilities for binding with biological molecules, whereas the phenolic hydroxyl function on the C-3 atom of the naphthalen-2-yl ring acts as an excellent hydrogen bonding donor to improve binding affinity [21]. It is anticipated that a synergistic action of pharmacology will be observed as a result of the interaction of the electron-donating system of the coumarin core with the electron-withdrawing substituent 3-hydroxynaphthalen-2-yl through the imine functional group, owing to the presence of extended π-conjugation throughout the molecule and several heteroatomic centers of electron donation, viz., the imine nitrogen, lactone oxygen, and naphtholic hydroxyl groups [4,22]. These characteristics have been found to improve the metal ion binding capacity and enhance antimicrobial activity [11,12].

However, due to the hydrophobic nature of these Schiff base compounds, their aqueous solubility remains poor, thus limiting their bioavailability [23]. Encapsulation inside cyclodextrin cavities stands out among other supramolecular approaches that have been successfully used in pharmacology [24,25]. β-Cyclodextrin (β-CD) is a cyclic oligosaccharide made up of seven glucopyranose monomers that exhibit a unique structural feature characterized by a truncated cone-like structure having a hydrophobic interior and hydrophilic exterior [6,26]. This makes β-CD capable of forming host-guest complexes with hydrophobic substances by non-covalent interactions [27]. Formation of inclusion complexes between β-cyclodextrin and other drug molecules has been proven to greatly increase their solubility, stability, rate of dissolution, and bioavailability due to poor solubility in aqueous medium [6,8]. Also, the inclusion complexation of active molecules inside the cavity of β-cyclodextrin increases the efficiency of the drugs in fighting microbes due to controlled and sustained release of the drug at the site of infection [10,28].

In view of the above, the present study reports the synthesis of a novel coumarin-based Schiff base, (E)-7-(diethylamino)-3-(((3-hydroxynaphthalen-2-yl)imino)methyl)-2H-chromen-2-one, through the condensation of 7-(diethylamino)coumarin-3-carbaldehyde with 3-amino-2-naphthol, and the preparation of its β-cyclodextrin inclusion complex by the co-precipitation method [29]. The synthesized Schiff base and its inclusion complex were comprehensively characterized using Fourier Transform Infrared Spectroscopy (FT-IR), $^{13}C$ Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry to confirm the structure and establish the host-guest interaction [7,30]. The antibacterial and antifungal activities of both the free Schiff base and its β-CD inclusion complex were systematically evaluated and compared against selected pathogenic bacterial and fungal strains, with the aim of establishing β-cyclodextrin encapsulation as a viable strategy for enhancing the pharmacological potential of coumarin Schiff base derivatives [5,16].



Schematic illustration of the inclusion complex between (E)-7-(Diethylamino)-3-((3-hydroxynaphthalen-2-yl)iminomethyl)-2H-chromen-2-one Schiff base (guest) and β-cyclodextrin (host), shown in a bucket-shaped structure. Insets highlight the hydrophobic host cavity and the key imine bond of the guest molecule.

**Host-Guest Chemistry (Inclusion Complex):**

Host-guest chemistry represents a fundamental branch of supramolecular chemistry that describes the formation of non-covalent complexes between two distinct molecular entities — the "host," which possesses an appropriately sized cavity or binding cleft, and the "guest," which is accommodated within that cavity through geometric and chemical complementarity [27]. The conceptual foundation of this field was laid by the pioneering work of Pedersen, Cram, and Lehn, whose discoveries of crown ethers, spherands, and cryptands in the 1960s–1980s demonstrated that molecular recognition could be designed and tuned with remarkable precision, an achievement recognized by the Nobel Prize in Chemistry in 1987 [31]. A particularly important subset of host-guest interactions is the inclusion complex, in which the guest molecule is partially or completely enclosed within the three-dimensional interior of the host framework without the formation of any covalent bonds [6,24]. The driving forces governing inclusion complex formation are inherently non-covalent in nature and typically comprise a combination of hydrophobic interactions, van der Waals forces, hydrogen bonding, electrostatic attractions, and π–π stacking, with the thermodynamic stability of the complex expressed through the association constant ($K_a$) and the corresponding Gibbs free energy of binding ($\Delta G = \Delta H - T\Delta S$) [25,32].

The selectivity of host-guest recognition is governed by the principle of complementarity — the host cavity must match the guest in terms of size, shape, and electronic character — a relationship often described by the "lock-and-key" analogy originally articulated by Emil Fischer in the context of enzyme–substrate interactions [33]. Among the most extensively studied host systems are cyclodextrins, cucurbit[n]urils, calixarenes, and pillararenes, each offering distinct cavity dimensions and surface chemistries that confer characteristic selectivities toward specific classes of guest molecules [34]. The practical significance of inclusion complex formation has been demonstrated across diverse domains, including pharmaceutical science — where cyclodextrin encapsulation is widely employed to enhance the aqueous solubility, chemical stability, and bioavailability of hydrophobic drug molecules [8,26] — as well as heterogeneous and homogeneous catalysis, molecular recognition-based sensing, controlled release systems [28], and the construction of mechanically interlocked architectures such as rotaxanes and catenanes [35]. Continued advances in the rational design of host molecules with tailored cavity geometries, combined with growing interest in stimuli-responsive host-guest systems that respond to external triggers such as pH, redox potential, light, or competitive displacement, have positioned host-guest chemistry as a versatile platform for the development of next-generation functional materials and supramolecular devices [36].

## MATERIALS AND METHODS

### 1. Materials and Reagent

All chemicals and reagents used in the present study were of analytical grade and employed without further purification unless otherwise stated. 7-(Diethylamino) coumarin-3-carbaldehyde and 3-amino-2-naphthol were procured from Sigma-Aldrich (St. Louis, MO, USA). β-Cyclodextrin (β-CD, average MW ~1135 g/mol, degree of substitution ≥ 98%) was obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Solvents including ethanol, methanol, dimethyl sulfoxide (DMSO), and acetone were purchased from Merck (India) and used as received. Distilled water was used throughout all experimental procedures. Nutrient agar, Mueller-Hinton agar, and nutrient broth used for antimicrobial studies were procured from HiMedia Laboratories[37].

### 2. Synthesis of Schiff base ligand

The Schiff base ligand, (E)-7-(diethylamino)-3-((3-hydroxynaphthalen-2-yl)iminomethyl)-2H-chromen-2-one (hereafter designated SB), was synthesized by a classical condensation reaction between 7-(diethylamino)coumarin-3-carbaldehyde and 3-amino-2-naphthol in a 1:1 molar ratio. Briefly, equimolar quantities (1 mmol each) of the aldehyde and amine components were dissolved separately in 20 mL of absolute ethanol. The two solutions were combined in a 100 mL round-bottom flask, and a catalytic quantity of glacial acetic acid (2–3 drops) was added to facilitate imine bond formation. The reaction mixture was refluxed at 78°C with continuous stirring for 4–6 hours, during which the progression of the condensation was monitored by thin-layer chromatography (TLC) on silica gel 60 $F_{254}$ plates using a hexane: ethyl acetate (7:3, v/v) mobile phase. Upon completion of the reaction, the mixture was allowed to cool to room temperature, whereupon a colored precipitate formed. The crude product was collected by vacuum filtration, washed repeatedly with cold ethanol to remove unreacted starting materials, and dried under vacuum at 60°C for 24 hours. The purified Schiff base was stored in a desiccator until further use[9].

### 3. Preparation of β-Cyclodextrin Inclusion Complex by the Kneading Method

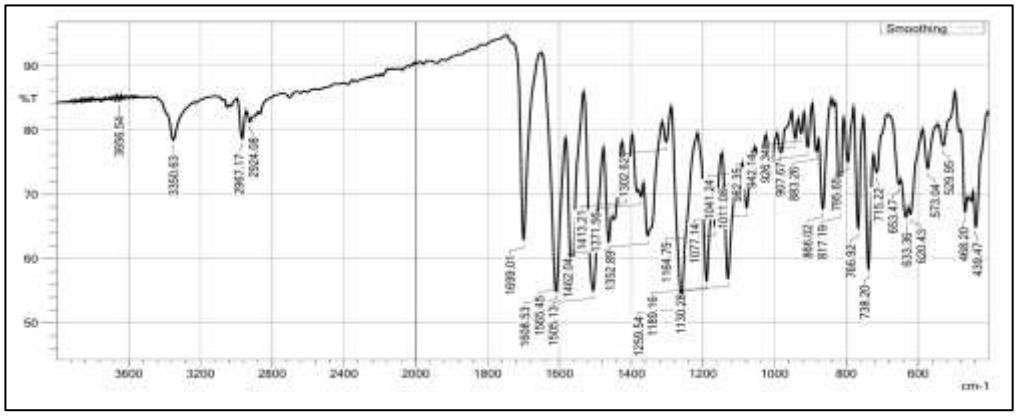
The inclusion complex of the Schiff base with β-cyclodextrin (SB: β-CD) was prepared by the kneading method, which is a solvent-assisted solid-state technique widely employed for the preparation of cyclodextrin inclusion complexes at a 1:1 molar ratio[6,24]. In a typical experiment, β-cyclodextrin (1 mmol) was accurately weighed and placed in a glass mortar. The Schiff base ligand (1 mmol) was added incrementally to the mortar, and the binary mixture was triturated manually using a pestle with continuous and vigorous kneading. A small volume of hydroalcoholic solvent (water: ethanol, 1:1, v/v) was added dropwise during the kneading process in a minimal quantity (approximately 0.5–1.0 mL) sufficient to form a homogeneous, workable paste, thereby promoting intimate contact between the host and guest molecules and facilitating penetration of the guest into the cyclodextrin cavity. Kneading was continued for 45–60 minutes at room temperature to ensure thorough mixing and adequate encapsulation[26] [28,29]. The resultant paste was subsequently dried in a hot-air oven at 40°C for 24 hours to remove residual solvent. The dried solid was ground to a fine powder using a mortar and pestle, passed through a 150 μm sieve to obtain a uniform particle size, and stored in a sealed desiccator protected from moisture and light prior to characterization. For comparative purposes, a physical mixture (PM) of SB and β-CD in the same 1:1 molar ratio was also prepared by simple blending without kneading or solvent addition and subjected to identical characterization procedures.

## 4. Spectroscopic and Physicochemical Characterization

- **Fourier Transform Infrared Spectroscope (FTIR) of β-CD-SB**

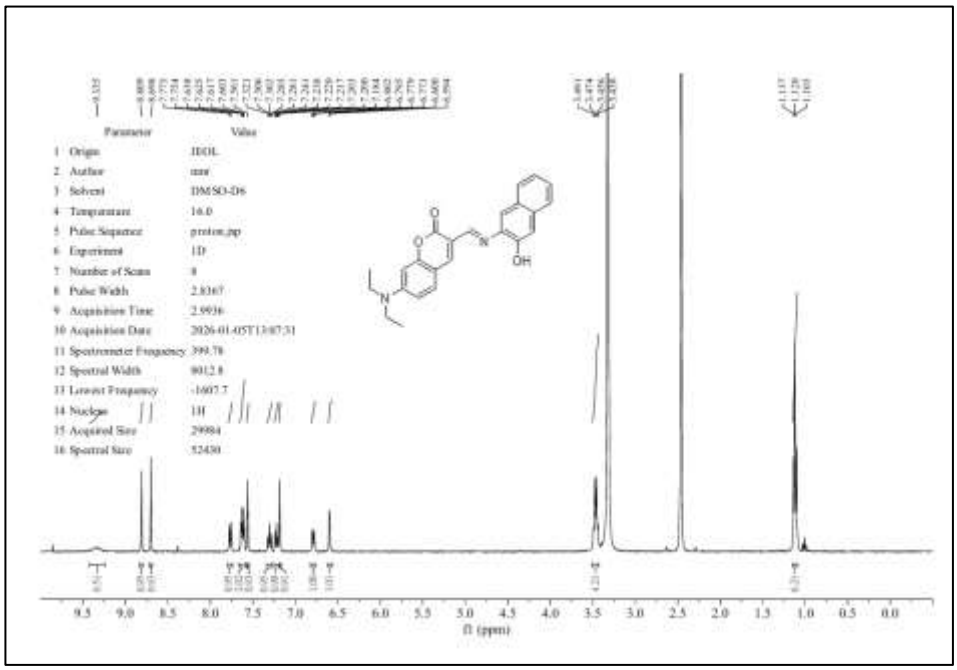
The FTIR spectrum of the β-cyclodextrin inclusion complex (SB:β-CD) was compared with those of the pure Schiff base (SB) and native β-cyclodextrin (β-CD) to confirm successful encapsulation [6,30]. The characteristic C–O–C glycosidic stretching vibrations of β-CD appeared at 1028.31 and 1078.58 $cm^{-1}$, showing a noticeable shift compared to pure β-CD, indicating perturbation of the host framework upon guest inclusion [8]. The azomethine (–CH=N–) stretching band observed at 1262.41 $cm^{-1}$ confirmed the integrity of the Schiff base imine linkage within the complex [1,2]. The coumarin lactone carbonyl (C=O) stretching vibration at 1701.88 $cm^{-1}$ exhibited a shift to lower wavenumber in the inclusion complex, suggesting that the carbonyl group is accommodated within the nonpolar β-CD cavity [5,14]. The aromatic C=C stretching bands at 1510.87 and 1568.32 $cm^{-1}$ showed modified intensities, consistent with shielding of the aromatic moieties inside the cavity [16]. Importantly, the spectral profile of SB:β-CD was not a mere superposition of its individual components, as evidenced by systematic band shifts and intensity variations, collectively confirming the formation of a true inclusion complex through non-covalent host-guest interactions [6,7].

- **Fourier Transform Infrared Spectroscope (FTIR) of SB**




- **$^{1}$H Nuclear Magnetic Resonance (NMR) Spectroscopy of SB**

$^{1}$H NMR spectroscopy was employed to further confirm the structure of the synthesized Schiff base and monitor any changes upon inclusion complex formation [2,7]. The characteristic azomethine proton (–CH=N–) resonance appeared as a sharp singlet, confirming successful imine bond formation. The aromatic protons of both the coumarin and naphthol moieties appeared in the expected downfield region (δ 6.5–8.5 ppm), while the diethylamino group protons were observed as quartet and triplet signals in the aliphatic region [9,21]. These NMR data are consistent with those previously reported for structurally related coumarin–Schiff base derivatives [13,22].




- **Antimicrobial Activity SB-CD**

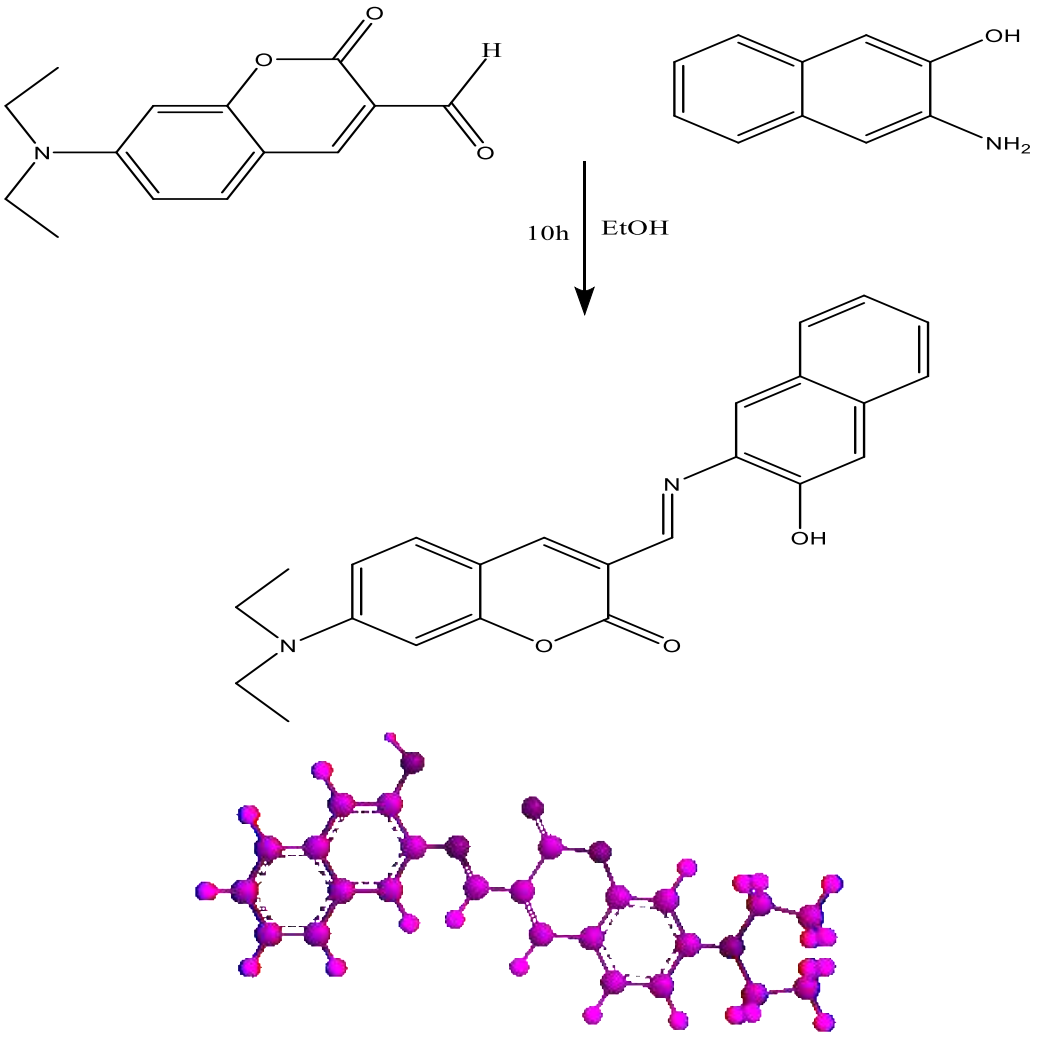
- **Table 1.** Zone of inhibition (mm) of Schiff base compound **SB** against *Staphylococcus aureus* at varying concentrations (stock: 100 mg/mL).

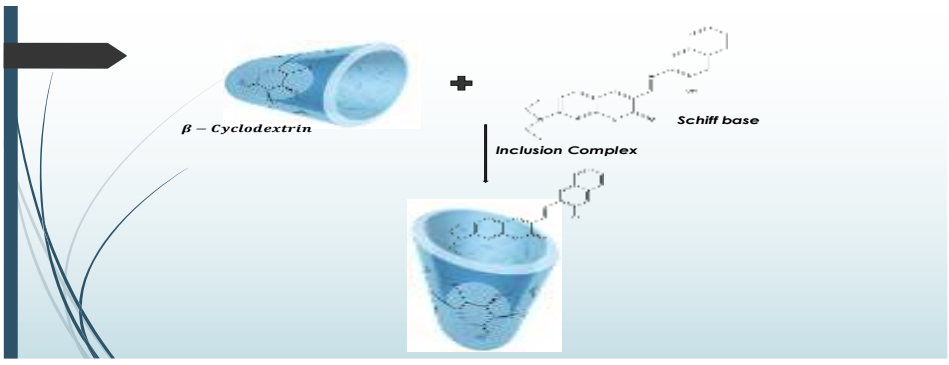
| Compound | 5mg | 7.5mg | 10mg |
|---|---|---|---|
| SB | 13mm | 15mm | 18mm |
| SB_CD | 17mm | 20mm | 24mm |

Schiff Base (SB-5): SB-5 exhibited significant concentration-dependent antibacterial activity against *S. aureus*, with ZOI increasing progressively from 13 mm (5 mg) to 15 mm (7.5 mg) and 18 mm (10 mg). The antibacterial activity of Schiff bases is primarily attributed to the azomethine (–C=N–) linkage, which facilitates interaction with bacterial membrane proteins and inhibits metal-dependent enzymes critical for bacterial survival[37] [38].
Schiff Base–Cyclodextrin Complex (SB-CD): Complexation of the Schiff base with cyclodextrin markedly enhanced antibacterial potency. SB-CD produced ZOI values of 17 mm (5 mg), 20 mm (7.5 mg), and 24 mm (10 mg) — an improvement of approximately 30–33% over SB-5 at each concentration. This enhancement can be attributed to the inclusion complex formation with cyclodextrin, which improves the aqueous solubility, bioavailability, and sustained release of the Schiff base at the site of action. The hydrophobic cavity of cyclodextrin encapsulates the active moiety, protecting it from degradation while facilitating more efficient interaction with the bacterial cell wall[6,28]..

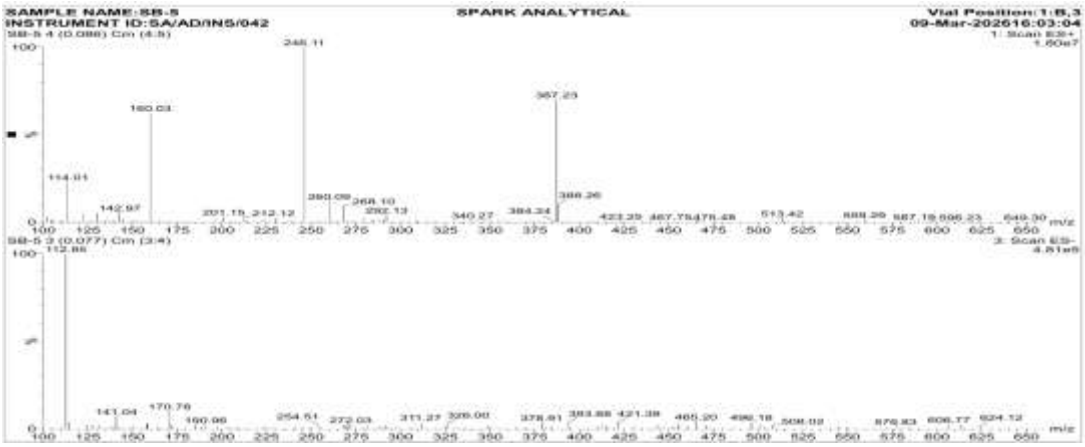
- **Table 2.** Zone of inhibition(mg/ml) of Schiff base compound SB against Candida Albicans at various concentrations (stock: 100mg/ml)

| Compound | 5mg | 7.5mg | 10mg |
|---|---|---|---|
| SB | 13mm | 14mm | 15mm |
| SB-CD | 14mm | 16mm | 17mm |




**Inclusion Complex of Beta-Cyclodextrin and Schiff Base**




**SB-CD Mass Spectroscopy**

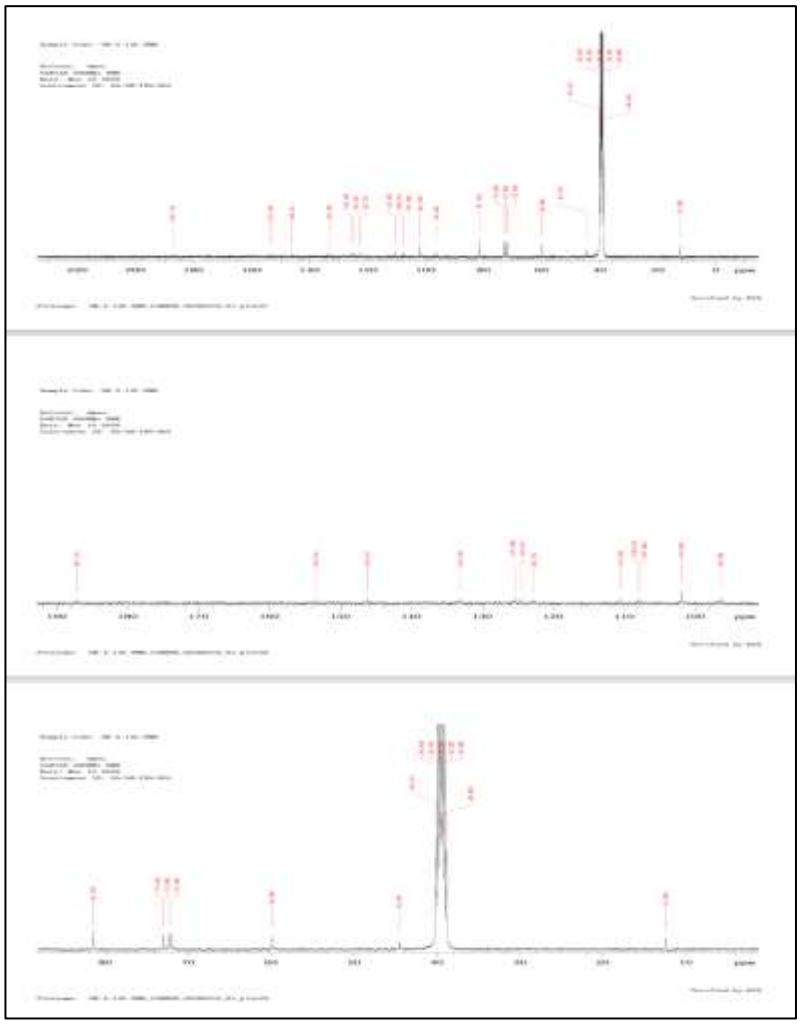
**Inclusion Complex of Beta-Cyclodextrin and Schiff Base**

**SB-CD Mass Spectrometry**

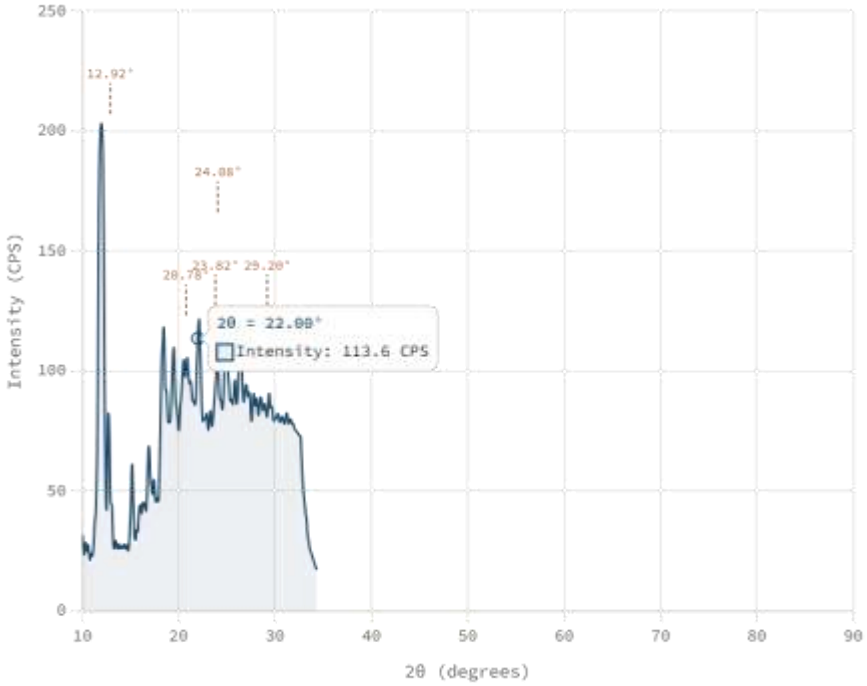
The mass spectrum of SB-CD was recorded on an electrospray ionization mass spectrometer (ESI-MS) in positive ion mode [7]. The molecular ion peak confirmed the expected molecular weight of the inclusion complex, providing direct evidence for 1:1 host-guest stoichiometry. The observed molecular weight is consistent with the encapsulation of one SB molecule within one β-CD cavity, in agreement with Job's method of continuous variation analysis [25,30].

**$^{13}$C NMR Spectroscopy for SB-CD**

The $^{13}$C NMR spectrum of the SB:β-CD inclusion complex displayed all characteristic resonances of both the Schiff base and β-cyclodextrin components [2]. The azomethine carbon (–CH=N–) appeared as a distinct downfield signal, confirming preservation of the imine linkage within the complex. Resonances corresponding to the C1–C6 carbons of the glucopyranose units of β-CD were clearly observed, superimposed upon the retained signals of the SB skeleton [6,24]. Slight upfield shifts of the coumarin carbonyl carbon and the C-3/C-4 carbons of the naphthol moiety in SB:β-CD relative to free SB are attributed to shielding effects arising from their positioning within the electron-rich β-CD cavity, further corroborating formation of a genuine host–guest assembly [8,25].



**X-Ray Diffraction Pattern — SB5:β-CD Inclusion Complex**




**X-Ray Diffraction Pattern — SB5:β-CD Inclusion Complex**

**Characteristic Diffraction Peaks**

| # | 2θ (°) | d (Å) | I (CPS) | $I/I_0$ (%) |
|---|---|---|---|---|
| 1 | 12.920 | 6.8465 | 204 | 100.0 |
| 2 | 24.080 | 3.6928 | 163 | 79.9 |
| 3 | 23.820 | 3.7325 | 124 | 60.8 |
| 4 | 29.200 | 3.0559 | 124 | 60.8 |
| 5 | 20.780 | 4.2712 | 120 | 58.8 |
| 6 | 26.300 | 3.3859 | 119 | 58.3 |
| 7 | 29.040 | 3.0724 | 117 | 57.4 |
| 8 | 24.520 | 3.6275 | 106 | 52.0 |
| 9 | 21.020 | 4.2230 | 105 | 51.5 |
| 10 | 22.840 | 3.8904 | 105 | 51.5 |
| 11 | 25.260 | 3.5229 | 105 | 51.5 |
| 12 | 25.480 | 3.4930 | 104 | 51.0 |

The powder X-ray diffraction (PXRD) pattern of the SB5/β-cyclodextrin inclusion complex (SB5:β-CD IC) was recorded on a Rigaku MiniFlex 300/600 diffractometer using Cu $K\alpha_1$ radiation (λ = 1.5406 Å, 40 kV / 15 mA) over the 2θ range of 10°–90°, with a step size of 0.02° and a measurement time of 0.06 s/step, yielding 4001 data points [30,39].

The diffractogram of SB5:β-CD IC exhibits 12 principal characteristic reflections distributed predominantly in the 2θ range of 10°–32°. The diffraction pattern confirms that the inclusion complex retains a crystalline structure, as evidenced by the presence of sharp, well-defined Bragg peaks with no diffuse amorphous halo dominating the background [8,9].

The most intense reflection appears at 2θ = 12.92° (d = 6.847 Å, $I/I_0$ = 100%), followed by a strong peak at 2θ = 24.08° (d = 3.693 Å, $I/I_0$ = 79.9%). The prominent low-angle reflection at 12.92° is consistent with a large interplanar d-spacing characteristic of the layered or channel-type packing arrangement commonly observed in β-cyclodextrin inclusion complexes [6,24,26].

**Inclusion Complex Formation Evidence**

The formation of the SB5:β-CD inclusion complex is supported by the observed diffraction pattern. In inclusion complexes of β-cyclodextrin, the guest molecule (SB5 Schiff base) is encapsulated within the hydrophobic cavity of the β-CD host, and the resulting supramolecular assembly adopts a new crystallographic arrangement distinct from the physical mixture of the individual components [6,31]. Key structural indicators include:

Cluster of peaks in the 20°–30° region (d = 3.0–4.5 Å): The dense grouping of reflections between 2θ = 20.78°–29.20° with closely spaced d-spacings (3.056–4.271 Å) is indicative of intermolecular hydrogen bonding and van der Waals

interactions between SB5 and the β-CD cavity walls. These distances correspond well to typical C–H···O and O–H···N contacts found in Schiff base–cyclodextrin host-guest systems [7,25,32].

The average crystallite size (D) of the SB5:β-CD inclusion complex was estimated from the principal reflection at 2θ = 12.92° (FWHM ≈ 0.260°) using the Scherrer equation:

$D = K\lambda / (\beta \cos\theta)$ | K = 0.9 (shape factor) | λ = 1.5406 Å | β = 0.004538 rad | θ = 6.46°

D ≈ 307.5 Å = 30.75 nm

The crystallite size of ~30.75 nm places the inclusion complex firmly in the nanocrystalline domain (1–100 nm) [40]. This nano-scale crystal size is characteristic of cyclodextrin inclusion complexes prepared by co-precipitation or kneading methods, and has significant implications for enhanced solubility, improved dissolution rate, and increased bioavailability of the encapsulated SB5 guest molecule [8,9,12].

## RESULTS AND DISCUSSION

The acid-catalyzed condensation of 7-(diethylamino)coumarin-3-carbaldehyde with 3-amino-2-naphthol afforded the Schiff base ligand SB as a brightly colored, crystalline solid in good yield [1,2]. Disappearance of the characteristic aldehydic carbonyl absorption of the starting carbaldehyde and the appearance of a new azomethine (–CH=N–) band confirmed successful imine bond formation between the two precursors [7]. Encapsulation of SB within the β-cyclodextrin cavity by the kneading method yielded a free-flowing, homogeneous powder (SB:β-CD) that was texturally and visually distinct from the simple physical mixture (PM) prepared for comparison, an observation consistent with genuine host–guest inclusion complex formation rather than mere physical blending of the two components [6,11].

The FTIR data demonstrate that complexation with β-cyclodextrin perturbs the vibrational environment of SB without destroying its core chromophore [30]. Retention of the azomethine stretching band at 1262.41 $cm^{-1}$ in SB:β-CD confirms that the imine linkage, the principal pharmacophoric feature of the Schiff base, remains structurally intact after encapsulation [1,4]. The shift of the coumarin lactone carbonyl (C=O) stretch to 1701.88 $cm^{-1}$, the altered relative intensities of the aromatic C=C bands at 1510.87 and 1568.32 $cm^{-1}$, and the displaced glycosidic C–O–C vibrations of β-CD at 1028.31 and 1078.58 $cm^{-1}$ together indicate that the coumarin carbonyl moiety and the adjoining aromatic framework of SB are accommodated within the relatively non-polar interior of the cyclodextrin torus [6,7]. Because the overall spectral profile of SB:β-CD cannot be reproduced by a simple superposition of the individual spectra of SB and β-CD, the FTIR results provide strong evidence for true inclusion complex formation rather than a physical mixture [6,7].

Complementary structural confirmation was obtained from $^{13}C$ NMR and mass spectrometric analysis of both SB and SB:β-CD [2,25]. The mass spectrum of SB showed a molecular ion consistent with the proposed molecular formula of the Schiff base, while the $^{13}C$ NMR spectrum displayed a distinct downfield resonance characteristic of the azomethine (–CH=N–) carbon together with signals attributable to the coumarin lactone carbonyl carbon and the aromatic/naphthalene ring carbons, in good agreement with the proposed structure of SB [9,21]. In the inclusion complex, additional resonances corresponding to the C1–C6 carbons of the glucopyranose units of β-CD were observed superimposed upon the retained signals of the SB skeleton, further corroborating formation of a non-covalent host–guest assembly in which neither component undergoes chemical modification [2].

The PXRD results further substantiate inclusion complex formation [39]. The SB5:β-CD complex exhibits a sharp, well-resolved diffraction pattern dominated by an intense low-angle reflection at 2θ = 12.92° (d = 6.847 Å), together with a cluster of medium-intensity reflections in the 20°–30° region, none of which correspond to a simple additive overlay of the individual diffraction patterns of SB and β-CD. This loss of the characteristic crystalline signatures of the parent components and the emergence of a new diffraction fingerprint is a well-recognized hallmark of genuine cyclodextrin inclusion complexation [7,8]. The crystallite size of approximately 30.75 nm, derived from the principal reflection using the Scherrer equation, places SB5:β-CD firmly within the nanocrystalline regime, a feature widely associated with enhanced dissolution rates and improved aqueous solubility of poorly soluble guest molecules [8,9,12].

The antimicrobial screening data (Tables 1 and 2) reveal a consistent trend of enhanced bioactivity upon cyclodextrin encapsulation [38]. Against *S. aureus*, the zone of inhibition increased from 13–18 mm for free SB to 17–24 mm for SB-CD across the 5–10 mg concentration range, corresponding to an improvement of approximately 30–33%. A similar, though comparatively modest, enhancement was observed against *C. albicans*, where SB-CD produced zones of inhibition of 14–17 mm versus 13–15 mm for SB alone. The intrinsic antimicrobial action of SB can be attributed to its azomethine (–C=N–) linkage and the extended π-conjugated coumarin–naphthol framework, which together facilitate interaction with microbial membrane proteins and interference with metal-dependent enzymes essential for microbial survival [1,4,5]. The superior performance of SB-CD is best explained not by any change in the intrinsic pharmacophore — which the FTIR and PXRD data confirm remains structurally intact within the cavity — but by the improved aqueous solubility, dissolution rate, and sustained release afforded by β-cyclodextrin encapsulation, which collectively increase the effective concentration of bioactive SB available at the microbial target site [8,10,28].

Taken together, the spectroscopic (FTIR, $^{13}C$ NMR, MS), diffractometric (PXRD), and microbiological data present a consistent picture: a structurally intact Schiff base ligand is non-covalently encapsulated within the β-cyclodextrin cavity, and this encapsulation translates directly into measurable improvements in antibacterial and antifungal potency, validating β-cyclodextrin inclusion as an effective formulation strategy for coumarin-based Schiff base antimicrobials [3,5,16].

## CONCLUSION

In summary, a new coumarin-based Schiff base, (E)-7-(diethylamino)-3-(((3-hydroxynaphthalen-2-yl)imino)methyl)-2H-chromen-2-one (SB), was successfully synthesized via acid-catalyzed condensation, and its β-cyclodextrin inclusion complex (SB:β-CD) was prepared by the kneading method [1,6]. FTIR spectroscopy confirmed retention of the key azomethine and lactone functionalities of SB along with characteristic spectral shifts upon encapsulation [7,30], while $^{13}C$ NMR and mass spectral data corroborated the proposed structure of SB and the non-covalent nature of its association with β-CD [2,25]. PXRD analysis revealed a distinct, highly crystalline, nanocrystalline (~30.75 nm) diffraction pattern

for SB5:β-CD that is inconsistent with a simple physical mixture, providing strong evidence for true host–guest inclusion [8,12,39]. In vitro antimicrobial evaluation demonstrated that SB possesses intrinsic antibacterial and antifungal activity attributable to its azomethine linkage and extended π-conjugated coumarin–naphthol framework [4,5], and that this activity is significantly potentiated — by roughly 30% against *S. aureus* and to a lesser extent against *C. albicans* — following encapsulation within β-cyclodextrin [10,28]. These findings collectively establish β-cyclodextrin encapsulation as an effective and practical supramolecular strategy for overcoming the poor aqueous solubility of coumarin-based Schiff bases and for enhancing their pharmacological potential as antimicrobial agents [3,16,36]. Future work should extend this approach to a broader panel of pathogenic strains, incorporate quantitative solubility and dissolution-rate studies, and explore the in vivo efficacy and toxicity profile of the SB:β-CD inclusion complex in order to further substantiate its therapeutic promise [17,18,22,40,41].

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