

Functionalization of Carboxymethyl Cellulose via Amino Acid Esterification and Schiff Base Formation: Synthesis, Characterization, and Biological Applications

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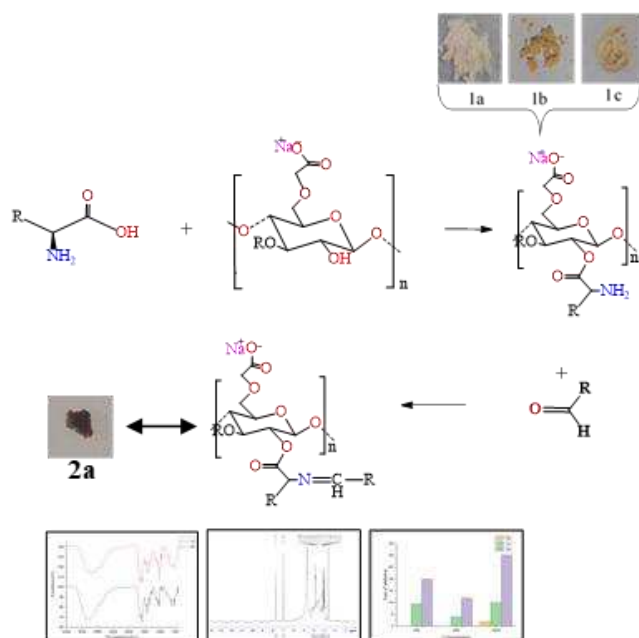
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Abstract:

A biodegradable and biocompatible polymer, carboxymethyl cellulose (CMC), is widely utilized in diverse sectors ranging from biomedical to industrial applications. This study aims to synthesize and characterize CMC derivatives incorporating three amino acids, L-Arginine(1a), L-Histidine(1b), and β -Phenylalanine(1c), as well as an amino acid-based Schiff base(2a). CMC was esterified with each amino acid, then cross-linked via Schiff-base formation with glutaraldehyde. Structural confirmation was performed using FTIR, ¹H/¹³C NMR, and GC-MS, while crystallinity was verified by XRD. Cytotoxicity was assessed via brine-shrimp lethality (LD₅₀), and antibacterial activity against *Aeromonas hydrophila*, evaluated by the well-diffusion method at varying concentrations. Schiff-base modification noticeably improves both cytotoxicity and antibacterial activity of CMC-based materials. This study represents the first integration of amino acid Schiff bases directly into the CMC backbone, yielding multifunctional biopolymers with superior bioactivity for potential application in wound dressings and targeted drug delivery.

Keywords: CMC, Amino acid, Schiff base, spectral analysis, biological activity



1. Introduction

Cellulose, the most abundant naturally occurring polymer, and its derivatives are valued for their strong physicochemical properties, high tensile strength, and biocompatibility, finding application in composites, coatings, packaging, and paper products [1-3]. Among these, carboxymethyl cellulose (CMC), prepared by etherification of cellulose with monochloroacetic acid [4], is particularly notable for its water solubility, biodegradability, and film-forming capability, making it attractive for pharmaceutical and biomedical use [5],[6]. However, unmodified CMC lacks intrinsic antimicrobial or cytotoxic activity, which limits its direct application in advanced wound dressings, antimicrobial coatings, and targeted drug-delivery systems [7]. Conversely, some inherently bioactive compounds have limited direct application due to challenges like instability, rapid degradation, or poor retention [8]. To address this limitation, chemical functionalization strategies have been employed to introduce bioactive moieties into the CMC backbone, aiming to

improve solubility and stability while enhancing the material's potential for targeted delivery or controlled release, additionally keeping the process affordable and sustainable [9],[10].

In recent years, various studies have shown that chemically modified CMC has valuable properties and can be used in different industries, including pharmaceuticals, food, and cosmetics [11]. CMC-based biomaterials are gaining popularity in the drug-delivery process [12], tissue engineering [13], and wound dressing [14].

On the other hand, amino acids are well known for their important biological actions, including neurotransmission, metabolic regulation, immune modulation, antioxidant defense, hormone synthesis, and tissue repair [15–19]. Notably, significant activity in biological applications has been observed in amino acid-based CMC and their metal complexes [20], [21].

Schiff-base chemistry is particularly appealing: imine linkages form readily between aldehydes and primary amines, enabling grafting of amine-containing molecules under mild conditions and have demonstrated a wide range of biological activities, including the inhibition of tumor and cancer cell growth, making them promising candidates for chemotherapy, as well as antiviral, anti-inflammatory, anti-HIV, antiprotozoal, and anthelmintic effects [22–24]. Amino acids, with their diverse side chains and inherent biological functions, serve as ideal partners in Schiff-base formation, potentially introducing zwitterion character, selective metal chelation, and antimicrobial or cytotoxic properties. As a result, amino acid-based Schiff bases have shown broad biomedical potential, including antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, and anti-parasitic activities [25–28]. Furthermore, their metal complexes have been explored to enhance these properties [29–32].

Since glutaraldehyde has two reactive aldehyde groups (-CHO) at each end of its five-carbon chain, both of them can react with primary amine groups in Amino Acids (the amine group

on the α -carbon), allowing it to crosslink two amino acid molecules by forming C=N bonds at each end [33], [34].

While Schiff base chemistry and CMC modification have been researched separately, no systematic study has integrated amino-acid Schiff bases directly into CMC and compared the influence of different side chains on material performance and bioactivity.

Here, new CMC derivatives were synthesized by modifying carboxymethyl cellulose with amino acids, and we report the first synthesis and comparative evaluation of three CMC derivatives bearing Schiff bases of L-Arginine (1a $\rightarrow$ 2a). Each derivative was prepared by esterification of CMC with the amino acid (1:1.5 wt/wt, 90 °C), followed by imine crosslinking with glutaraldehyde (70 °C, 20 h). Structures were confirmed by FTIR, $^1\text{H}/^{13}\text{C}$ NMR, and GC-MS analyses. X-ray diffraction (XRD) confirmed successful alteration, revealing stronger diffraction peaks and nanoscale crystallite production, indicating partial crystallinity within the polymer matrix.

We then assessed cytotoxicity via brine-shrimp lethality (LD_{50}) and antibacterial efficacy against *Aeromonas hydrophila* by well-diffusion assays. By elucidating structure–activity relationships, we demonstrate how amino-acid side-chain chemistry can be harnessed to generate multifunctional CMC-based biomaterials with enhanced bioactivity, and identify the most promising candidate for applications in antimicrobial coatings and wound-healing matrices.

2. Materials and Methods.

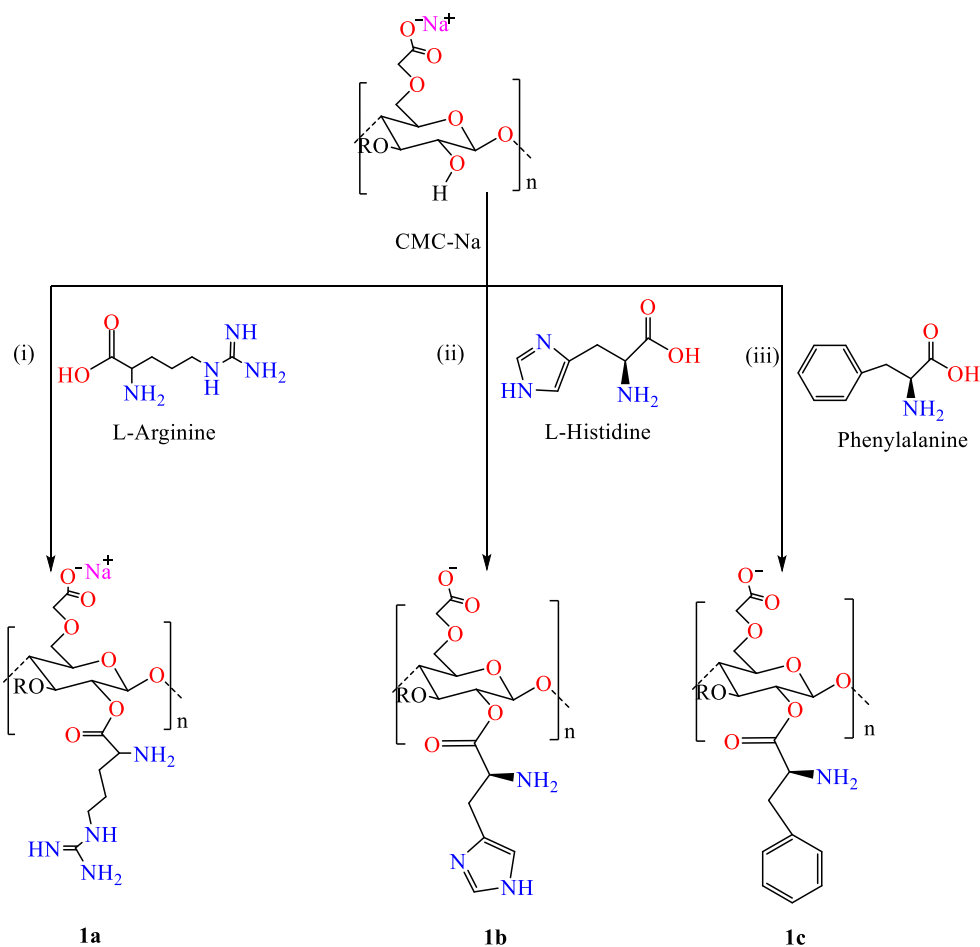
2.1 Materials

All reagents and chemicals, including carboxymethyl cellulose sodium salt (CMC-Na), amino acids (L-arginine, L-histidine, and β -phenylalanine), glutaraldehyde, ethanol, and DMSO, were of synthesis grade and used without further purification and purchased from Merck, Germany. M-endo agar was used as the medium for antimicrobial activity tests.

2.2 Synthesis of Amino Acid-based CMC

CMC (Na^+ , salt, 1 g) was activated in 5 ml of dimethyl sulfoxide (DMSO) by stirring at 50–60 °C for 30 min. The formed ‘gel suspension’ was used for esterification experiments [35].

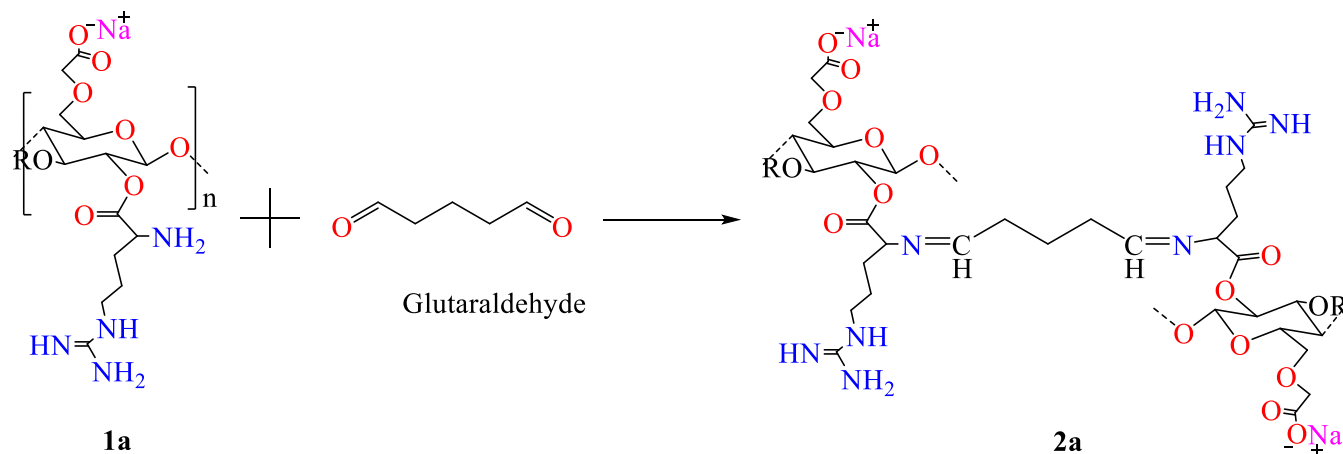
The esterification was carried out by mixing the CMC suspension with the Amino Acids in an ethanol medium. The mixer was then treated at ~90°C for 4-6 hours. The ratio of amino acids to CMC was 1:1.5 (wt./wt.), as shown in Scheme 1.



Scheme 1 Synthetic route for modification Of CMC with Amino Acids (i) L-Arginine:1a, (ii) L-Histidine:1b & (iii) Phenylalanine: 1c

2.3 Modification of Amino Acid-based CMC Derivative with Aldehyde

The esterified CMC (Arginine-based CMC-1a) was subsequently heated with aldehyde (glutaraldehyde) at 70°C for 3 hours, as illustrated in Scheme 2. The product obtained was a deep red, gummy-textured compound with a yield of 72%. The formation and success of the reactions were confirmed by the spectral analysis.



Scheme 2 Synthetic route for further modification of L-Arginine-based CMC with Glutaraldehyde

2.4 Characterization Techniques

The IR spectra of the obtained products were measured by a Perkin Elmer Fourier Transform Infra-red Spectrophotometer (FTIR) at the Department of Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur. ¹H-NMR spectra were recorded by a Bruker BPX-200 spectrophotometer at 400 MHz, and ¹³C-NMR spectra at 100 MHz at the Dr. M A Wazed Miah Science Research Center, Jahangirnagar University, Savar, Dhaka. Deuterium Water (D₂O) is used as the solvent. The GC-MS was performed by Agilent 5977C GC/MSD, where the output was depicted as a chromatogram, with retention time (RT), and as a spectrum, with mass-to-charge ratio (m/z) on the X-axis. X-ray diffraction (XRD) analysis was performed using an XRD instrument with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) at the

Institute of Materials and Mineral Research (IMMM), BCSIR, Bangladesh, to examine the crystalline structure and phase composition of the Schiff base-modified CMC samples.

2.5 Evaluation of Biological Activity

2.5.1 Cytotoxic Activity

The cytotoxic activity was measured through the brine shrimp lethality bioassay, which was conducted by exposing nauplii of *Artemia salina* to different concentrations of the test samples [36]. The LD₅₀ value from the brine shrimp lethality bioassay is convenient and commonly used in pharmacology as a preliminary toxicity screening for potential drugs before moving to advanced pharmacological [37], [38]. The LD₅₀ values (concentration of sample required to kill 50 % of brine shrimp) were calculated using Microsoft Excel 2013 by a plot of % of mortality against the logarithm of the sample concentrations. The percentage of mortality (rounded to the nearest whole number) at each dose and control was determined by using the following formula (1):

$$\% \text{ of mortality} = (\text{no. of dead nauplii} / \text{total number}) \times 100 \text{ ----- (1)}$$

$$\text{Corrected \% mortality} = \{(M_{\text{obs}} - M_{\text{control}}) / (100 - M_{\text{control}})\} \times 100 \text{ ----- (2)}$$

Where, M_{obs} = observed % mortality; M_{control} = control % mortality [39].

2.5.2 Anti-bacterial activity

Anti-bacterial activity of synthesized compounds against a gram-negative bacterium, *Aromonous hydrophila*, was evaluated via the well diffusion method by measuring the zone of inhibition [40]. The data were analyzed by two-way analysis of variance (ANOVA), and zones of inhibition of the compounds against the tested microorganisms were presented as the mean $\pm$ SD derived from two trials.

3. Results and Discussion

3.1 Structure Elucidation

3.1.1 FTIR Spectral Analysis

In the FTIR spectrum of CMC, a broad band at 3400 cm^{-1} corresponds to O-H stretching (νOH), and sharp bands at 1584 cm^{-1} and 1410 cm^{-1} represent the asymmetric and symmetric stretching of the carboxylate group ($\nu\text{C=O}$ & $\nu\text{C-O}$ respectively) in $-\text{COONa}$. Additionally, 1015 cm^{-1} is attributed to the glycosidic linkage present in the CMC polymer.

Following modification of CMC with L-Arginine (1a), the νOH band shifted from 3400 cm^{-1} to 3349 cm^{-1} , indicating a decrease in the mean strength of the hydrogen bond compared with CMC and suggesting that the free $-\text{OH}$ group of CMC was involved in the reaction. At 1629 cm^{-1} , a new absorption band appeared corresponding to C=O stretching, which supports the formation of an amino acid ester. Furthermore, the presence of an absorption peak at 1453 cm^{-1} indicates the formation of the ester C-O-C bond. Meanwhile, absorption peaks near 1584 cm^{-1} ($-\text{COONa}$) and

1016 cm^{-1} (glycoside bond) remained unchanged, confirming that they were not involved in the reaction (Fig1-a).

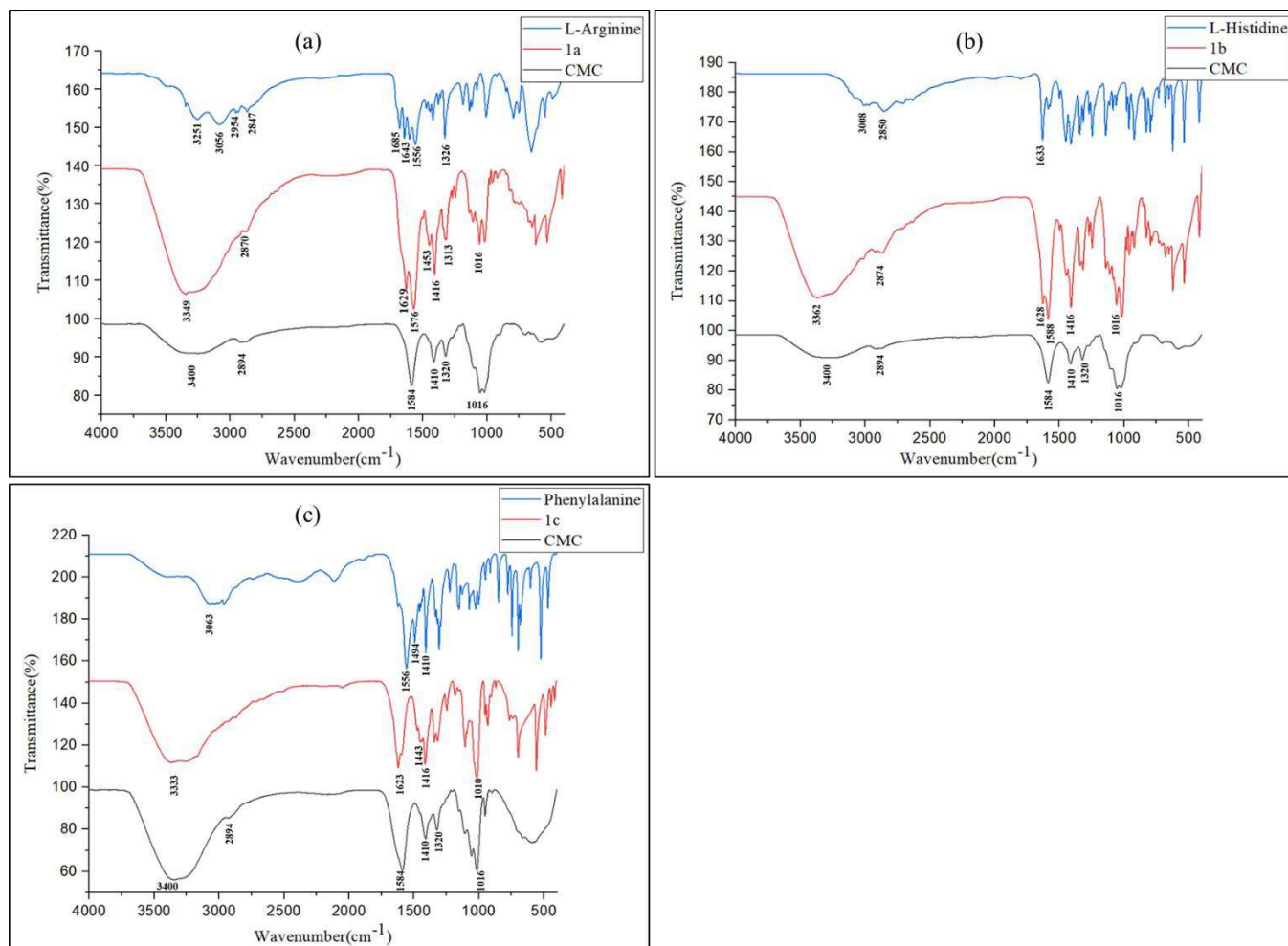


Fig1 FTIR spectra of Amino acids modified CMC and comparison with their starting materials for (a) Arginine modified CMC, (b) Histidine modified CMC, (c) Phenylalanine modified CMC

Similarly, in the spectra of CMC modified with Histidine (1b) and Phenylalanine (1c), new absorption bands at 1622 cm^{-1} and 1628 cm^{-1} , respectively, indicate a new bond formation due to

the esterification of the -OH group of CMC. (Fig1-b, c). These observations align well with previously reported FTIR spectra of modified CMC [20].

After modification of 1a with glutaraldehyde (2a), an additional band appeared at 1558 cm⁻¹ due to the formation of an imine (C=N) bond (Fig2).

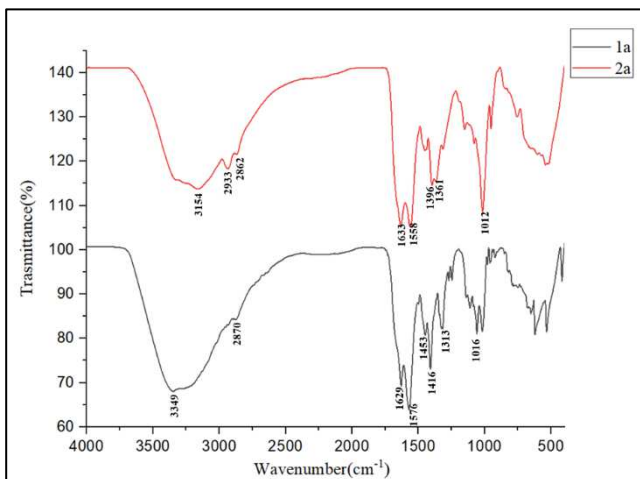


Fig2 FTIR spectra of amino acid based Schiff base modified CMC (2a), compared with the unmodified intermediate (compound 1a)

3.1.2 NMR Analysis

In ¹H-NMR, a peak appeared at 4.69ppm corresponding to the solvent HOD, arising from proton exchange between D₂O and trace water or labile protons in the sample. The ¹³C NMR spectrum of the modified CMC derivative showed peaks attributed to the newly introduced functional groups; however, characteristic peaks of the CMC backbone were absent. The absence of CMC backbone signals in ¹³C NMR was likely due to several factors, including the low natural abundance of ¹³C (¹³C has only ~1.1% natural abundance, which results in significantly lower sensitivity compared to ¹H), the complex relaxation behavior of polysaccharides, and solubility limitations of the sample.

^1H - NMR (D_2O , 400 MHz, δ ppm) analysis of 1b revealed a doublet at 4.18ppm (1H), corresponding to the anomeric proton. A broad, overlapped signal, in the range of 3.58-3.67 ppm (4H), was attributed to the protons of the glucose ring, which is consistent with a previous report [41]. δH = 2.64ppm (s), 3.9 ppm (dd, 2H, J_0 = 4.8), 3.15 ppm (dd, 1H, J_0 = 4.4), 3.04 ppm (dd, 1H, J_0 = 7.6), 6.98 ppm (s,1H), 7.70 ppm (s,1H) assigned for 7, 8, 10a, 10b, 11, and 13 protons respectively.. Exchangeable protons of $-\text{NH}_2$ and $-\text{OH}$ did not appear because of proton-deuteron exchange. Furthermore, the absence of a peak corresponding to the $-\text{COOH}$ proton suggests its involvement in esterification (Fig3-a).

The ^{13}C NMR and DEPT-135 spectra of compound 1b were recorded. The imidazole ring carbons appeared at δ 116.69 ppm, δ 136.32 ppm, and δ 132.09 ppm. The carbon adjacent to the imidazole ring exhibited a chemical shift at δ 28.11 ppm, while the α -carbon of the amino acid backbone was observed at δ 54.82 ppm. The carbonyl carbon of the ester showed a signal at δ 173.92 (Fig3- b, c).

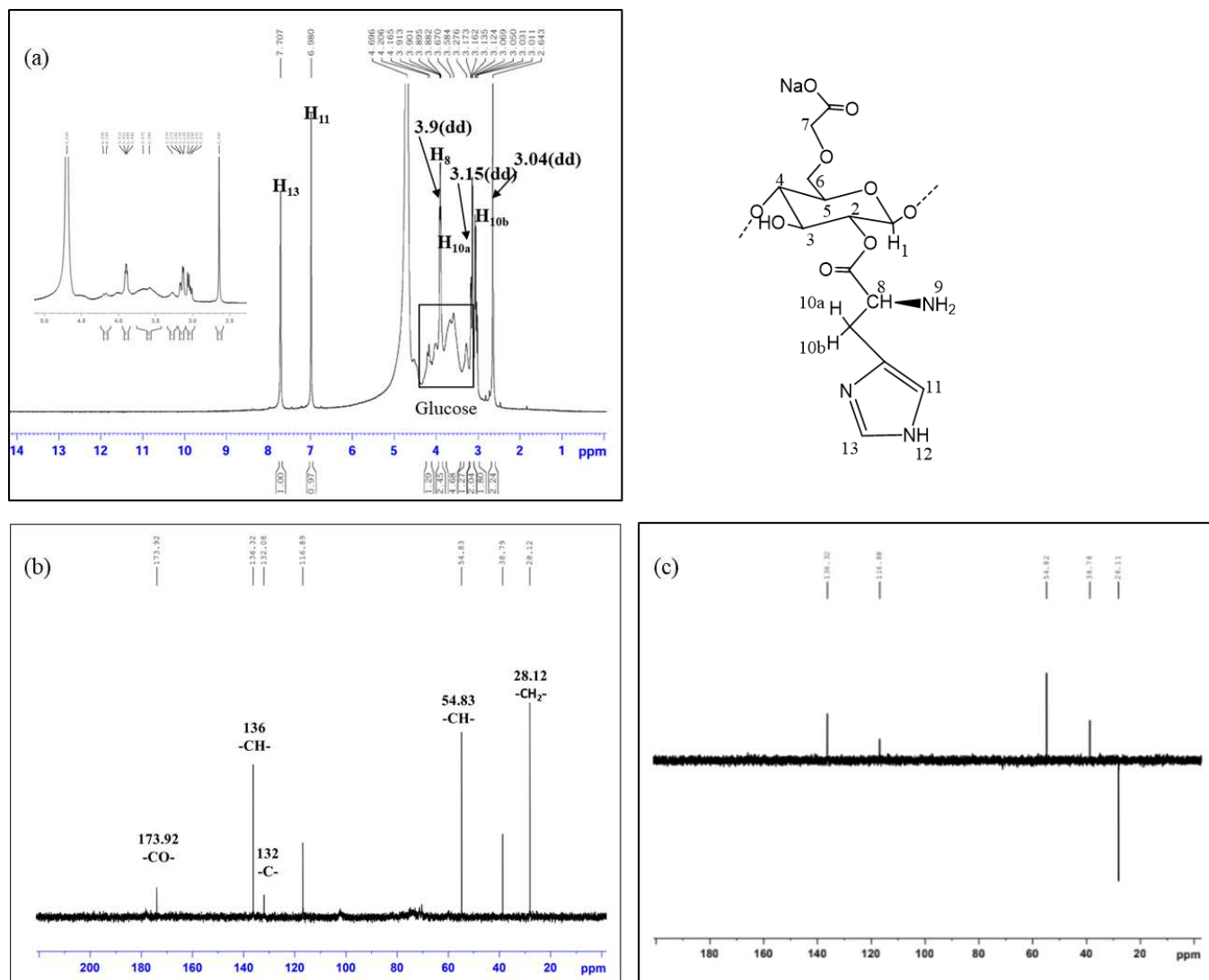


Fig3 NMR analysis: NMR spectra of compound 1b recorded in D₂O (a) ¹H NMR at 400MHz, (b) ¹³C NMR, (c) DEPT-135 at 100MHz. Chemical shifts(δ) were recorded in ppm related to TMS, confirms the structure of synthesized compound

For compound 1c, in the range of 3.58-3.67 ppm (4H), a broad, overlapped peak appeared for the proton of glucose ring. Additional peaks were recorded at 2.64 (s), 2.63 ppm (s, 2H), 3.91 ppm (dd, 1H, J₀= 5.2), 3.21 ppm (dd, 1H, J₀= 5.2), 3.04 ppm (dd, 1H, J₀= 8), observed for 7, 8, 9a,

9b respectively and a multiplet at 7.30 ppm (5H, $J_0 = 7.6$) was attributed to the protons of the benzene ring (Fig 4-a).

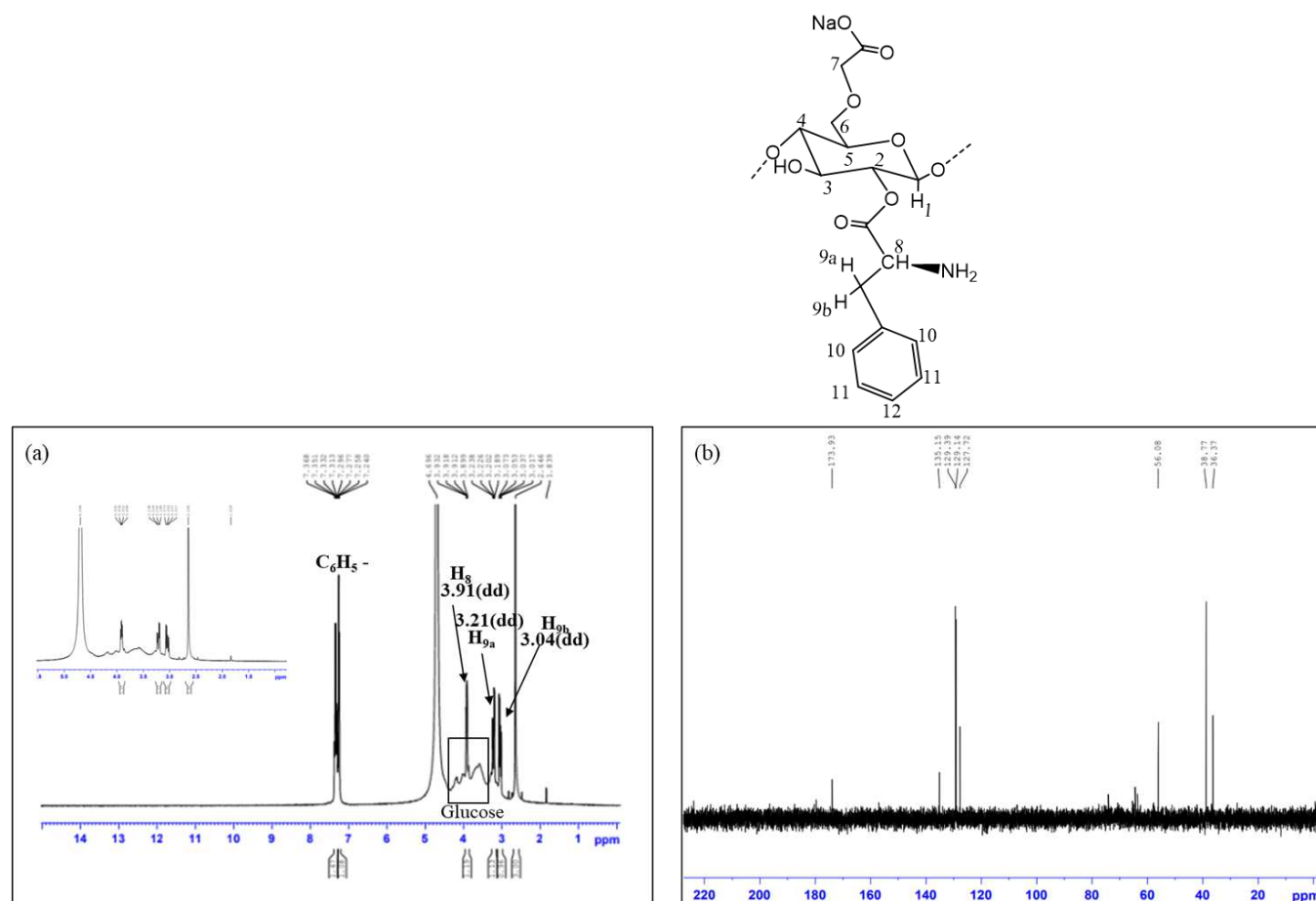


Fig4 (a) ^1H , & (b) ^{13}C NMR spectra of compound 1c recorded in D_2O : confirms the presence of aromatic ring, sugar and aliphatic chain and indicates the formation of ester by characteristic peak for carbonyl carbon of ester

The ^{13}C NMR spectrum of 1c, δ 173.93 ppm, is assigned to the carbonyl carbon of the ester. Signals at δ 129.39 ppm, δ 129.14 ppm, and δ 127.72 ppm are attributed to the aromatic carbons of the phenyl ring. The α -carbon of the amino acid appeared at δ 56.08 ppm, while the CH_2 group adjacent to the phenyl ring was observed at δ 36.37 ppm (Fig4-b)

In the ^1H -NMR Spectrum of 2a, the proton signals from the cellulose backbone, i.e., protons on the glucose unit, have appeared in the range of 3.5 to 3.771 ppm. ^1H - NMR (D_2O , 400 MHz, δ ppm): $\delta\text{H} = 3.84$ ppm (t, 1H, $J_0 = 6.8$) indicates the H8, The ^1H -NMR spectrum shows peaks at 1.64 ppm (m, $J_0 = 6.8$, 2H), 1.54 (m, 2H, $J_0 = 6.4$), 3.14 ppm (t, 2H, $J_0 = 6.4$), 3.33ppm (t, 1H, $J_0 = 5.6$), 2.27 ppm (dt, 2H, $J_0 = 5.6$, 8), 2.17 ppm (t, 2H, $J_0 = 7.6$), corresponding to protons 16, 10, 11,15, 6, and 17 respectively shown in fig5.

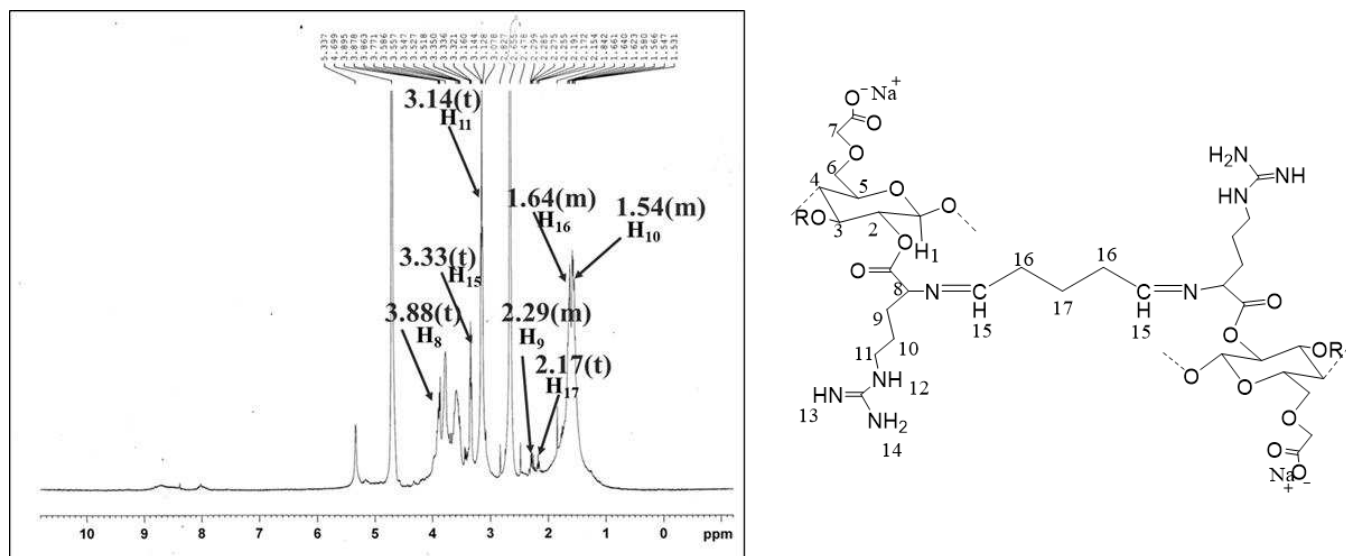


Fig5 ^1H NMR spectrum of Schiff base modified compound 2a, recorded in D_2O , showing characteristic chemical shift and multiplicities

No proton signal corresponding to the aldehyde group was observed, indicating that both $-\text{CHO}$ groups of glutaraldehyde were consumed in the reaction, likely forming a cross-linked skeleton.

3.1.3 GC-MS Analysis

Due to the high molecular weight of the polymer and its highly polar, non-volatile nature, it was challenging to analyze using traditional Gas Chromatography-Mass Spectrometry (GC-

MS). Therefore, it is likely to undergo thermal degradation in the GC column. The GC chromatograms of 2a (Fig6) exhibit one distinct peak at RT 3.889 minutes, which was not present in the standard chromatogram.

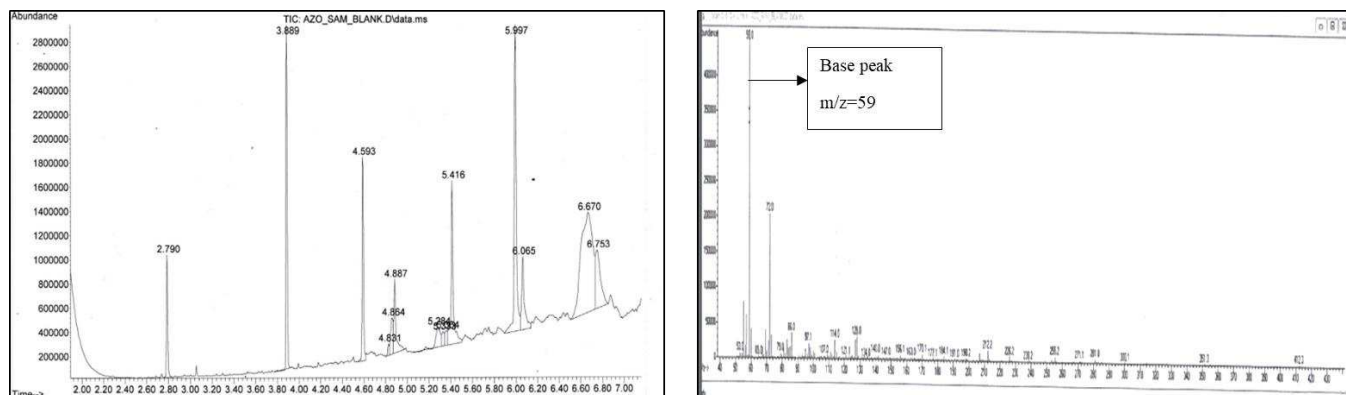


Fig6 GC-MS chromatogram of Schiff base modified CMC derivatives (compound 2a), showing a distinct peak at RT 3.889min and the base peak at $m/z=59$, attributed to the guanidine group from L-Arginine moiety

The presence of all expected structural components was verified by the distinctive fragmentation pattern observed in the corresponding mass spectra of the CMC-L-arginine Schiff base derivative. However, the molecular ion (M^+) peak was not observed due to the high molecular weight and low volatility of the derivative.

The most intense ion (base peak) appears at m/z 59, indicating the most stable or abundant fragment ion, which is attributed to the guanidine ion ($C(NH_2)_2NH_2^+$) formed from the cleavage of L-arginine's side chain (Fig6).

3.1.4 XRD Analysis

X-ray diffraction (XRD) patterns were used to examine the crystallinity and phase composition of the synthesized Schiff base-modified CMC derivatives. Unmodified CMC generally shows a mostly amorphous structure, which depends on the degree of substitution and the source of cellulose. Several studies have reported a broad diffraction peak around 19°–21° (2 θ) for carboxymethyl cellulose, which indicates a disorganized arrangement of polymer chains [42], [43]. In this study, the XRD patterns (Fig7) reveal a broad peak centered at approximately 19.52° (2 θ) for the CMC component, consistent with prior reports. After Schiff base modification with amino acids and glutaraldehyde crosslinking, additional peaks appeared at 23.33° and 27.81°, with slightly sharper profiles. These features suggest the formation of localized crystalline regions and increased molecular ordering within the polymer matrix. The changes in diffraction intensity and peak sharpness indicate that Schiff base crosslinking may limit polymer chain mobility and encourage partial crystallinity.

The crystallite sizes of the modified CMC derivatives were calculated using the Debye–Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D is the crystallite size in nanometers (nm), K is the shape factor (taken as 0.93), λ is the X-ray wavelength (1.5406 Å), and θ is the Bragg angle ($\theta = \frac{1}{2}$ of 2 θ value). The Full Width at Half Maximum (FWHM), denoted as β , was obtained from the XRD peak profiles using Origin software. The broader the peak (larger β), the smaller the crystallite size, indicating a more disordered or nanostructured material.

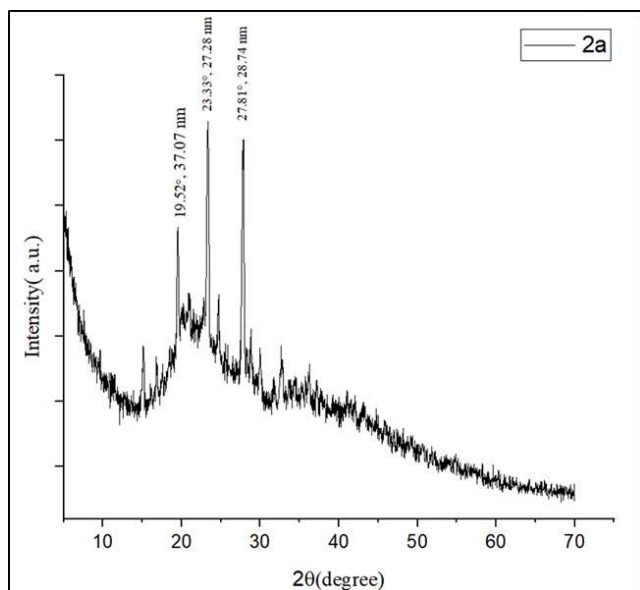


Fig7 X-ray diffraction (XRD) pattern of Schiff base-modified CMC (compound 2a), suggesting localized crystalline regions formed due to Schiff base crosslinking and enhanced molecular ordering

The calculated crystallite sizes for the 19.52° , 23.33° , and 27.81° peaks were approximately 37.07 nm, 27.28 nm, and 28.74 nm, respectively. These values confirm the presence of crystalline domains within the Schiff base-modified CMC.

The development of partial crystallinity can influence material performance, potentially enhancing mechanical stability, thermal resistance, and interaction with biological environments—qualities beneficial for applications such as antimicrobial coatings or wound dressings.

3.2 Biological Activity Evaluation

3.2.1 Cytotoxicity measurements

Compound 1a exhibited relatively low cytotoxicity with an LD_{50} of $1.94 \mu\text{g/ml}$, whereas compound 1b demonstrated significant cytotoxic activity with an LD_{50} value of $1.34 \mu\text{g/ml}$, and

1c showed moderate cytotoxicity against nauplii with an LD₅₀ value of 1.54 µg/ml. In contrast, no mortality was detected in the control/blank throughout the observation period.

Compound 2a, derived from the Schiff base modification of 1a, exhibited enhanced cytotoxicity, with an LD₅₀ value of 1.19 µg/mL, indicating that chemical modification significantly improved its bioactivity (Fig8).

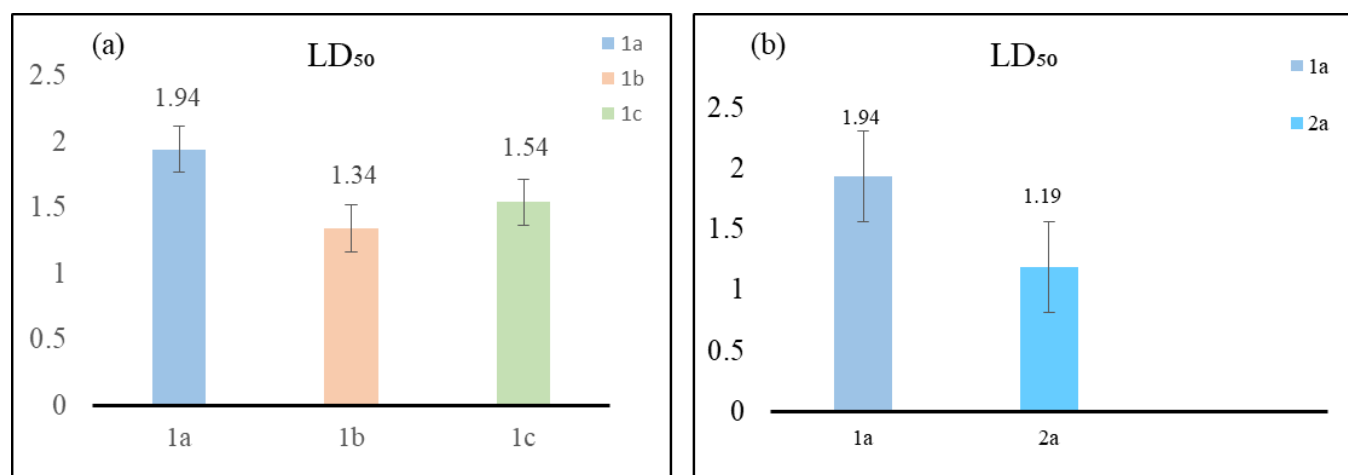


Fig8 Graphical representation of Cytotoxic Activity of synthesized compounds: (a) Comparison of LD₅₀ values for three amino acid-modified CMC derivatives (1a, 1b & 1c). (b) Significant change in cytotoxicity of 1a after further modification with glutaraldehyde (2a)

3.2.2 Antibacterial Activity

The ability of the synthesized compounds to kill bacteria was tested by measuring the inhibition zones against *Aeromonas hydrophila* (Table 1). Distilled water, which served as a negative control, showed no inhibition. Although at 100 ppm, amino acid-modified CMC did not show any antibacterial activity, at 500 ppm, compounds 1b and 1c had zones of 9.6 ± 0.1 mm and 4.05 ± 0.15 mm, which grew to 20 ± 0.1 mm and 11.95 ± 0.15 mm at 1000 ppm, respectively. However, Schiff base formation significantly enhanced the antibacterial activity, with zones of

inhibition of 2 ± 0.3 mm at 100 ppm, 10.05 ± 0.05 mm at 500 ppm, and 30.25 ± 0.25 mm at 1000 ppm (Fig9).

Table 1: Zone of inhibition of compounds 1a, 1b & 1c for three different dose (100, 500 & 1000 ppm)

Sample Code	Zone of Inhibition(mm)		
	100ppm	500ppm	1000ppm
Control	0.00	0.00	0.00
1b	0	9.6 ± 0.1	20 ± 0.1
1c	0	4.05 ± 0.15	11.95 ± 0.15
2a	2 ± 0.3	10.05 ± 0.05	30.25 ± 0.25

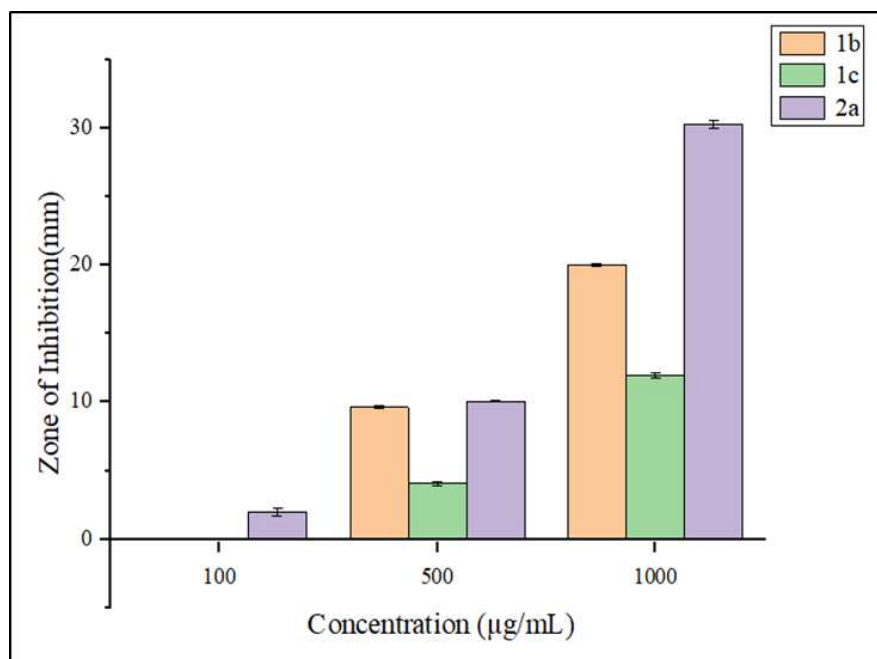
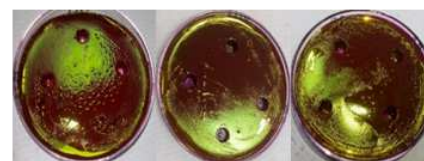


Fig9 Graphical representation of zone of inhibition (mm) observed for compound 1b, 1c & 2a against *Aeromonas hydrophila* at concentration of 100, 500 and 1000 µg/mL. Compound 2a exhibited the highest activity in all doses

The zone of inhibition exhibited by our product (at 1000 ppm) is significantly greater than that produced by several commonly used antibiotics, including Levofloxacin (24 mm), Chloramphenicol (23 mm), Nalidixic acid (23 mm), Norfloxacin (22 mm) [44]. A two-way ANOVA was performed for each experiment, revealing that bacterial growth rates varied significantly ($p < 0.001$).

Overall, spectroscopic analysis (FTIR and NMR), along with XRD and GC-MS characterization, confirmed the successful synthesis and structural modification of CMC. In addition, the strong antibacterial activity against *A. hydrophila* and low cytotoxicity ($LD_{50} = 1.19 \mu\text{g/mL}$ in the brine shrimp assay) suggest that modified CMC-Schiff base could be a promising candidate for wound dressing materials. Furthermore, its hydrophilic nature and biocompatibility further support its suitability as a drug carrier.

4. Conclusion:

The biological activities of Carboxymethyl cellulose were effectively improved in this study through the incorporation of amino acids and Schiff base of amino acid through an efficient synthetic route and Structural characterization using FTIR confirmed imine ($-\text{C}=\text{N}$) bond formation, while ^1H & ^{13}C NMR and GC-MS supported the presence of expected functional groups and XRD data indicated increased molecular ordering. These modifications contribute to the development of multifunctional CMC-based materials with improved stability and bioactivity for potential biomedical applications.

The modified CMC derivatives—1a (L-Arginine), 1b (L-Histidine), and 1c (β -Phenylalanine), exhibited significant cytotoxicity with LD_{50} values of 1.94, 1.34, and 1.54 $\mu\text{g/mL}$, respectively. Derivative 1b was the most cytotoxic, while 1a was the least. Further modification

of 1a with glutaraldehyde yielded 2a, which demonstrated enhanced cytotoxicity ($LD_{50} = 1.19 \mu\text{g/ml}$) and the strongest antibacterial activity at 1000 ppm against *A. hydrophila*.

These findings suggest that developed CMC derivatives exhibit considerable potential as a wound healing agent or drug carrier due to their notable b. However, more in vivo research is necessary to completely determine its efficacy and safety for clinical application. This ability to customize the properties of CMC derivatives opens up opportunities for their application in targeted fields, where specific toxicity profiles are required.

Further Studies will focus on identifying optimal conditions for incorporating other amino acid-based Schiff bases and synthesizing their corresponding metal complexes, and investigating their anti-cancer and anti-tumor activities, as well as anti-fungal and antioxidant properties.

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