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Microwave-Assisted Carboxymethylation of Guar Gum Improves Antioxidant and Antibacterial Activity

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Abstract:

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Guar gum (GG), a naturally occurring polysaccharide derived from *Cyamopsis tetragonoloba*, possesses notable physicochemical and biological properties, but its practical applications are limited by poor solubility, instability, and microbial susceptibility. In this study, microwave-assisted carboxymethylation was employed to efficiently modify GG, yielding carboxymethyl guar gum (CMGG) with a high degree of substitution (DS = 0.64) in 15 minutes. Fourier-transform infrared, proton and carbon-13 nuclear magnetic resonance analyses confirmed functionalization by incorporation of carboxymethyl groups, with a distinct carboxyl peak at 1723 cm^{-1} and a new signal at δ 184.2 ppm corresponding to the carbonyl carbon. Additionally, X-ray diffraction, scanning electron microscopy and thermogravimetric analysis revealed increased crystallinity, morphological alterations including the formation of smaller aggregates and a spongy-like texture, and improved thermal stability. CMGG demonstrated significantly increased antioxidant activity (IC_{50} = 4.78 ± 0.12 mg/mL) and exhibited pronounced antibacterial effects against *Bacillus cereus*, *Streptococcus thermophilus*, *Staphylococcus aureus*, and *Escherichia coli*, outperforming native GG. Molecular docking simulations indicated potential inhibitory interactions between CMGG and dihydropteroate synthase, suggesting a possible mechanism for its antimicrobial activity. Overall, CMGG emerges as multifunctional, biocompatible material with promising potential for biomedical, pharmaceutical, and industrial applications.

Keywords: Guar gum; Carboxymethylation; Microwave Functionalization; Antioxidant activity; Antibacterial activity; Molecular docking.

1. Introduction

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Cyamopsis tetragonoloba (guar) is the natural source of GG, a nonionic polysaccharide composed of linear (1→4)-β-D-mannose units randomly substituted with galactose via (1→6)-glycosidic linkages [1]. Due to its renewable origin, biocompatibility, and high viscosity, GG has been widely used in food, pharmaceutical, textile, and petroleum industries [2]. However, its native form exhibits limitations such as excessive swelling, uncontrolled hydration, and microbial instability [3]. To overcome these drawbacks, chemical modifications are commonly applied to tailor the physicochemical and functional characteristics of GG [2]. Among several derivatization techniques, such as sulfation [4], acetylation [5], phosphorylation [6], and selenylation [7], carboxymethylation has gained particular attention for enhancing solubility, viscosity, and microbial resistance through the introduction of carboxymethyl (-CH_2COOH) groups [8,9]. Conventional heating methods to introduce these carboxymethyl groups often require prolonged reaction times and high energy input, leading to heterogeneous reactions and limited scalability [10,11].

Microwave-assisted synthesis for the functionalization of polymers has recently emerged as an efficient, eco-friendly alternative to conventional heating methods. This method allows uniform, rapid heating and improved reaction kinetics by coupling electromagnetic radiation with dipolar molecules [11,12]. Guar gum has been successfully functionalized with acrylic acid through microwave-assisted polymerization, enabling higher grafting yields and cleaner reaction profiles than traditional thermal methods [13]. Microwave-assisted techniques have also been applied to carboxymethyl guar gum (functionalized with carboxymethyl groups by conventional heating methods first), resulting in enhanced polyacrylamide chain grafting efficiency and improved functional performance [14]. However, despite these advances, microwave-assisted carboxymethylation of native guar gum itself has not yet been reported. Existing studies focus on microwave-enabled grafting onto pre-modified guar derivatives or other polysaccharides [15], leaving a clear gap regarding the direct introduction of

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carboxymethyl groups onto the native galactomannan backbone. Beyond structural optimization, carboxymethylation can enhance biological activities such as antioxidant and antimicrobial effects by introducing electron-donating groups that promote free radical scavenging and bacterial membrane disruption [16–21]. However, mechanistic insights into these bioactivities remain limited.

In this study, we report a microwave-assisted carboxymethylation strategy for the rapid and energy-efficient derivatization of guar gum. The resulting CMGG exhibits enhanced physicochemical stability, antioxidant, and antimicrobial properties. Furthermore, molecular docking simulations provide insights into the antibacterial mechanism of CMGG at the molecular level. This integrated green synthesis and computational analysis framework highlights CMGG as a promising biopolymer for biomedical and environmental applications.

2. Materials and Methods

2.1 Materials

GG (G4129) with a molecular weight of 220 kDa, monochloroacetic acid (MCA, 402923), and 2,2-diphenyl-1-picrylhydrazyl (DPPH, D9132) were obtained from Sigma-Aldrich (Germany). All chemicals utilized were of analytical grade and were used as received without any further purification. Ultrapure water with a resistivity of 18.2 MΩ·cm was used to prepare all solutions.

2.2 Preparation of CMGG using microwave irradiation

The carboxymethylation of GG was carried out using microwave irradiation based on a previously established method for a different chemical functionalization [13,22], with slight modifications. Briefly, GG (1 g) was dispersed in 2-propanol (15 mL), followed by the addition of 10 M aqueous NaOH (15 mL). The mixture was stirred for 1 hour at 50 °C to allow an initial alkalization. Subsequently, monochloroacetic acid (MCA, 1 g), pre-dissolved in 2-propanol (12 mL) and pre-heated at the same temperature (50 °C), was added to the alkaline GG mixture. A

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range of process parameters were screened in this next microwave assisted step, including oven temperature (35 – 65 °C), time (10 – 20 minutes), monochloroacetic acid amount (0.6 – 1.4 g) and microwave power (100 – 600 W), details on **Table S1**. The following conditions were deemed optimal to achieve microwave-assisted carboxymethylation of GG and the CMGG characterized as described in the following methods was produced using these optimal conditions. Therefore, the reaction mixture was then subjected to microwave irradiation at 50 °C for 15 minutes, with 30-second intervals of electromagnetic irradiation at an optimized power of 600 W. After irradiation, the product was cooled to room temperature, washed thoroughly with ethanol, filtered, repeatedly rinsed with deionized water and dried under vacuum at 50 °C for 24 hours. As a control, a separate sample was prepared by treating GG with NaOH solution under the same conditions but without the addition of MCA, to account for any changes due to protonation of intermediate GG species in the absence of MCA. The microwave irradiation protocol is schematically illustrated in **Figure 1**, and the screened reaction conditions, including temperature, reaction time, MCA amount, and irradiation power, are provided in **Supplementary Information**. Biocompatibility tests with human dermal fibroblast cells are also reported (**Supplementary Figure S1**).

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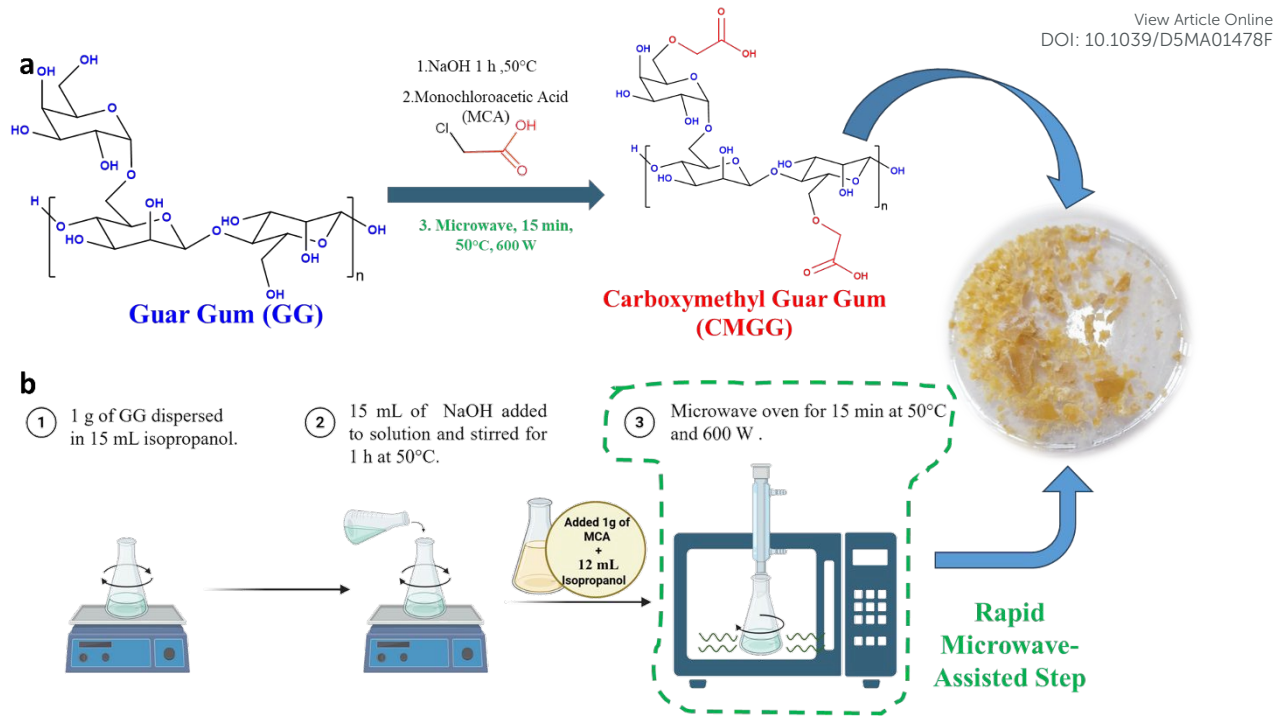

Figure 1. a. Representative chemical reaction with carboxymethyl groups highlighted in red, introduced by monochloroacetic acid (MCA) and details of the optimized microwave-assisted conditions in green. **b.** Schematic representation of the carboxymethylation process for the natural GG powder. Highlighted in green the rapid microwave-assisted step.

2.3 Characterization techniques

Various characterization techniques were employed to investigate the structural, morphological, thermal, and crystalline properties of GG and CMGG. Scanning electron microscopy (FESEM, JEOL JSM-7200F, USA) was used to analyze surface morphology without gold coating. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) (Bruker 400 MHz NMR spectrometer) spectra were recorded in deuterated water (D_2O) to confirm structural modifications and determine the degree of substitution after carboxymethylation. Fourier transform infrared spectroscopy (FTIR, Bruker Optics GmbH, Ettlingen, German) was applied in attenuated total reflectance (ATR) mode to identify functional groups and verify chemical changes. Thermogravimetric analysis coupled with differential scanning calorimetry (TGA/DSC using a PerkinElmer STA8000 instrument) was performed to assess thermal stability and degradation behavior, using a heating rate of 10 °C min^{-1} with samples placed in

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aluminum pans. Lastly, powder X-ray diffraction (PXRD) was performed using a Rigaku Miniflex II diffractometer equipped with CuKα radiation (λ = 1.5406 Å) to evaluate the degree of crystallinity of GG and CMGG and assess changes in molecular ordering following chemical modification. Diffraction patterns were collected over a 2θ range of 10–80°, using an accelerating voltage of 40 kV and a scanning rate of 10° min^{-1}.

Degree of substitution determination

The DS of CMGG was evaluated using a titrimetric method, as outlined in previously reported protocols [23]. For this, 1 g of CMGG was mixed with 50 mL of methanol, then 5 mL of 0.5 M aqueous solution of nitric acid was added, and the mixture was stirred for 10 min at room temperature. The sample was boiled for 5 min and stirred for 20 min at 300 rpm. After that, the sample was allowed to stand for 30 min. The sample was filtered and washed with ethanol (3 × 50 mL) to remove salt and acids. Afterward, the precipitate was washed with methanol (1 × 50 mL), transferred into a beaker, and heated until the alcohol evaporated. The CMGG was dried in a conventional oven at 90 °C for 3 h. Then, CMGG was added to 100 mL of distilled water and 25 mL of 0.5 M aqueous solution of NaOH in an Erlenmeyer flask. The solution was heated at 98°C for 1 h. Finally, the heated solution was titrated with 0.3 M HCl using phenolphthalein indicator until neutralization (when the color of the solution changed from magenta to transparent). The same procedure was carried out for a blank solution (0.5 M aqueous solution of NaOH without CMGG). The carboxymethyl group (-COOH content) and the DS were calculated as follows:

$$n_{COOH} = C_{NaOH} \times V_{NaOH} - C_{HCl} \times V_{HCl} \qquad (1)$$

$$DS = \frac{162 \times n_{COOH}}{m - 58 \times n_{COOH}} \qquad (2)$$

Where n_{COOH} is the number of moles of COOH groups in the sample, C_{NaOH} and C_{HCl} are the molar concentrations of standard NaOH and HCl solutions, respectively, V_{NaOH} is the volume of NaOH, and V_{HCl} is the volume of HCl used for the titration of the excess of NaOH, DS is the

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degree of substitution, 162 is the molecular weight of anhydrous glucose unit (g.mol^{-1}), 58 is the molecular weight of the carboxymethyl (-CH_2COO^-) group that replaces the hydroxyl (-OH) group (g.mol^{-1}) in the GG polymer, and m is the amount of CMGG used for the test (g). Each titration was repeated at least three times to confirm reproducibility.

DPPH• Free Radical Scavenging investigation

The antioxidant activity of CMGG and GG was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, following the protocol described by Moussa *et al.* [24]. Ethanolic suspensions/solutions of GG and CMGG were prepared at various concentrations ranging from 0.2 to 200 mg/mL. GG exhibited limited solubility under these conditions. Each solution was mixed with a DPPH solution at a final concentration of 0.023 mg/mL in ethanol. The mixtures were incubated at 25°C in the dark for 30 min to allow the reaction to proceed without interference from light. The percentage of DPPH• radical scavenging was obtained by measuring the absorbance at 517 nm using a UV-Vis spectrophotometer. The scavenging activity percentage was calculated using equation 3 ($\mathbf{A}_{control}$ = absorbance of the DPPH solution without the sample and $\mathbf{A}_{sample}$ = absorbance of the DPPH solution with the sample):

$$Radical\ Scavenging\ Activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (3)$$

Antibacterial Activity

The antibacterial properties of native GG and CMGG were evaluated against several microbial strains [25]: *Bacillus cereus* (gram positive, American Type Culture Collection (ATCC 14579), *Streptococcus thermophilus* (gram positive, ATCC 33023), *Staphylococcus aureus* (gram positive, ATCC 6538), and *Escherichia coli* (gram negative, ATCC 8739). Briefly, the

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antibacterial activity of the samples was evaluated using a disc diffusion method based on the principles of the standard antibiogram. Sterile Whatman paper discs (6 mm diameter) were impregnated with 2 µL of each test solution (100 mg/mL in DMSO) and placed onto the surface of agar plates previously inoculated with 100 µL of microbial suspension. The turbidity of the bacterial suspensions was adjusted to 10^8 CFU/mL.

After placement of the discs using sterile forceps, the agar plates were kept at room temperature for 1 hour to allow diffusion of the samples, followed by incubation at 37 °C for 24 hours. Antimicrobial activity was assessed by measuring the diameter of the inhibition zones formed around the discs. Larger inhibition zones (diameter) indicate greater antimicrobial effectiveness. The statistical analysis was made using two-ways analysis of variance (ANOVA) using OriginLab software. Statistical significance is indicated as follows: $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***), based on Tukey's test.

Molecular docking study

To investigate the binding interactions of GG and CMGG within the DHPS pocket, molecular docking simulations were conducted using the Schrödinger Suite. For representing the GG, the (1,4)-β-D-mannose unit bound to 1,6-galactose unit was used. For the CMGG, the same structure was used with carboxymethyl groups at the C6 of mannose and galactose, as shown in the monomer structure of **Figure 1**. The structural data of DHPS was obtained from the RCSB Protein Data Bank (http://www.pdb.org) under the PDB ID 3TZF [26]. This enzyme was chosen as the docking target, and it was optimized through energy minimization using the Protein Preparation Wizard in the Schrödinger Suite [27]. The co-crystallized ligand (sulfamethoxazole) and the monomers of GG and CMGG were prepared using LigPrep [28], ensuring appropriate protonation states, correct atom types, the addition of hydrogen atoms and the assignment of bond orders to refine their molecular structures for docking. The docking grid was generated using the Receptor Grid Generation protocol in Maestro [29], with the centroid

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of the bound ligand set as the grid center. Default parameters were applied for grid generation. The flexible docking simulations were carried out in single precision (SP) mode [30], enabling accurate ligand-protein interaction predictions while considering molecular flexibility.

3. Results and Discussion

3.1 Carboxymethylation of Guar Gum

Carboxymethylation of guar gum (GG) has been well reported in the literature but only by conventional heating methods[8,10,11]. Here, from the range of process parameters screened (**Table S1**), the highest degree of carboxymethylation of one gramme of GG via a microwave-assisted method was obtained when one gramme of monochloroacetic acid was used with microwaves of 600 W at a temperature of 50°C for 15 minutes.

Using this method, ^{1}H NMR and ^{13}C NMR analysis confirmed the successful carboxymethylation of CMGG (**Figure 2** and **S2)**. The ^{1}H NMR spectrum of CMGG (**Figure 2a**) displays characteristic up field signals in the range of δ 3.0 – 5.0 ppm, which correspond to the sugar protons of the polysaccharide backbone. These protons are associated with hydroxyl and ring hydrogen atoms in the GG structure (**Figure 2c**) [10,31,32]. Additionally, the strong signal at δ 3.86 and 8.38 ppm in the CMGG spectrum is attributed to the methylene protons (-CH_2-) and hydroxyl group of the carboxylic groups (-COOH), respectively. The peak at δ 8.38 ppm is difficult to corroborate with previous literature, as most studies report only the δ 3.0–5.0 ppm region [10,31,32].

The ^{13}C spectrum of CMGG (**Figure 2b**) shows signals at δ 179.9 –168.3 ppm corresponding to the carbonyl carbon of the carboxylic groups (-COOH) introduced through the carboxymethylation process. Additionally, carbon signals in the CMGG ^{13}C spectrum appear in the range of δ 61.1 – 69.5 ppm, corresponding to the carbons C2–C6 of the polysaccharide

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backbone. The shift in proton signals and the appearance of the carboxylic carbon signal is consistent with successful carboxymethylation of the GG [33,34].

A distinct absorption peak observed at 1587 cm^{-1} and 1416 cm^{-1} in the FTIR spectrum of CMGG (**Figure 2e**) corresponds to the stretching vibrations of the >C=O bonds in the carboxylic acid groups, confirming the successful chemical modification of GG by the introduction of carboxymethyl functionalities. Notably, in the comparative spectra of GG and CMGG, differences in characteristic bands are observed in the region between 1725 and 1000 cm^{-1}, suggesting structural changes associated with carboxymethylation. A detailed assignment of the characteristic absorption bands for the individual spectra of GG and CMGG is provided in supplementary information (**Figure S3)**, which further supports the incorporation of carboxymethyl groups. Thus, the NMR and FTIR spectroscopic analyses provided strong evidence for the successful carboxymethylation of guar gum, aligned with previous reports in the literature [10,11,35]

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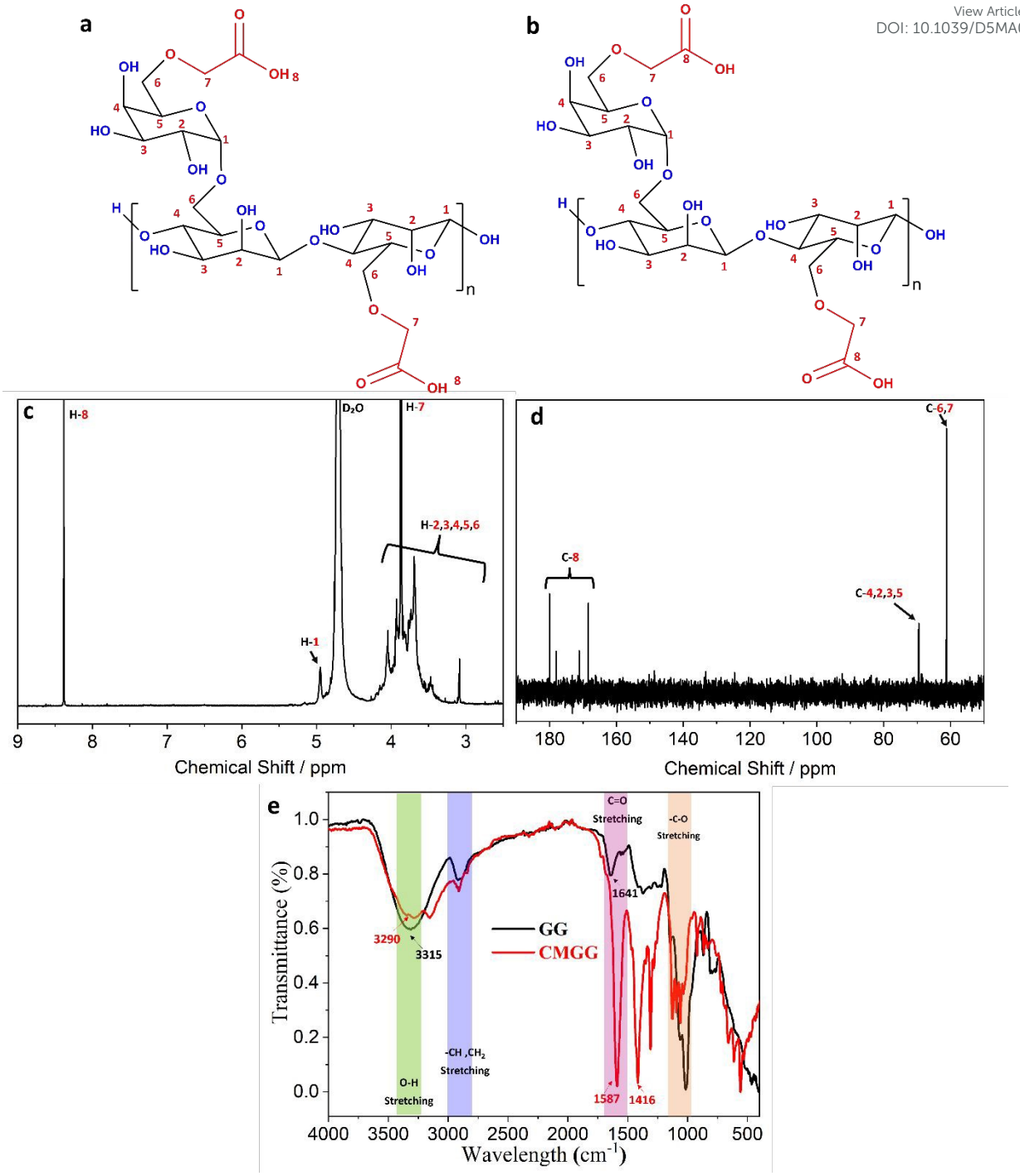

Figure 2. (a, c) ^{1}H NMR and (b, d) ^{13}C NMR spectra of CMGG with the ^{1}H and ^{13}C assignments, respectively, and (e) FTIR comparative spectra of unmodified GG (black line) and CMGG (red line), highlighting the structural changes upon modification.

The carboxymethylation of guar gum using this microwave assisted functionalization method also caused macroscopic morphological changes to the polymer, including the formation of

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smaller aggregates and a spongy-like texture compared to its original smooth, uniform particles. **Figure 3**. The CMGG particles exhibit agglomeration and increased porosity, compared to GG. These structural changes suggest enhanced surface activity, which may improve the material's reactivity and functionality, particularly for applications involving adsorption or molecular interactions. Similar morphological trends have been observed in other chemically modified polysaccharides [36]. This sponge-like texture was absent in GG when it was exposed to the process conditions for carboxymethylation in the absence of MCA (**Figure S4**), confirming that the structural changes were due to the carboxymethylation reaction. The increased porosity and agglomeration observed in CMGG may contribute to improved solubility, surface area, and reactivity, further supporting its potential applications in biomedicine, food packaging, and water treatment.

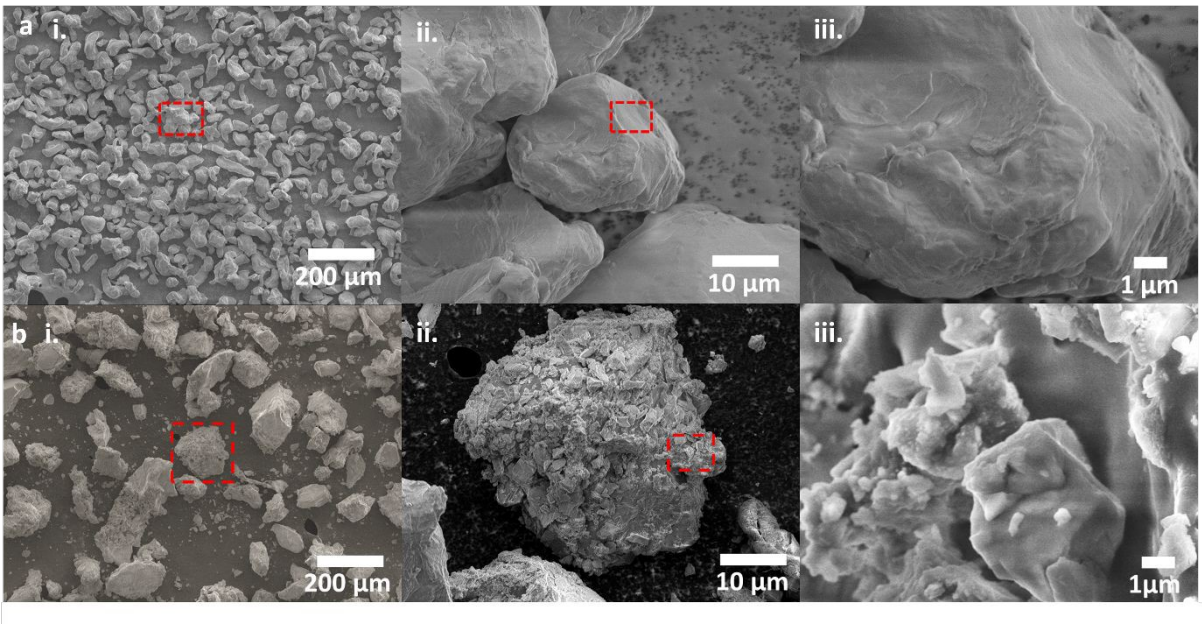

Figure 3. SEM images of (**a**, top) GG and (**b**, bottom) CMGG, respectively. The red dashed squares represent the region where higher magnification was executed. Scale bars are 100 µm (**i**, left), 10 µm (**ii**, center) and 1µm (**iii**, right), respectively.

GG undergoes two major weight loss events upon heating: an initial loss around 100°C due to the evaporation of adsorbed water [37] and a second step at 264°C, attributed to polymer

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degradation, **Figure 4**. The char residue at 600°C is approximately 5%, likely due to the presence of inorganic fillers or stabilizers [38]. For CMGG, a more gradual weight loss with increasing temperature is observed, leading to a final loss in weight of 47% between room temperature and 600°C, accompanied by a higher char residue compared to GG, **Figure 4a**. This gradual loss in mass suggests improved thermal stability due to the incorporation of carboxymethyl groups, which enhance crosslinking and reduce the formation of volatile degradation products. The presence of residual inorganic salts from the carboxymethylation reaction may also contribute to this stability [38,39]. The derivative thermogravimetric (DTG) (**Figure 4b**) shows that GG exhibits a degradation peak at 303°C, consistent with previous findings [40] while CMGG displays a smaller peak at 265°C, possibly due to the presence of uncross-linked chains. Tamba *et al.* reported a similar degradation temperature at 241 °C (57% mass loss) for CMGG (261 kDa), functionalized via a conventional heating method (50 °C for 4h) and 262 °C (70% mass loss) for native GG (220 kDa).[41]

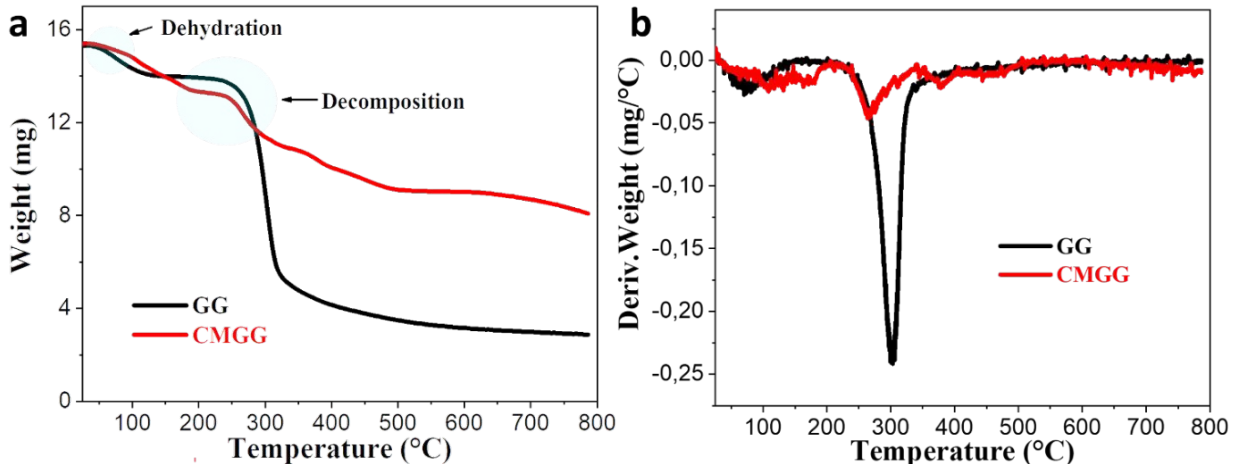

Figure 4. Thermograms TGA (**a**) and DTG (**b**) profiles of GG (black line) and CMGG (red line).

Powder X-ray diffraction analysis of both unmodified GG and CMGG, indicated structural changes induced by the chemical modification, **Figure 5**. The diffractogram of unmodified GG (black line) displays a predominantly amorphous nature, characterized by a broad diffraction

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peak centered at a 2θ angle of ~20°. In contrast, the diffractogram of CMGG (red line) reveals a significant transformation in its structural profile, transitioning from the predominantly amorphous nature of unmodified GG to a semi-crystalline state. This transition is evidenced by the emergence of sharper and more intense diffraction peaks, indicating enhanced molecular ordering due to the carboxymethylation process. This was also evidenced from the observation of distinct melting peaks in DSC thermograms for CMGG which were absent for GG, **Figure S5**. The introduction of carboxymethyl groups likely disrupts the random coil conformations of the GG backbone, facilitating partial alignment of polymer chains and the formation of more organized crystalline regions. Additionally, these modifications enhance intra- and intermolecular interactions, including hydrogen bonding and electrostatic forces, which further stabilize the crystalline domains.

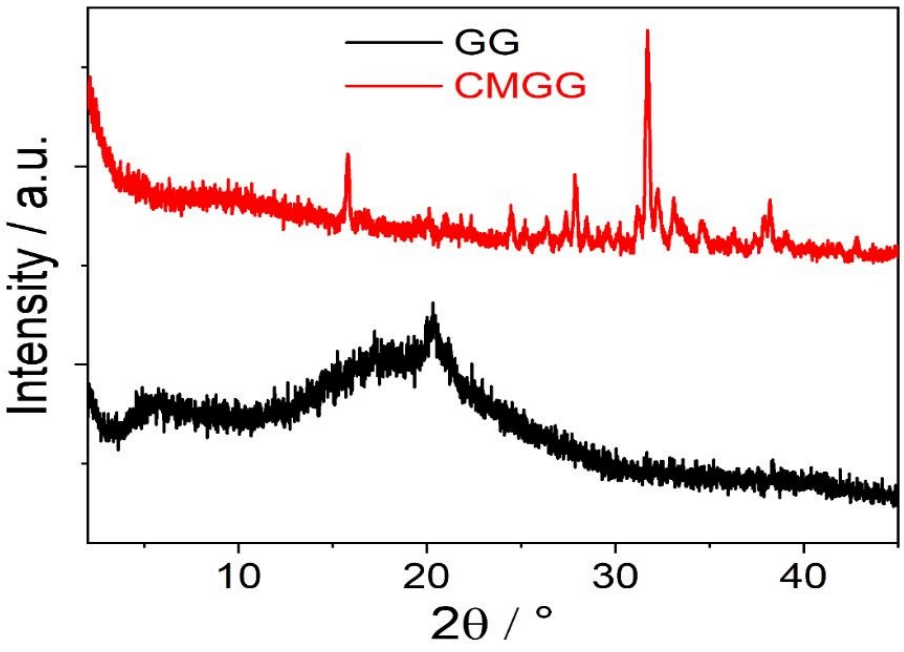

Figure 5. PXRD Diffractograms of unmodified GG (black line) and CMGG (red line).

Microwave-assisted carboxymethylation of GG at 600 W in 15 minutes (optimized condition, **Table S1**) achieved a DS of 0.64, significantly improving reaction efficiency over conventional

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heating at 50 °C, which required 2-4 hours for similar results, **Table 1** [42]. In the microwave-assisted esterification of guar gum, Nazir *et al.* reported degradation of GG only at more than 15 minutes of reaction time at a power of 1000 W.[43] Given that the microwave exposure time in our experimental conditions was only up to 15 minutes, no polymer degradation is expected.

Table 1. Degree of carboxymethylation of CMGG compared with values reported.

Reference	Polymer	DS	Carboxymethylation method	Time
Gong et al.[44]	Guar gum	0.6	Conventional method 60°C	10 h
G. Dodi et al [42]	Guar gum	0.64	Conventional method 50°C	4 h
S. Pal [8]	Guar Gum	0.4-0.7	Conventional method 50°C	2 h
Present work	Guar gum	0.64	Microwave-assisted 50°C	15 min

The superior performance of microwave-assisted synthesis is due to its unique heating mechanism, where electromagnetic waves interact directly with polar molecules, ensuring rapid and uniform temperature increase. This process overcomes diffusion limitations, improving reaction kinetics and molecular transport [45]. Additionally, microwave irradiation enhances selectivity and efficiency by (i) generating localized heating, reducing side reactions; (ii) increasing reagent solubility and accessibility; and (iii) rapidly activating molecular bonds, accelerating the reaction. The optimized power level of 600 W (**Table S1**) ensures efficient energy transfer while minimizing polysaccharide degradation, making the process reproducible and scalable for industrial applications. Beyond efficiency, microwave-assisted synthesis aligns with green chemistry principles, reducing energy consumption and reaction times. These findings underscore its potential as a sustainable and high-performance approach for polysaccharide modification, with broad applications in materials science.

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3.2 Antioxidant Activity of CMGG and GG

CMGG, with a DS of 0.64, exhibited high antioxidant activity, achieving approximately 80% DPPH radical inhibition at a concentration of 200 mg/mL, with an IC_{50} of ~4.78 mg/mL (**Figure 6**). This activity was found to be concentration-dependent, with higher concentrations leading to greater radical scavenging. In comparison, an IC_{50} value could not be achieved against DPPH radicals at a DPPH concentration of 0.023 mg/mL with unmodified GG, even with up to 200 mg of GG added per mL, likely due to the poor solubility of GG. This suggests that the introduction of carboxylic groups through modification enhances the antioxidant capacity of GG [46], and is likely due to a combination of optimized DS, enhanced water solubility, and the electron-donating ability of carboxymethyl groups. Previous studies have shown that increasing the concentration of CMGG from 3 to 8 wt% leads to a marked increase in viscosity and the emergence of non-Newtonian, pseudoplastic behavior. [11,32] This behavior is commonly attributed to enhanced polymer–solvent interactions and improved chain disentanglement resulting from carboxymethyl substitution. Pal et al. (2009) compared the rheological properties of native guar gum (GG) and CMGG prepared via a conventional carboxymethylation method (DS ≈ 0.6). Both polymers were evaluated at a low concentration of 0.5 wt%. However, at this concentration, the complete solubilization of native GG is questionable, which complicates a direct comparison of intrinsic rheological properties. Despite this limitation, Pal et al. reported a similar non-Newtonian behavior for both systems, with CMGG exhibiting a slightly higher viscosity.[8]

This DPPH scavenging activity assay primarily reflects the ability of a compound to donate electrons or hydrogen atoms to neutralize the stable DPPH radical, providing an initial indication of antioxidant potential at the chemical level. The enhanced scavenging activity observed for CMGG therefore suggests that the carboxymethylation of guar gum introduces structural features that improve its radical quenching capability in solution. However, the

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potential demonstrated by DPPH assay do not fully represent antioxidant activity in cellular system.

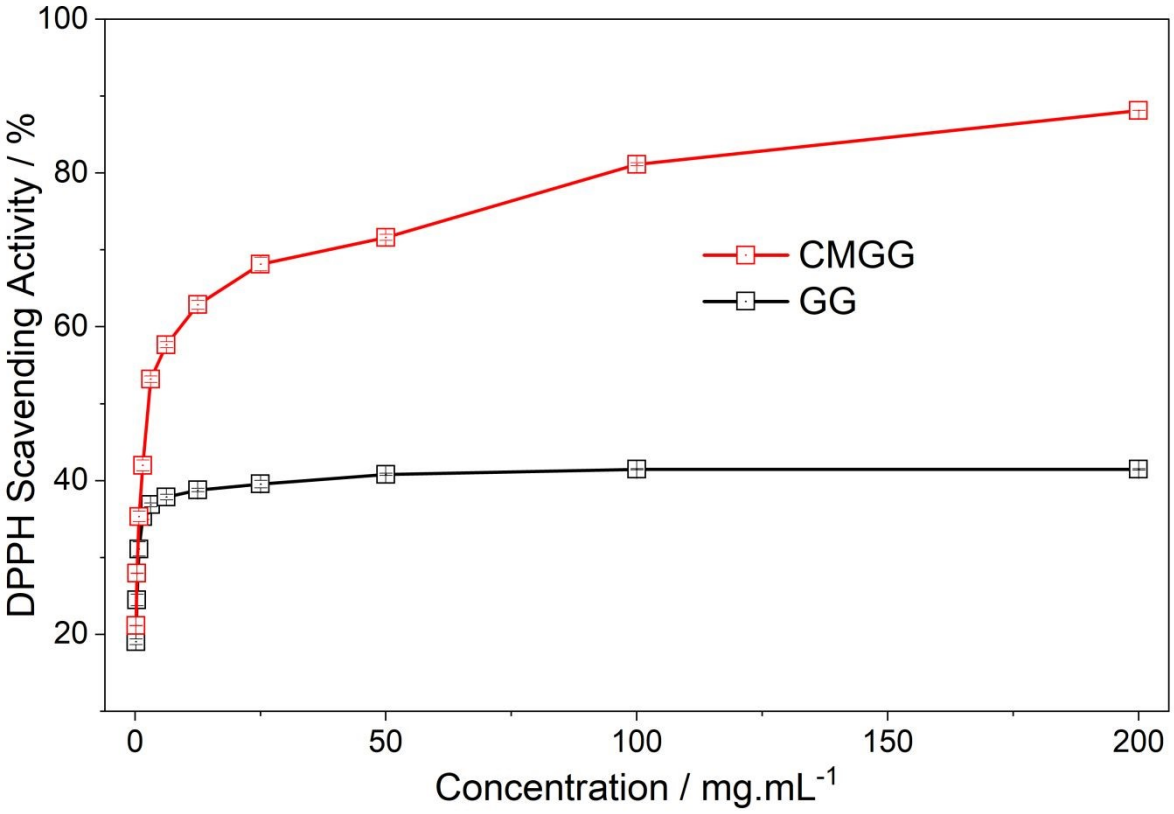

Figure 6. Evaluation of antioxidant activity of GG (black) and CMGG (red) using· a DPPH solution at a final concentration of 0.023 mg/mL in ethanol.

The effect of chemical substitution on antioxidant performance has been extensively studied in GG derivatives. Phosphorylated GG, with DS values of 0.37, 0.43 and 0.52, showed improved DPPH scavenging activity, with IC_{50} values of 1.74, 1.45, and 1.32 mg/mL, respectively [47]. Sulfated GG variants such as SGG_{cata3} and SGG_{cata7} achieved high inhibition levels of 80.32% and 79.35% at 5 mg/mL. Among these, SGG_{cata3}—featuring a higher DS and lower molecular weight—demonstrated the most potent activity [48]. Additionally, sulfated GG has also been reported to exhibit strong antioxidant potential due to the incorporation of sulfate groups, which increase negative charge density and facilitate radical stabilization. In another study, sulfated GG showed approximately 75% scavenging at 1 mg/mL against DPPH radicals [49].

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Furthermore, phosphorylated GG achieved around 78% scavenging with an IC_{50} of ~5.5 µg/mL, attributed to the phosphate groups enhancing hydrogen atom transfer mechanisms [50,51]. Bioactive films produced by CMGG have demonstrated over 95% DPPH scavenging at concentrations above 500 µg/mL [52], highlighting the significant enhancement of antioxidant functionality through carboxymethylation. The present study indicates that the IC_{50} of 4.78 mg/mL achieved with CMGG is consistent with the range of antioxidant activities reported for GG-based derivatives in the literature[53].

Outside of GG-based systems, the influence of DS on antioxidant activity has been similarly observed. Carboxymethylated polysaccharides from *Amana edulis* with DS values ranging from 0.605 to 0.783 exhibited high in vitro antioxidant activity (77.3–99.9%), while native polysaccharides showed much lower activity (20–75%) [54]. Carboxymethylated cashew gum also displayed strong DPPH scavenging with an IC_{50} of 0.43 mg/mL [55]. Similarly, carboxymethylated *Sargassum fusiforme* polysaccharides showed improved antioxidant performance, increasing from 60.05% in the native form to 66.6% upon modification [56]. Phosphorylated pumpkin polysaccharides with DS values ranging from 0.33 to 0.52 demonstrated 55% to 70% radical scavenging activity, confirming the trend of DS-dependent antioxidant efficiency in non-GG polysaccharides as well [57].

Comparing the antioxidant performances of various modified polysaccharides, CMGG stands out by offering a strong balance of solubility and functional group reactivity. The carboxymethylation process introduces carboxymethyl groups (-CH_2COOH), which not only enhance solubility but also provide hydrogen atoms that facilitate electron donation and radical stabilization [56], confirming preliminary antioxidant potential of the modified polymer.

3.7 Antibacterial Activity

In this work, CMGG exhibited significantly larger inhibition zones, compared to native GG ($p < 0.05$), for all four tested bacterial strains (*Bacillus cereus*, *Streptococcus thermophilus*, *Staphylococcus aureus*, and *Escherichia coli*), indicating an enhanced antimicrobial effect, **Figure 7**. For *Bacillus cereus*, the zone expanded from 6.2 ± 0.8 mm (GG) to 15.2 ± 0.2 mm (CMGG); for *Streptococcus thermophilus*, from 10.4 ± 0.6 mm to 15.1 ± 0.3 mm; for *Staphylococcus aureus*, from 11.1 ± 1.0 mm to 13.6 ± 0.4 mm; and for *Escherichia coli*, from 11.5 ± 0.5 mm to 13.1 ± 0.2 mm, upon treatment with GG or CMGG respectively.

This enhancement suggests that the carboxymethylation of guar gum introduces functional groups that improve its interaction with bacterial cell membranes, thereby increasing its antibacterial efficacy. The modification likely enhances the solubility, charge distribution, and molecular interactions of the polysaccharide, all of which can contribute to the disruption of microbial growth. Representative images of the inhibition zones from the disc diffusion assays are provided in **Figure S6**.

Carboxymethylation has been widely reported to enhance the antimicrobial properties of various polysaccharides by introducing functional groups that improve solubility, charge distribution, and molecular interactions with bacterial membranes. For instance, carboxymethyl β-glucans exhibited significant antibacterial activity against *Staphylococcus aureus*, with maximum inhibition zones reaching 19.37 ± 0.45 mm [58]. Similarly, carboxymethylated derivatives of degraded *Sargassum fusiforme* polysaccharide (DPSF) showed inhibition zones of 11.10 ± 0.04 mm and 14.68 ± 0.05 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively. In contrast, neither the native polysaccharide nor its degraded form exhibited any noticeable antibacterial activity, highlighting the critical role of carboxymethylation in its activity [59]. Liu *et al*. also reported that chloroacetic acid-modified *Catathelasma ventricosum* polysaccharides displayed elevated antibacterial activity against *E. coli*, *Salmonella typhimurium*, *S. aureus*, and *Bacillus subtilis* [60]. Furthermore, carboxymethylated kappa-

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carrageenan with degrees of substitution ranging from 0.8 to 1.6 demonstrated markedly increased antimicrobial efficacy against *S. aureus*, *B. cereus*, and *E. coli* when compared to the native kappa-carrageenan [61].

CMGG exhibited markedly higher antibacterial activity against the Gram-positive strains *B. cereus* and *S. thermophilus*, with inhibition levels significantly greater than those observed for the Gram-negative species ($p \leq 0.001$). This enhanced susceptibility of Gram-positive bacteria is commonly attributed to their cell-wall architecture: they lack an outer membrane and possess a more permeable peptidoglycan layer, allowing the negatively charged carboxylate groups of CMGG to interact more effectively with positively charged components of the cell wall. Such interactions are likely to destabilize the membrane and promote leakage of intracellular materials.

In contrast, *E. coli* and *S. aureus* showed lower sensitivity to CMGG, with statistically significant but smaller reductions in bacterial growth ($p \leq 0.01$ for *E. coli* and $p \leq 0.05$ for *S. aureus*). For Gram-negative species, the outer membrane acts as an additional barrier that restricts penetration of high-molecular-weight or polyanionic compounds such as CMGG. The reduced activity against *S. aureus*, compared with the other Gram-positive strains, may further be explained by its dense peptidoglycan layer, characteristic teichoic acid composition, and its ability to form biofilms, all of which can hinder polymer–cell wall interactions.[62]

Overall, these results indicate that the antibacterial performance of CMGG is strongly influenced by both its carboxymethyl substitution and the structural features of the target microorganisms, which together dictate cell-wall accessibility and susceptibility.

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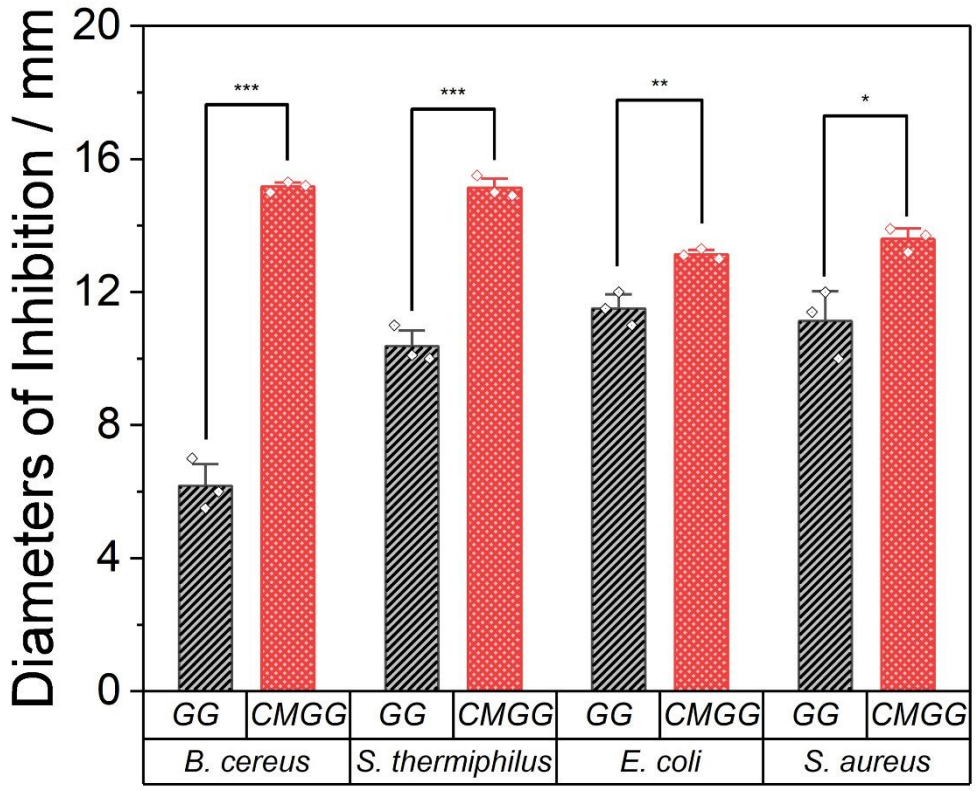

Figure 7. Antibacterial activity of GG and CMGG against *Bacillus cereus, Streptococcus thermophilus, Escherichia coli* and *Staphylococcus aureus*, respectively. Statistical significance is indicated as follows: $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***), based on Tukey's test.

DHPS is an example of an enzyme that is targeted by existing antimicrobial agents, such as sulfonamides, trimethoprims and aminosalicylic [63,64]. When DHPS is inhibited, the synthesis of folic acid in bacteria is inhibited, causing death [65]. To investigate the feasability of DHPS inhibition as the antibacterial mechanism of action of GG and CMGG, molecular docking simulations targeting DHPS, utilizing both the GG unit and the carboxymethyl modified version CMGG, were conducted. The goal was to compare their binding affinities and interactions with the enzyme and hypothesise the mechanism behind the observed antibacterial activity. Our docking protocol was validated by redocking the co-crystallized ligand (sulfamethoxazole), achieving an RMSD value of less than 1 Å, as shown in **Figure S7**. Although docking a single disaccharide unit inherently oversimplifies the complexity of a polymeric system, it represents a well-established strategy in the literature for gaining initial

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insights into polysaccharide–protein interactions.[66] Additionally, the higher binding affinity demonstrated for the modified guar gum unit suggests DHPS might play a role in the observed antibacterial activity.

The molecular docking results reveal a significant improvement in the binding affinity of the modified polymer unit (CMGG) compared to the original unmodified unit (GG), with docking scores of -8.125 kcal/mol and -4.652 kcal/mol, respectively. For reference, the docking score is -6.100 kcal/mol for the co-crystalized ligand typically designed to interact with the specific enzyme site. This difference can be attributed to the presence of the carboxyl group, which introduces additional interactions with the bacterial DHPS target. Specifically, seven hydrogen bonds were formed between the hydroxyl (OH) groups and the residues SER222, GLY189, ARG63, ARG235, and PHE28, three of which were created through the added carboxyl functionality, as illustrated in **Figure 8**.

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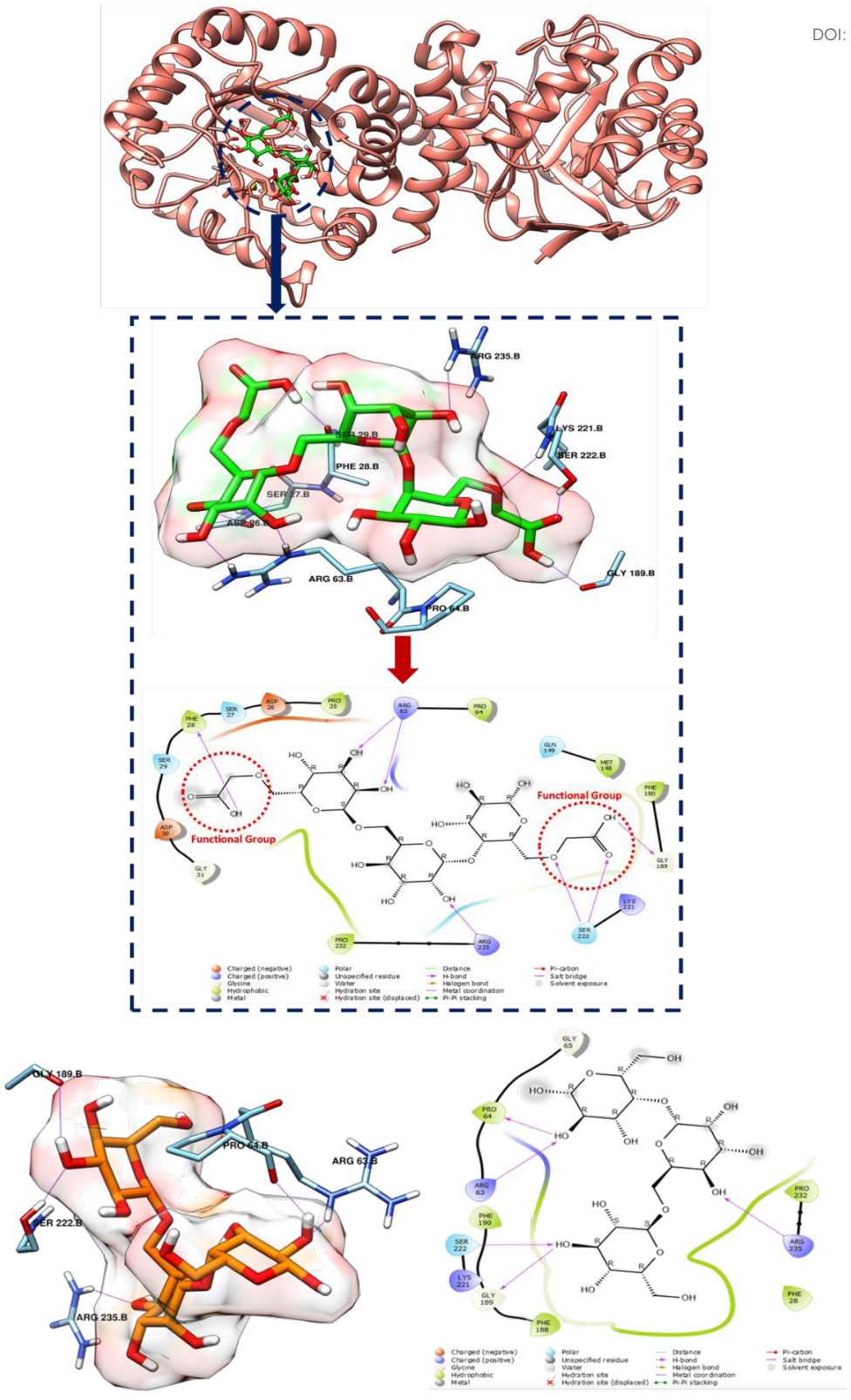

1

2 **Figure 8.** Binding interaction of CMGG (top) and GG (bottom) within the active site of DHPS, highlighting the
3 calculated structure and interactions with groups in the active site (dark blue dashed square).

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In contrast, the unmodified unit (GG) exhibits weaker affinity and limited interactions, forming only five hydrogen bonds with the residues PRO64, ARG63, SER222, GLY189, and ARG23, suggesting poorer recognition by the active site of the DHPS target, **Figure 8**.

The enhanced molecular interactions due to carboxyl functionalization can be explained by the ability of this group to form strong hydrogen bonds and interact with key residues of the bacterial DHPS receptor. These results suggest that chemical modification of the guar gum polysaccharide could enhance its antibacterial activity by promoting better binding to the active site of the target, and that DHPS inhibition is a feasible mechanism of action for the observed CMGG antibacterial activity (**Figure 7**). The overall biocompatibility of CMGG, which is like GG, showed no toxicity towards human dermal fibroblasts (**Figure S1**) combined with its' enhanced antioxidant and antibacterial activity, indicates its' potential for use in biomedical applications.

4. Conclusion

This study confirmed our hypothesis that a microwave-assisted method can be used to achieve carboxymethylation of GG quickly and with a high degree of substitution. The resulting carboxymethylated polymer, CMGG, exhibited significantly enhanced antioxidant and antibacterial properties due to improvements in the polymer solubility, charge distribution, and functional group accessibility. The microwave-assisted synthesis route enabled rapid and efficient carboxymethylation, achieving an optimal DS of 0.64, while drastically reducing reaction time compared to conventional heating methods. Structural and thermal characterization revealed that the modified biopolymer CMGG exhibits higher crystallinity, distinct morphological changes, and improved thermal stability, confirming its enhanced structural robustness. Critically, CMGG demonstrated superior bioactivity compared to native GG, with a notable antioxidant capacity (IC_{50} = 4.78 ± 0.12 mg/mL) and strong antibacterial

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effects against both Gram-positive (*Bacillus cereus*, *Streptococcus thermophilus*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) strains. These findings support our hypothesis that carboxymethylation enhances bioactivity by facilitating electrostatic interactions with microbial membranes and improving functional group availability. Furthermore, molecular docking simulations suggested that CMGG may inhibit DHPS, a key enzyme in microbial folate biosynthesis, providing a plausible mechanism for its antimicrobial action. Collectively, these results demonstrate that microwave-assisted carboxymethylation is a promising strategy to functionalize GG for biomedical and industrial applications.

CRediT authorship contribution statement

Hayet Telli: Conceptualization, methodology, formal analysis, visualization, and original draft preparation. **Hamida Maachou:** Conceptualization, methodology, visualization Supervision, reviewing, and editing. **Yamina Zouambia:** Conceptualization, methodology, visualization Co-Supervision, reviewing and editing. **Redouane Chebout:** Conceptualization, visualization. **Hodhaifa Derdar:** Conceptualization, visualization. **Adh'ya-eddine Hamitouche:** Conceptualization, visualization. **Ali Dekir:** Conceptualization, Data curation, visualization. **Youssef Larbah:** Conceptualization, visualization. **Abdullah A. Ghawanmeh:** Conceptualization, visualization. **Eliza E. Brett:** Conceptualization, methodology, visualization. **Aruã C. Da Silva:** Conceptualization, Supervision, methodology, visualization, formal analysis, Data curation, original draft preparation, critical revision, substantial rewriting, and final editing. **Sarah Hudson:** Conceptualization, methodology, visualization, Supervision, reviewing, and editing.

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Data availability

The data given in the current research are available upon request from the corresponding author.

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- **The data supporting this article have been included as part of the Supplementary Information.**