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Study of the antibacterial activity of chitosan from insects against Gram-positive and Gram-negative bacteria

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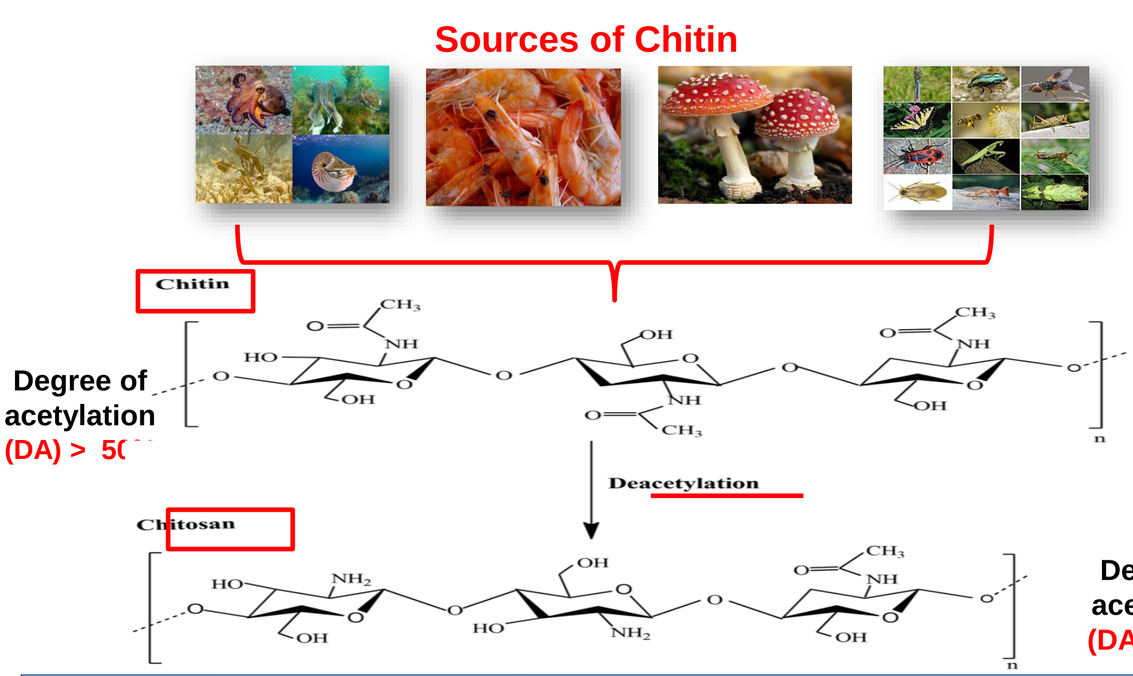
INTRODUCTION & AIM

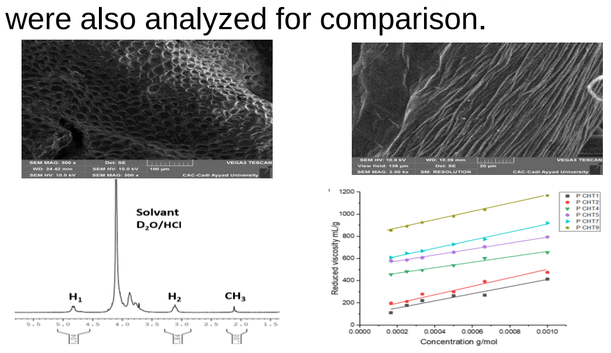
The rise of antibiotic resistance highlights the need for new antimicrobial solutions. Chitosan, a biopolymer derived from chitin, exhibits antibacterial properties influenced by its molecular weight (Mv), degree of acetylation (DA), and structural modifications. It disrupts bacterial membranes, with low Mv chitosan being more effective against Gram-negative bacteria and high Mv chitosan targeting Gram-positive bacteria. A lower DA enhances its interaction with bacterial surfaces. While traditionally sourced from crustaceans, insect-derived chitosan presents a sustainable and potentially more bioactive alternative. This study evaluates its antibacterial efficiency against Gram-positive and Gram-negative strains, offering insights into biomedical and food safety applications.



METHOD

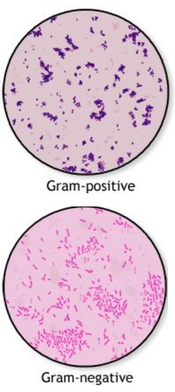
1.Chitosan Selection and Characterization

Nine chitosan samples were extracted from Schistocerca gregaria and Hermetia illucens using Broussignac (KOH-based) and Kurita (NaOH-based) processes. These samples had a degree of acetylation (DA) between 0% and 10% and molar masses ranging from 15,000 to 188,000 g/mol. Their surface structures varied from fibrous to porous. Three commercial chitosans (Sigma-Aldrich) were also analyzed for comparison.



2.Bacterial Strains and Culture Conditions

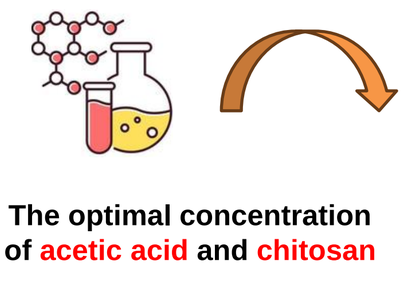
The antibacterial activity of chitosans was tested against five Gram-positive and Gram-negative strains from the Mohammed VI University Hospital Center (Marrakech, Morocco). Strains were cultured on Mueller-Hinton agar at 37°C for 24 h.



Staphylococcus aureus ATCC 29213
Enterococcus faecalis ATCC 29219
Escherichia coli ATCC 27835
Pseudomonas aeruginosa ATCC 25922
Klebsiella pneumoniae ATCC 700 603 ESBL (Extended Spectrum Beta-Lactamase)

3.Antibacterial Activity of Chitosan Samples in Solution

The antibacterial activity of chitosan in solution was evaluated using the disk diffusion method. After incubation, inhibition zone diameters were measured. Gentamicin and ciprofloxacin served as positive controls, while 0.5% acetic acid was used as a negative control. Tests were performed in triplicate, and results are expressed as mean ± standard deviation.



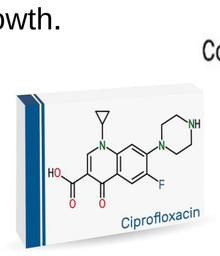
The optimal concentration of acetic acid and chitosan

The disk diffusion method

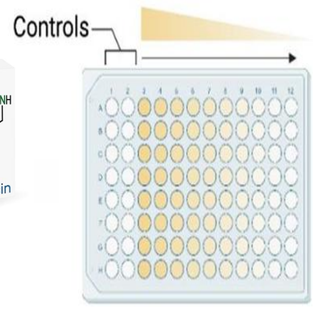
4.Determination of MIC and MBC

The MIC was determined by the microdilution method in a 96-well plate with serial dilutions of chitosan (2.50–0.08 mg/mL). After incubation at 37°C for 24 hours, the MIC was the lowest concentration preventing visible growth. The MBC was determined by plating MIC-negative samples on Mueller-Hinton agar, with the lowest concentration (mg/mL) completely inhibiting bacterial growth.

(GMC, 30 µg/disc)



(CIP, 5 µg/disc)



RESULTS & DISCUSSION

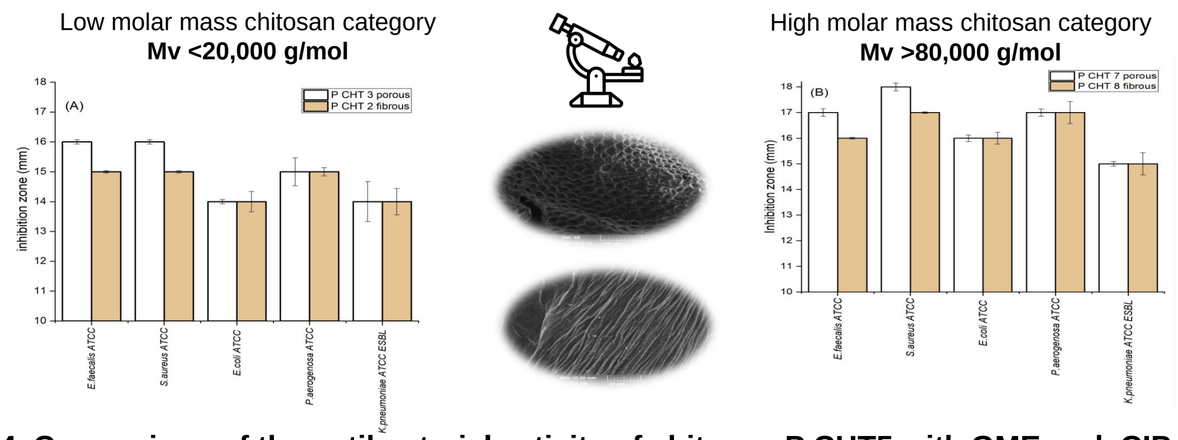
1. Effect of the molar mass Mv of chitosan

Chitosan with a higher molar mass (> 80,000 g/mol) exhibits larger inhibition zones (16–18 mm for Gram-positive bacteria, 15–18 mm for Gram-negative bacteria) compared to low molar mass chitosan (≤ 20,000 g/mol). The efficiency is influenced by its mode of action: deeper penetration for low molar masses and surface activity for higher molar masses.

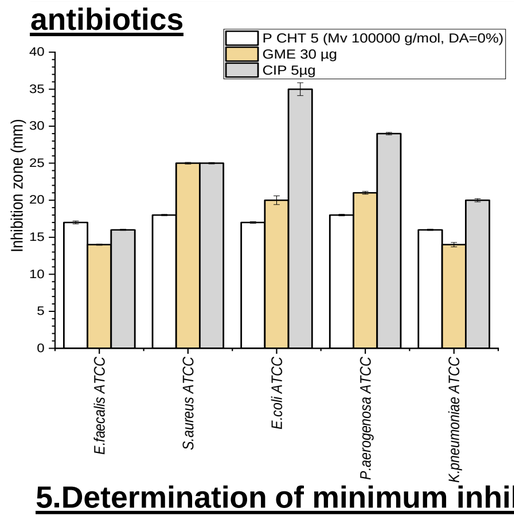
2. Effect of the degree of acetylation DA of chitosan

Chitosan with DA ≤ 10% shows significantly higher antibacterial activity, improving inhibition by up to 100% for Gram-negative bacteria compared to high DA chitosan (30–43%). Low DA enhances cationic interactions with bacterial membranes, leading to stronger effects.

3.Role of surface structure in improving the antibacterial activity of chitosan



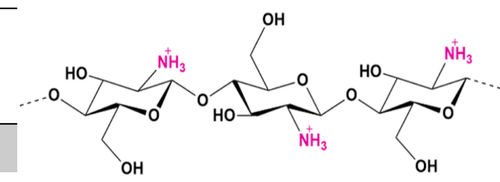
4. Comparison of the antibacterial activity of chitosan P CHT5 with GME and CIP antibiotics



Bacteria	Antibacterial activity of PCHT5 compared to GME and CIP (%)	
	%R1= $\frac{Ø_{P\ CHT5}}{Ø_{GME}} \times 100$	%R2= $\frac{Ø_{P\ CHT5}}{Ø_{CIP}} \times 100$
E. faecalis	**121**	**106**
S. aureus	68	68
E. coli	85	49
P. aeroginusa	86	62
K. pneumoniae	**114**	80

5.Determination of minimum inhibitory and bactericidal concentrations (MIC/MCB)

Bacteria	P CHT 5 DA= 0%		C CHT 2 DA= 30%	
	MIC	MBC	MIC	MBC
E. faecalis	0.16	0.31	0.62	1.25
S. aureus	0.08	0.16	0.62	1.25
E. coli	0.16	0.31	0.62	1.25
P. aeroginosa	0.16	0.31	1.25	1.25
K. pneumoniae	0.08	0.16	0.62	1.25



The total deacetylation of chitosan **(DA=0%)** strengthens the interaction of cations **(NH^{3+})** with the negatively charged cell membranes of bacteria

CONCLUSION

Chitosan demonstrates antibacterial activity against Gram-positive and Gram-negative strains, with effectiveness influenced by strain and physicochemical properties. Chitosan with **DA ≤ 10%** and controlled Mv shows superior performance compared to commercial samples with high DA. Fully deacetylated chitosan **(P CHT 5)** exhibits the best activity, sometimes surpassing antibiotics, making it a promising alternative in combating antibiotic resistance.

FUTURE WORK / REFERENCES

- Expanding studies to chitosan derived from other insect species to compare antibacterial efficiencies.
- Conducting clinical trials to validate its medical applications as a biocompatible antimicrobial agent.

[1] S. Elkadaoui, J. Desbrieres, Y. El Hachimi, A. Hamdaoui, A. Tolaimate, from different parts of desert locust (Schistocerca gregaria), Polym. Bull. (2024). **https://doi.org/10.1007/s00289-024-05411-w.**

[2] S. Elkadaoui, M. Azzi, J. Desbrieres, J. Zim, Y. El Hachimi, A. Tolaimate, International Journal of Biological Macromolecules Valorization of Hermetia illucens breeding rejects by chitins and chitosans production . Influence of processes and life cycle on their physicochemical characteristics, Int. J. Biol. Macromol. 266 (2024) 13131. **https://doi.org/10.1016/j.ijbiomac.2024.131314.**

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