NOVEL ANTIMICROBIAL NANOPARTICLES MADE WITH HUMIC SUBSTANCES FROM GREEN COMPOSTS

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INTRODUCTION

- **Compost-derived Humic Substances (HS)** are well known plant regulators.
- Technologically advanced uses of HS could be also devised, then valorising a waste product.
- We synthesized novel **nanomaterials with antibacterial activities** by exploiting HS and chitosan.
- This issue is of concern, due to the raising **bacterial resistance** to the classical antimicrobial agents.

MATERIALS & METHODS

COMPOST PRODUCTION AND HS EXTRACTION

- Residues from fennel (FEN), artichoke (CYN) and coffee husks (COF) were employed as composting materials.
- HS were isolated from composts by alkaline solutions.

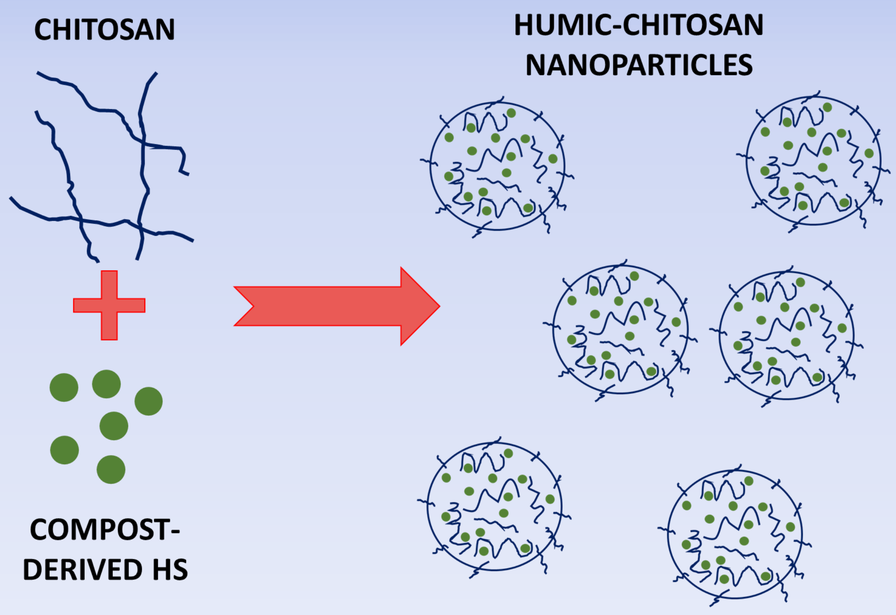
NANOPARTICLES SYNTHESIS AND CHARACTERIZATION

- Nanoparticles (NP) were produced by adding HS to a chitosan solution (**Figure 1**). The NP were studied by Infrared Spectrometry and thermogravimetry. Also, the size and ζ potential of NP were assessed.

NANOPARTICLES ANTIMICROBIAL ACTIVITY

- The NP bioactivity against *Pseudomonas aeruginosa* (ATCC27355) and *Staphylococcus aureus* (ATCC6538P) was evaluated by diffusion disc method, wherein ampicillin served as positive control.

Simplified scheme of the preparation of humic-chitosan nanoparticles by ionotropic gelation.



The humic solution is dropwise added to the chitosan solution under stirring. Then, a sonication cycle is applied to separate the NP from each other.

RESULTS

INFRARED SPECTROMETRY

Similar chemical features were found for the various NP, therefore, only one spectrum is reported. Signals attributed to carbohydrates in both chitosan and HS-can be identified at 1060 and 1380 cm^{-1}. Amide peaks (1555 cm^{-1}) as well as C=C or COO^- (1630 cm^{-1}) signals were also observed.

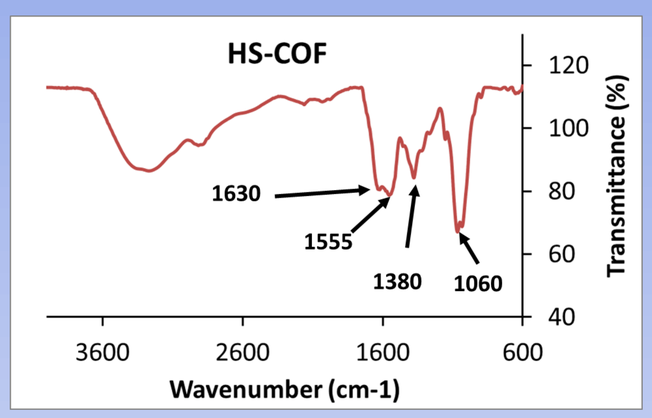


Figure 2. Infrared spectrum of NP obtained from HS-COF.

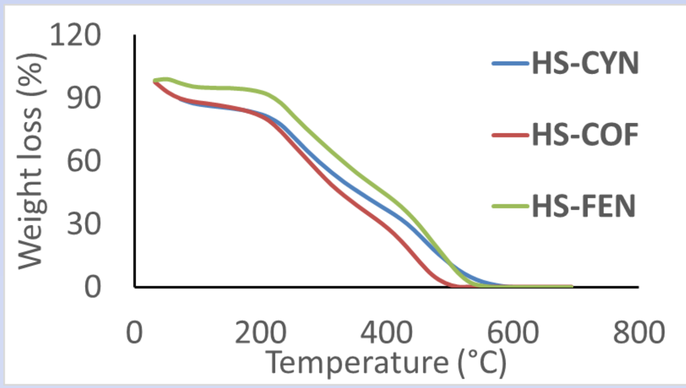


Figure 3. Weight loss (%) of NP derived from the green compost HS.

THERMOGRAVIMETRY

Nanomaterials from HS-FEN showed the largest thermal stability. Instead NP from HS-COF and HS-CYN showed similar thermal behaviour until ~200 °C, then the thermal degradation was faster for NP from HS-COF. The different thermal decompositions are likely due to the molecular differences of the HS used for NP synthesis.

NP SIZE AND ζ POTENTIAL

Nanomaterials from HS-FEN also showed the largest size (**254** nm), while the smallest was found for HS-CYN (**231** nm). The lowest ζ potential was detected for NP from HS-COF (**21.8** mV), followed by HS-CYN (**20.2** mV) and by HS-FEN (**15.5** mV).

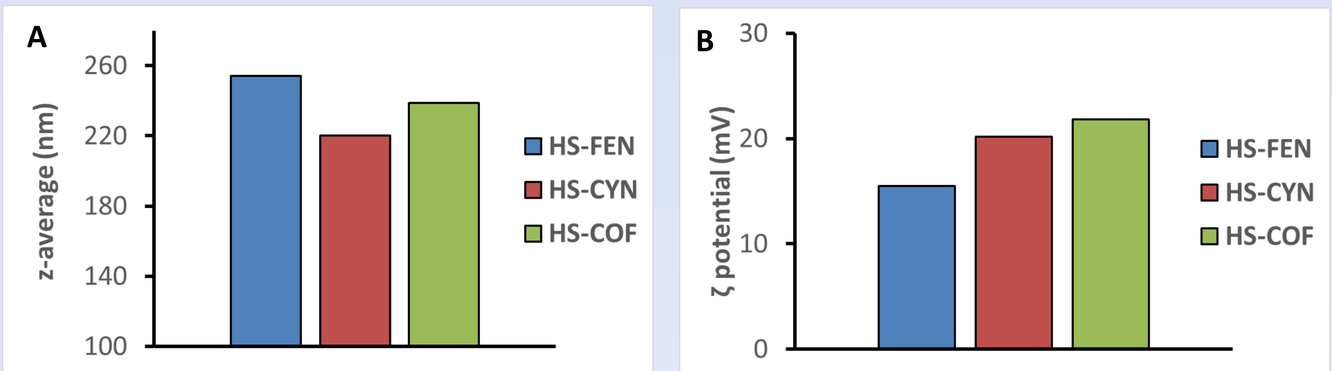


Figure 4. z-average (nm-A) and ζ potential (mV-B) of NP derived from the green compost HS.

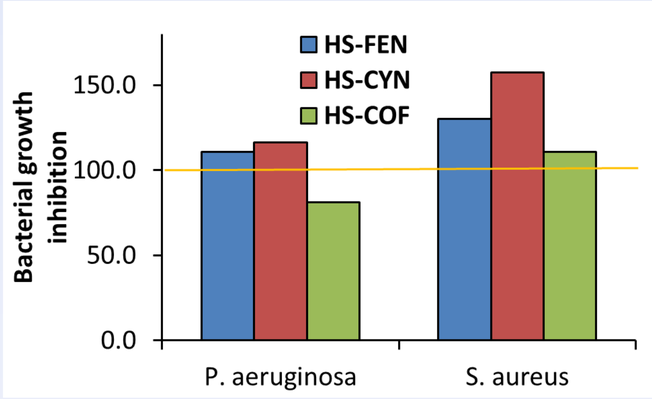


Figure 5. Antibacterial activity of HS-derived NP. Data has been calculated by setting the inhibition zone of ampicillin to 100

ANTIBACTERIAL TEST

Only NP from HS FEN and HS-CYN showed an antimicrobial effect larger than ampicillin (yellow line-inhibition zone = 100) against *P. aeruginosa*. In the case of *S. aureus*, also HS-COF induced an inhibition zone larger than for ampicillin.

CONCLUSIONS

- The antibacterial effect of HS-based nanomaterials is **species-specific** and could be due to their physical chemical features, especially their **size and ζ potential**, which are known to **influence bio-adhesion and penetration of NP** across bacterial cell walls.
- HS-derived NP could help to **reduce the dependence from more expensive and less efficient antimicrobials**.
- **More research** is needed to fine-tune the physical-chemical characteristics of HS-based NP and to extend their application to **other bacterial strains**.