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Bioactive solids obtained from montmorillonite functionalization by biogenic compounds to be used as antimicrobial filler

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ABSTRACT

The growth of fungi and bacteria on material surfaces negatively impacts human health and produces economic losses, and harmful waste for the environment. In the present research, new eco-friendly and low-cost antimicrobial fillers are actively sought by the functionalization of montmorillonite using biogenic compounds. The synthesized hybrids from montmorillonite, natural surfactant, and geraniol were characterized and showed effective antifungal and antibacterial activity.

1. Introduction

The biodeterioration of material surfaces causes serious problems, especially in indoor environments where people are predominantly exposed for longer periods to the effects of biological contaminants generated from biofilms [1]. Adding to this the waste generated from cleaning and removing the spoilage material or coating has a negative environmental impact besides economic and energy losses [2]. Therefore, efforts are made to obtain eco-friendly materials that exhibit antimicrobial activity to prevent biofilm growth, extend the service life of the materials, and prevent the transmission of infections [3]. Although these new materials show potential, further research is necessary to fully understand their effectiveness and safety over extended periods or in different environmental conditions.

The present research aimed to obtain a bioactive hybrid from montmorillonite (Mt) functionalization by biogenic compounds. For the first time, the essential oil (EO) of geranium (EG) and the terpenoid compound geraniol (GL) were evaluated for the modification of a Mt to obtain a bioactive natural filler. Geraniol acyclic monoterpene alcohol (3,7-dimethyl-2,6-octadien-1-ol) is one of the major geranium EO compounds frequently extracted from *Pelargonium* sp. [4]. This terpenoid is used commercially as an ingredient in perfumes, cosmetics, personal care products, and pharmaceuticals [4]. It is categorized by the Food and Drug Administration (FDA), recognized as safe (GRAS) for specific uses, and has been intensely studied for cancer treatments [5]. On the other hand, clay minerals (e.g. montmorillonites, halloysite, and sepiolite) can be modified to nanoscale to be used as carriers for inorganic and organic compounds [3,6].

2. Materials and methods

2.1. Antimicrobial potentialities of biogenic compounds

The EO and the terpenoid were obtained commercially from the Alfredo Francioni S. A. company (Buenos Aires, Argentina). Plates with malt extract agar (MEA) culture were prepared with different concentrations of EG and GL (0.15, 0.3, 0.6 and 1.2 mg/mL) and 20 μL of the spore suspension (10^5 spores/mL) was inoculated in the center of each plate. Fungal strains used were *Cladosporium cladosporioides* (MG731215), *Chaetomium globosum* (KU936228) and *Aspergillus versicolor* (MG725821). Plates were incubated at 28 °C and the colony diameters were measured at the end of the assay. In the case of bacteria, *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11229) were used and the reduction in the number of colony-forming units (CFU) was evaluated on Luria-Bertani Broth (LB) agar supplemented with different concentrations of EG and GL (0.15, 0.3, 0.6 and 1.2 mg/

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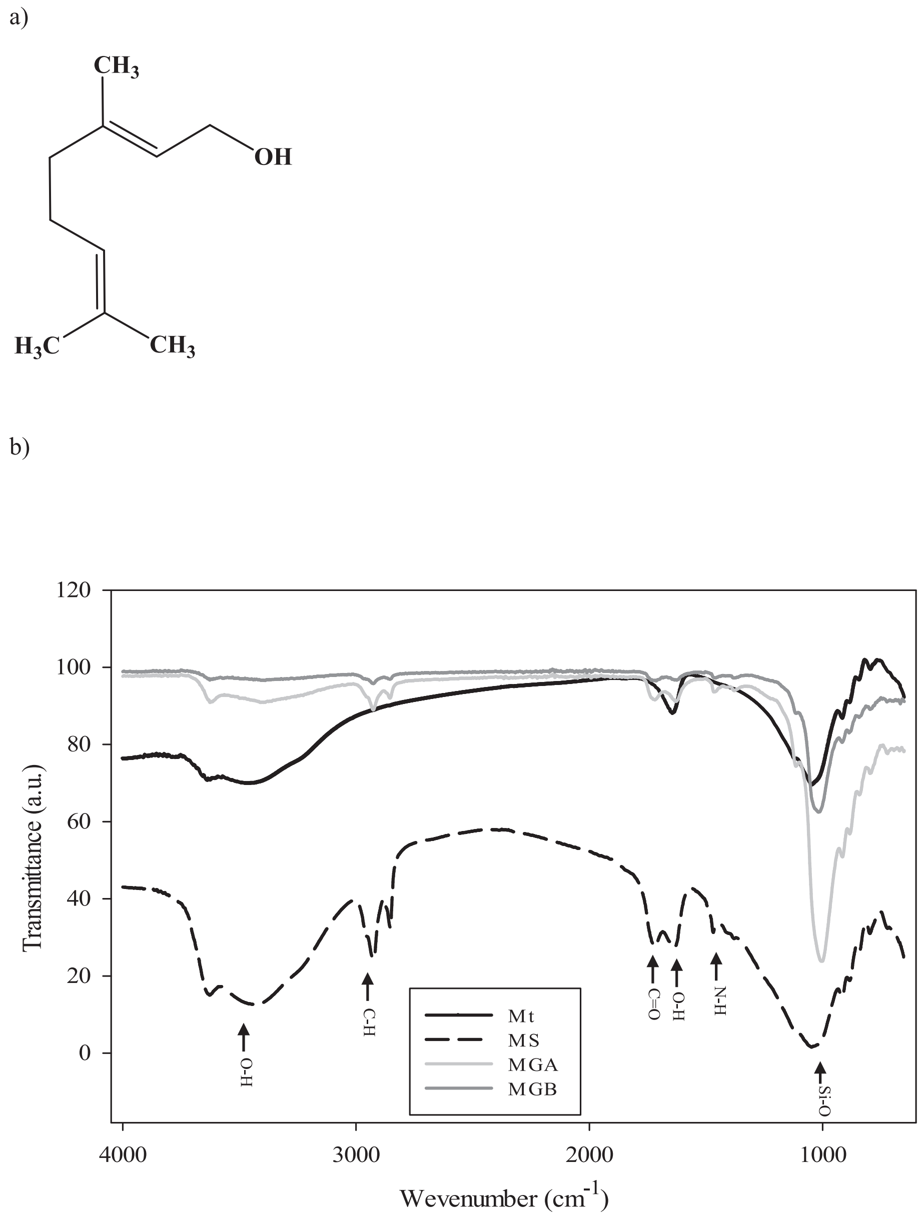

Fig. 1. A) chemical structures of the geraniol; b) ftir spectra: raw montmorillonite, mga and mgb.

mL). The plates were inoculated by spreading 100 μL containing 10^6 CFU/mL. The plates were incubated at 30 °C for 24 h. At the end of the assays, the minimum inhibitory concentration (MIC) was determined.

2.2. Mt functionalization by biogenic compounds

The Na-montmorillonite (>99 %) was from Lago Pellegrini, Río Negro, Argentina, and provided by Castiglioni Pes and Cia. The corresponding Mt structural formula is $[(Si_{3.89}\ Al_{0.11})\ (Al_{1.43}\ Fe_{0.26}\ Mg_{0.30})]\ M^{+}_{0.41}$. Total specific surface area (TSSA) = 621 m^2/g and cationic exchange capacity (CEC) = 82.5 cmol(+)/Kg. The Mt functionalization was performed following a reported procedure [6]. Clay minerals by exchange of ions can be modified to a nanoscale level to incorporate organic or inorganic compounds [7]. Soy lecithin (SL) could be used as a natural low-cost surfactant. In acid conditions, SL is positively charged due to its amino groups, this allows the Mt ion exchange [8]. The hydrophobic characteristics of organo-clay (Mt-SL) improve the adsorption of hydrophobic biogenic compounds such as terpenoids [6]. SL was dissolved in ethanol, and the pH was adjusted to 2.3 by the addition of 0.1 M HCl. SL (food quality) was commercially obtained. The bioactive biogenic compound was mixed with the SL (20 and 40 % wt). The mix was incorporated into the synthesis system with the pre-swollen Mt under constant stirring (SL:Mt ratio was 0.33:1). Finally, the functionalized Mt was separated by centrifugation and dried. The products obtained were labeled as MGA and MGB, respectively.

2.3. Characterization of the functionalized Mt

The FTIR spectra (4000–400 cm^{-1}) were performed using KBr pellet technique in a Perkin Elmer (Spectrum ONE) spectrometer.

Crystallographic analysis by XRD was carried out with a Philips 3710 diffractometer using Cu Kα radiation 40 kV, 20 mA, Ni filter and patterns collected from 3 to 12° (2θ).

Thermogravimetric (TG) analysis was carried out using a Rigaku Thermo plus EVO instrument, with alumina as a reference. The temperature was increased to 1000 °C at a constant rate of 10 °C/min.

Morphological observations, semiquantitative surface analysis, and elemental mapping were performed using a ZEISS Evo 10 scanning

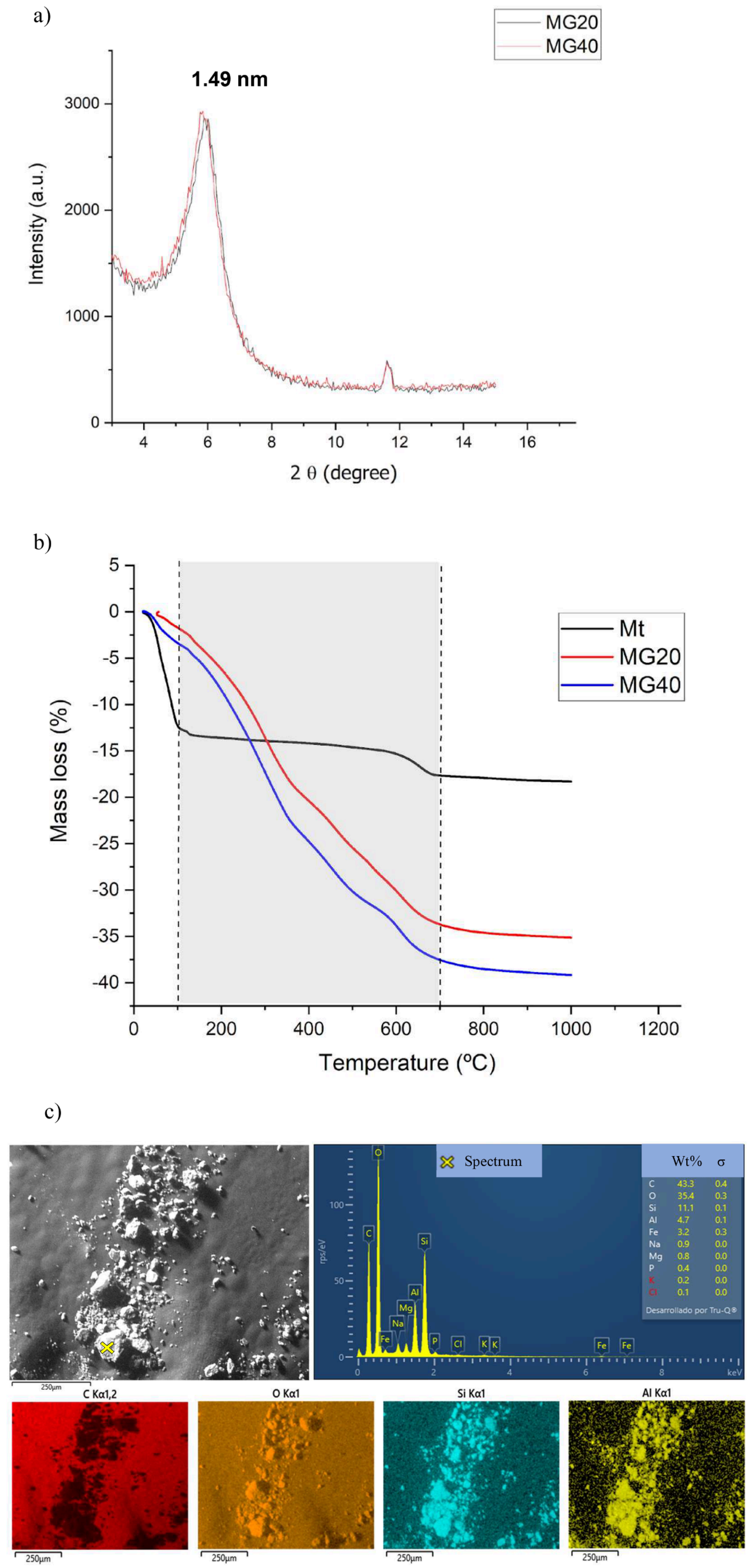

Fig. 2. A) xrd patterns: mga and mgb; b) tga: mt, mga and mgb; c) sem micrographs and eds mapping of majority elements from mgb.

Table 1
Agar well diffusion test: diameters of inhibition zone (mm).

	Control	MGA	MGB
C. cladosporioides	< 7	15.2 ± 1.2	15.0 ± 1.5
C. globosum	< 7	< 7	< 7
A. versicolor	< 7	14.8 ± 0.6	15.1 ± 0.4
S. aureous	< 7	15.7 ± 0.9	16.0 ± 0.6
E. coli	7	7	7

Note: D < 7 and = 7 indicate the negative fungal or bacterial activity, respectively.

electron microscope (SEM-EDS).

2.4. Antifungal and antibacterial activity

The antimicrobial activity was assessed by the agar well diffusion method with the same strains employed previously [6]. In the case of the fungi, 15 mL of inoculated MEA was used, and 7 mm diameter (D) wells were made on each one to be filled with 20 mg of the solids. The plates were incubated at 28 °C and after 48 h inhibition zones diameters were measured. It was considered that samples with D < 7 mm had no activity and those with D ≥ 7 mm, were active. The bacterial strains were seeded by swabbing plates with LB agar culture medium before making the wells. The plates were incubated at 30 °C and after 24 h inhibition zone diameters were registered, son that samples with D = 7 mm had no activity and those with D > 7 mm, were active. In all cases, three replicates were made.

3. Results and discussion

3.1. Antimicrobial potentialities of biogenic compounds

The antimicrobial performance of EG and GL was assessed. The MIC determined resulted in 0.3, 0.3, 0.6, 0.3 and > 1.2 mg/mL for the case of EG while that to GL was ≤ 0.15, 0.3, 0.3, ≤ 0.15 and 0.6 mg/mL related to *C. cladosporioides*, *C. globosum*, *A. versicolor*, *S. aureus* and *E. coli*, respectively. GL showed lower MIC against *C. cladosporioides*, *A. versicolor*, *S. aureus* and *E. coli* than EG. In general, GL presented higher activity against both fungal and bacterial strains. *C. cladosporioides* was the fungal strain more sensitive to the biogenic compounds studied while *S. aureus* was the more sensitive bacteria. Gram-negative bacteria, such as *E. coli*, possess an outer membrane that serves as a barrier to hydrophobic compounds. However, small molecules with polar functional groups can access the cell through porin protein transmembrane channels [9]. Consequently, Gram-negative bacteria could be more resistant to some antimicrobial agents than Gram-positive bacteria [10]. Considering these results, GL (Fig. 1a) was selected for the functionalization of the clay mineral.

3.2. Characterization of the functionalized Mt

FTIR spectra (Fig. 1b) show that once Mt was modified new peaks appeared, 2930 cm^{-1} and 2852 cm^{-1}, in MS (Mt-SL), MGA and MGB these correspond with aliphatic C—H presented in the organic compounds, SL and GL. Peaks in 1728 and 1465 cm^{-1} in the functionalized clays are related to C═O and N—H groups presented in SL. All samples showed peaks in 1640 and 1040 cm^{-1} corresponding with stretching vibration of O—H and Si—O groups, respectively from the mineral [11]. Diffractograms in Fig. 2a show reflection (0 0 1) peaks corresponding to 1.49 nm. The adsorption of the biogenic compounds in MGA and MGB expanded the interlayer space 0.22 nm because the basal spacing for the Mt used is 1.27 nm [6]. Fig. 2b illustrates the TG analysis curves of Mt and the hybrids (MGA and MGB) showed distinct stages of mass loss percentage. Mass loss is associated with the organic fraction, being more pronounced in the case of MG40.

SEM from the MGB sample shows micrometric particle size in Fig. 2c. Semiquantitative analysis revealed the presence of C, O, Si, Al, Fe, Na, Mg, and P. The presence of Si, Al, Fe, Na, and Mg corroborates the chemical structure of Mt. The C and O are related to the incorporated organic compounds (SL and GL).

3.3. Antifungal and antibacterial activity

The diameters of the inhibition zones obtained are presented in Table 1, data are expressed as mean ± SD of three experiments. The control (Mt without modification) did not show antimicrobial activity with any of the five strains tested. Photographic records can be seen in Fig. 3. Both MGA and MGB showed similar antifungal and antibacterial activity. The different structures of MGA and MGB resulted in strong activity against *C. cladosporioides*, *A. versicolor* and *S. aureus* with zones of inhibition higher than 14 mm. *C. globosum* and *E. coli* proved to be more resistant.

4. Conclusions

Bioactive fillers derived from montmorillonite and biogenic compounds can be obtained. Geraniol has demonstrated a broad spectrum of antimicrobial activity, which persists even after its incorporation into

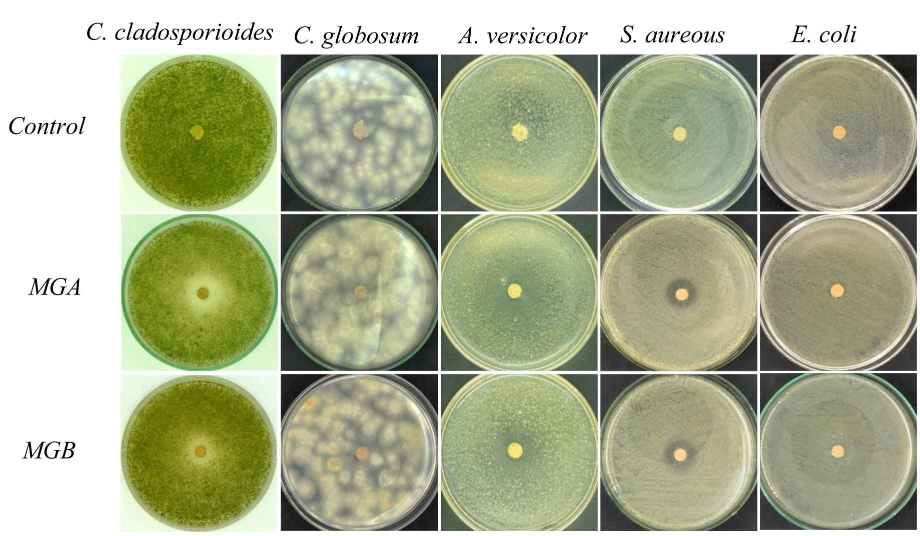

Fig. 3. Agar well diffusion test: control (Mt without modification), MGA and MGB.

the clay matrix for the first time to be used as a bioactive filler. This research highlights the viability of utilizing low-cost natural materials as carriers for environmentally friendly antimicrobial agents, offering promising applications across various fields.

CRediT authorship contribution statement

Guillermo P. Lopez: Methodology, Investigation. **Leyanet Barberia Roque:** Methodology, Investigation. **Katerine Igal:** Investigation, Writing – review & editing. **Erasmo Gámez Espinosa:** Investigation. **Mariela A. Fernández:** Investigation. **Natalia Bellotti:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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