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Ginger intercalated sodium montmorillonite nano clay: assembly, characterization, and investigation antimicrobial properties

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ABSTRACT

In this work, we have successfully incorporated ginger particles into the sodium montmorillonite (Na^+-MMT) structure. A new nanoparticles (G-MMT) were characterized using Fourier transform infrared (FT-IR) spectroscopy, ultraviolet-visible-near infrared (UV-VIS-NIR), X-ray diffraction analysis (XRD), energy-dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). Also, the antimicrobial properties of G-MMT nanoparticles were investigated using agar diffusion method. The results showed that the spherical particles of ginger were placed between the layers, and also slightly on the surface. Montmorillonite (MMT) layers, such as heat shields, protect the ginger from degradation. The results of antibacterial test showed that G-MMT inhibits 8 lethal types of gram-positive and gram-negative bacteria, as well as one type of yeast. Due to the antibacterial properties of G-MMT and the fact that ginger is protected at high temperatures, this nanoparticle can have a suitable place in various applications.

1. Introduction

Products based on non-renewable resources such as oil and coal with unsustainability feature in the long run can harm the environment [1]. Meanwhile, green chemistry can be a savior to remove these problems. Effective use of renewable resources, waste minimization, and preventing the use of toxic reagents and solvents in the manufacture of chemical products are the properties of green chemistry [2,3]. In order to this technology for being sustainable, natural resources must be used at some rates which are not able to go away in the big term, and the residues are easily biodegradable without causing pollution [4]. The use of plants, bacteria, and fungi in the preparation of nanoparticles and their application in various fields are parts of green chemistry [5-8]. Clay minerals are extensively consumed due to properties such as high surface region, high thermal resistance, low price, and availability [9-11]. Among various types of clays, MMT is the most widely used cause of its cation exchange capacity and huge porosity [12-14]. One molecule of MMT consists of an octahedral layer of magnesium or aluminum hydroxide, which is located between the two tetrahedral layer silica [15-17]. MMTs have hydrophilic properties due to the presence of hydrated mineral cations on the surface [18-20]. The modification of the MMT surface by organic matter causes the MMT to change from a hydrophilic to an organophilic / hydrophobic, resulting in an increase in *d*-spacing [21,22]. MMT nanoparticles due to important and bold features like high thermal performance, chemical

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resistance, excellent mechanical strength and resistance to abrasion and corrosion, have many applications in the food packaging industry, polymer composites, surface coatings, and drug delivery [23-29]. In recent years, the increasing resistance of bacterial infectious agents to antibiotic treatment has raised many concerns to these infectious agents in the future [30,31]. Bacteria are resistant to antibiotics through a variety of mechanisms, if they survive in the presence of antibiotics, with a high rate of proliferation can improve their resistance mechanism [32-35]. Given the above, it is important to find new ways to overcome the increasing resistance of pathogens. In recent years, amazing advances in medicinal plants have led researchers to use them to combat pathogenic microorganisms [36-38]. Ginger (Zingiber officinale), Zingiberaceae family, named as a famous spice that is applied widely particulary in most of the Asian countries like Iran, South Korea and etc [39]. Main chemical analysis of ginger demonstrates that it includes more than 400 various compounds. Most of constituents in ginger herb rhizomes include carbohydrates (50-70%), lipids (3-8%), terpenes, and phenolic compounds [40]. Terpene components of ginger are *zingiberene*, *β-bisabolene*, *α-farnesene*, *β-sesquiphellandrene*, and *α-curcumene*, while phenolic compounds contain gingerol, paradols, and shogaol. Gingerols (23-25%) and shogaol (18-25%) are shown in upper quantity compare as other ones. Other items such as; amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also discovered [41,42]. Ginger is a kind of natural anti-radical and anti-cancer dietary mixture. Also, ginger has been using in local remedies for many years cause of its antibacterial effect [43,44]. In this research, we have used an easy way to insert ginger particles into the space between the Na^+-MMT layers. The results completely confirm the placement of ginger particles in the interlayer space. One of the unique properties of these new nanoparticles is their high thermal stability. Antibacterial results also showed that these nanoparticles can be very effective against 8 types of gram-positive and gram-negative bacteria and one type of yeast.

2. Results and Discussion

FTIR spectra of samples are illustrated in Figure 1. In Na^+-MMT spectrum, peaks appearing at 456 and 1044 cm^{-1} is related to the bending and stretching Si-O vibration [45,46]. The peaks at around 525, 621, 799, and 917 cm^{-1} are corresponding to the Si-O-Al vibration and MgO groups, Mg-O-Si or Fe-O-Si groups, AlMgOH vibration groups, and Al_2OH bending groups, respectively [45-47]. Also, peaks appearing at 1638-3448 cm^{-1} are attributed to scissoring vibrations and symmetric vibrations of OH units [45,48,49]. Moreover, peak at around 3632 cm^{-1} is corresponding to Stretching of OH (SiOH groups) [45,46,50]. In ginger spectrum, peaks at around 583, 777, 899, 1139, 1337, 1458, 2970-2983, 3218, 3254, 3705, and 3752 cm^{-1} are corresponding to the pyridine ring vibration, H stretching, C-H stretching vibration, CH_3 asymmetric stretching vibrations (pectin groups), C-N stretching vibration, aromatic skeletal combined with C-H in-plane deforming and stretching, C-H stretching vibrations (6-gingerol, 8-gingerol, and 10-gingerol), C-H stretching vibrations (6-shogoal), OH stretching vibration (lignin groups), OH stretching vibration (10-gingerol), and OH stretching vibration related to the 6-shogoal, respectively [47,51-54]. Absorption peak that appeared at 1279 cm^{-1} is characteristic peak of C-O vibrations (lignin groups) [55-57]. In addition, peaks appearing at 1783 and 1798 cm^{-1} are attributed to C=O stretching related to the 6-shogoal and gingerol groups (6-gingerol, 8-gingerol, and 10-gingerol), respectively [54]. Furthermore, peaks at around 2121, 2851, and 2863 cm^{-1} are attributed to CH_2 asymmetric stretching vibrations related to cellulose and hemicellulose [47,51]. Finally, in G-MMT spectrum, peaks at around 502-638, 885, 979-1008, 1169-1336, 1783, 1799, 2157-2855, 2972-2984, 3216, 3226-3631, and 3752 cm^{-1} are corresponding to Si-O-Al vibration and MgO groups, AlMgOH vibration groups, Al_2OH groups, Si-O in-plane stretching vibrations, C=O stretching (6-shogoal), C=O stretching (gingerol groups), CH_2 asymmetric stretching vibrations (cellulose and hemicellulose), C-H stretching vibrations (6-gingerol, 8-gingerol, and 10-gingerol), C-H stretching vibrations (6-shogoal), OH stretching vibration (lignin groups), and OH stretching vibration (6-shogoal), respectively [45,46,50-54].

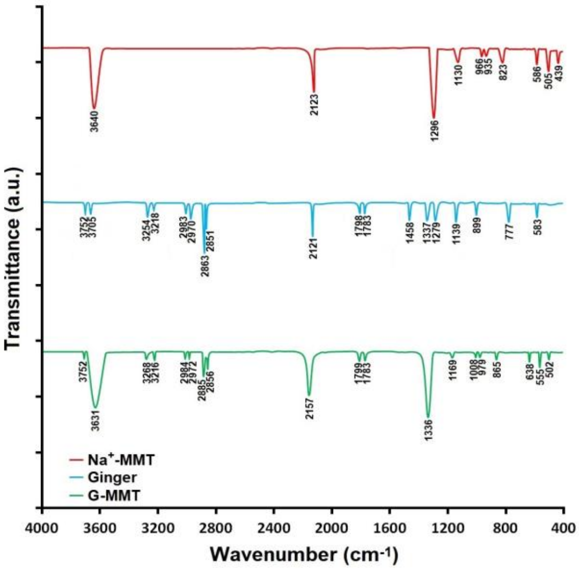

Figure 1. FT-IR spectra of Na^+-MMT, Ginger, and G-MMT.

Ginger, Na^+-MMT, and G-MMT were characterized by UV-VIS-NIR spectroscopy (Figure 2). In ginger spectrum, peak at 280 nm is corresponding to the cellulose, hemicellulose, lignin, and pectin groups [58-61]. Moreover, peak observed at 225 nm for

Na^+-MMT assigned to the charge transfer transition of Fe-OH group [50]. In addition, G-MMT showed two peaks at 225 and 278 nm, indicating the presence of ginger in structure.

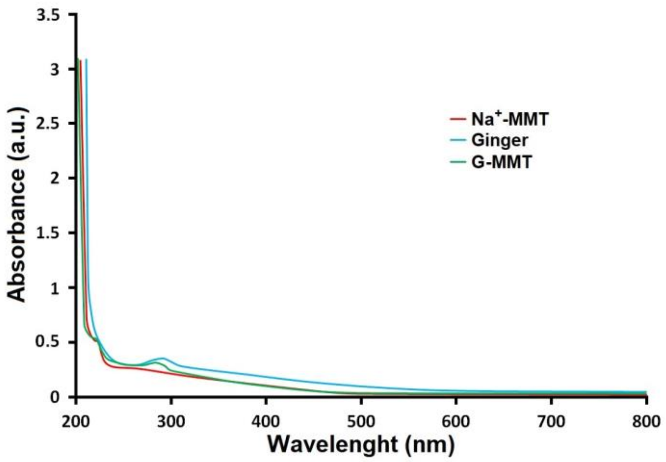

Figure 2. UV-VIS-NIR spectra of Na^+-MMT, Ginger, and G-MMT.

XRD results of the Na^+-MMT and G-MMT samples are presented in Figure 3. The peak with a center at 2θ = 7.7° (*d*-spacing = 11.37 Å) in the XRD pattern of Na+-MMT is due to Na^+ cations [62]. Compared with the Na^+-MMT, the d-spacing value of G-MMT sample increased to 16.76 Å. The existence of ginger in the gallery space of clay structure increased the MMT interlayer space. But, the observed diffraction for G-MMT at around 6° was detected. In fact, this diffraction change is due to the lack of Na^+ cations and the presence of new intercalated moieties between ginger and MMT.

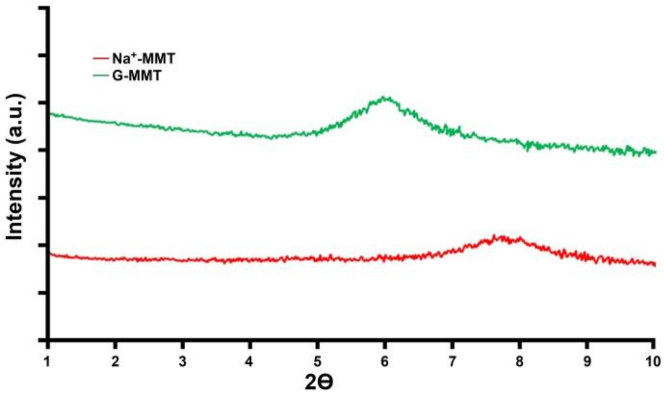

Figure 3. XRD patterns of Na^+-MMT and G-MMT.

EDX analysis was employed to investigate the elementals before and after modification as shown in Figure 4. There are a total of 115 polyphenolic components in ginger [63-65]. All types of ginger contain moisture, ash, protein, fat, fiber, neutral detergent fiber, and acid detergent fiber, but the amount of their presence depends on many factors such as the type of ginger and storage conditions [65]. Also, the cell wall consists of two types, primary cell walls that contain cellulose (9-25%), hemicellulose (25-50%), and pectin (10-35%) and secondary cell walls contain cellulose (40-80%), hemicellulose (10-40%), and lignin (5-25%) [51,64,65]. But, the cell wall is partly destroyed by grinding. As shown in Figure 4, Na^+-MMT lacks carbon (C) and sulfur (S). It is clearly observed that after modification, C and S have been added to the structure and the content of the other elements has been changed. The results confirm the success of the modification and show that ginger is present in the MMT structure.

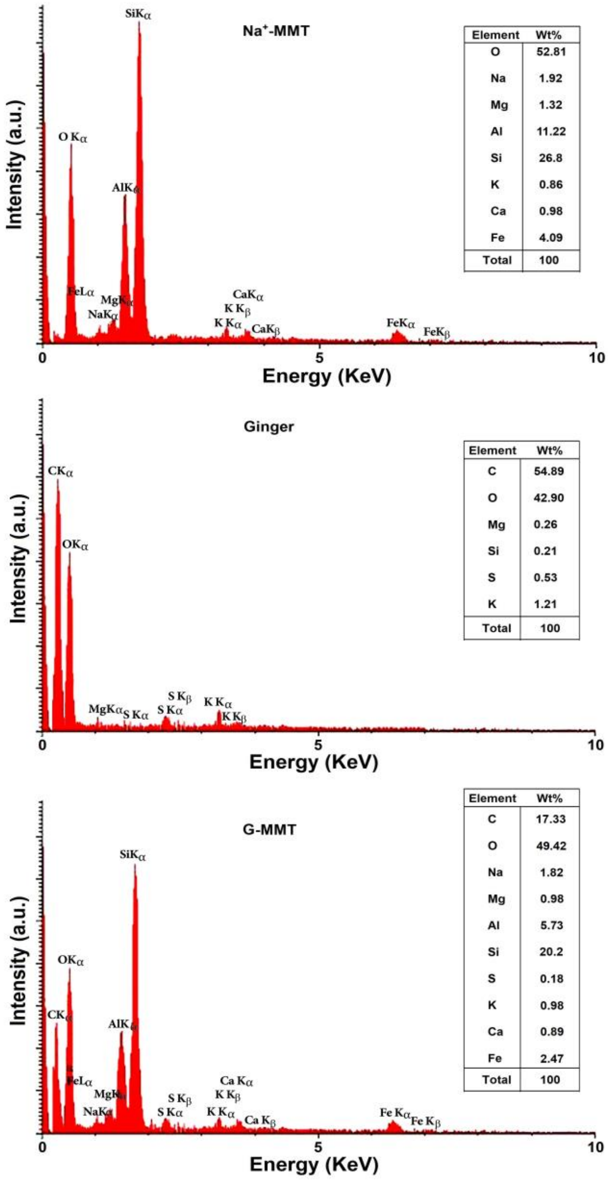

Figure 4. Elemental analysis of Na^+-MMT, Ginger, and G-MMT using EDX.

Figure 5 represents SEM and TEM observations of Na^+-MMT, as well as G-MMT, which were used to investigate the position of ginger in nanoparticles. As the SEM images have shown, Na^+-MMT has a plate structure, but after modification, the structure is almost different, which is due to the presence of ginger in the interlayer space and to some extent outside the interlayer space of Na^+-MMT. This added structure appears (indicated by the arrow in Figure 5) to be related to the destroyed cell wall of ginger, which contains cellulose and hemicellulose [66]. Also, TEM images clearly show that ginger particles, which are almost spherical, are located between the MMT plates. TEM and SEM images and XRD results clearly confirm the desired product (G-MMT).

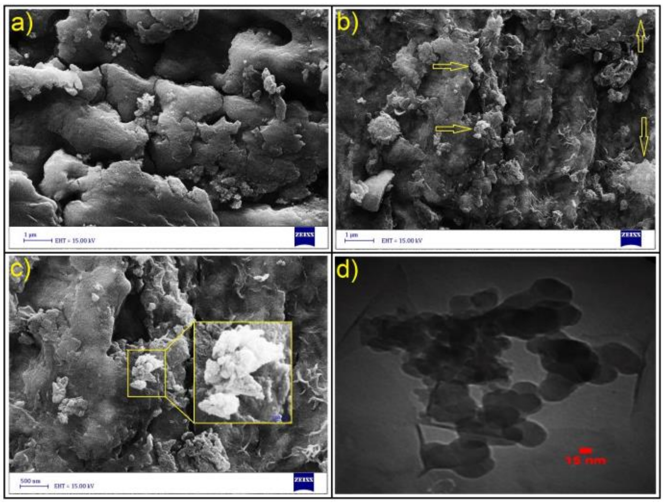

Figure 5. SEM and TEM images of samples: (a) Na^+-MMT, (b), (c), and (d) G-MMT

TGA of Na^+-MMT, ginger, and G-MMT give further evidence regarding the presence and content of ginger on MMT, the results are summarized in Table 1 and Figure 6.

Table 1. Degradation behavior of Na^+-MMT, Ginger, and G-MMT.

Sample	Weight loss[%] at Temperature [ºC]			Total Weight loss [%]
	30-200	200-450	450-700	
Na^+-MMT	8.3	0.9	1.2	10.4
Ginger	19.3	67.8	10.3	97.4
G-MMT	6.6	10.1	0.6	17.3

As the results show, Na^+-MMT exhibit only a slight weight loss, which is due to dehydration inside the interlayer space and dehydroxylation of inorganic of clay [67-71]. However, ginger is almost completely degradation, which can be related to moisture loss, decomposition of organic material, and oxidation of the organic matter, respectively [72]. The results for G-MMT compared to Na^+-MMT show that 7.1 *wt*.% more weight loss. Based on the results of the EDX analysis, TEM, and SEM it can be said that this is 7.1 *wt*.% which is related to those parts where the ginger is placed on the surface of the clay and is exposed to heat and is degradation. But the rest of the ginger is between the plates, and the clay acts as a heat shield to protect the ginger from degradation. This heat resistance creates the golden opportunity for G-MMT to be used in a variety of fields, for example in the process of polymers, which is often done at high temperatures, ginger is not degraded and is present in nanocomposite.

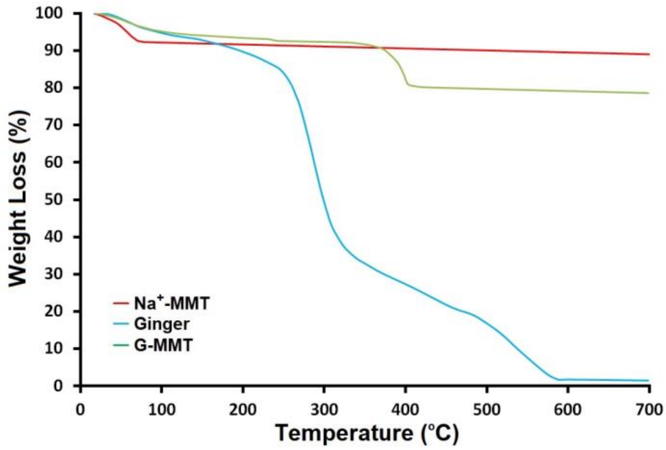

Figure 6. TGA curves of Na^+-MMT, Ginger, and G-MMT.

Ginger release in neutral, acidic, and alkaline solutions was investigated by UV spectroscopy at 280 nm wavelength and release profiles at different pH values are shown in Figure 7. Ginger release occurs in approximately three stages with different slopes. As can be seen in the first stage (start time up to 3 h) the release is wonderfully high, which is mainly related to the part of the ginger that is placed on the MMT surface. The beginning of the second stage at different pH values is same (3 h), but the ending is various. This issue for alkaline solution is 24 h and for neutral and acidic solutions is 30 h. In fact, at this stage, the solution penetrates the MMT layers and the ginger is released. In the third stage, the concentration of ginger probably reaches equilibrium, and for this reason, the release profiles have stabilized.

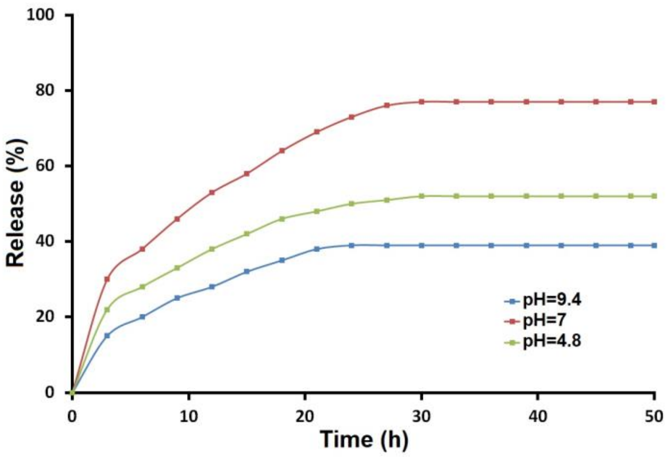

Figure 7. Release diagram of ginger at different pH values.

Ginger has long been used as a medicinal plant for various human diseases such as nausea, colds, dyspepsia, fever, and rheumatism [73]. Ginger contains many compounds that have antioxidant, anti-inflammatory, antidiabetic, and anticancer effects. Among them, the contents of 6-, 8-, and 10-gingerol, and 6-shogaol, which are related to antibacterial and anticancer activities, are different in different types of ginger [74]. Also, the amount of these contents in fresh, dried, extract, and steamed ginger is different [73]. It is usually highest in fresh ginger and lowest in extracts. The quality of the extract is related to the extraction method, pressure, and temperature. In extraction usually uses high temperatures, which can reduce or eliminate 6-, 8- and 10-gingerol [74]. However, it has been reported in the literature that the amount of 6-shogaol, on which the anti-cancer properties of ginger depends, increases greatly with steamed ginger (120 °C for 4 h), but the amount of 6-, 8-, and 10-gingerol decreases [73]. This method can be used to treat cancer. Some researches on the treatment of ginger on ovarian cancer cells in vitro showed the inhibition in growth of cells positively by 6-Shogaol and also inhibition of NF-κB (necrosis factor kappa B) activation and decreases VEGF (vascular endothelial growth factor) and IL-8 secretion (IL8 or chemokine (C-X-C motif) ligand 8, CXCL8) [75,76]. Ginger elements modulate secretion of angiogenic agents in ovarian cancer cells in vitro and perform as potent chemopreventive dietary candidate [77]. Regard to anticancer effects, ginger and its contents have been known to protect or ban the proliferation of and induce apoptosis of a kind of cancer cell types in vitro [78,79]. Increasingly, using ginger as chemo preventer of colorectal cancer has been noted [80,81]. But for antibacterial properties, 6-, 8-, and 10-gingerol is much more important [73,82,83]. Moreover, ginger contains chemical compounds such as saponin, alkanoids, and flavonoids that have antifungal activity [84,85]. Plant extracts are subject to degradation and decomposition in storage, as well as a decrease in the decrease in potency of ginger extract upon storage has been reported, and this has been attributed to the volatile nature of the active principles in ginger [86]. Therefore, the contents of 6-, 8-, and 10-gingerol as well as the stability of ginger properties are very important points. In this study, unlike other methods, we used dried ginger to achieve the highest levels of gingerol compounds. The antimicrobial properties of G-MMT nanoparticles against 8 types of bacteria (gram-positive and gram-negative), 1 type of fungus and 1 type of yeast were investigated using agar diffusion method, determination of tiny growth inhibitory concentration (MIC) and determination of minimum bacterial lethal concentration (MBC) has been done. Also, their effectiveness was compared with the antibiotics rifampin, gentamicin, and nystatin. Antibacterial results are shown in Tables 2 and 3. The results showed that G-MMT has the ability to inhibit the lethal yeast and all bacteria. It just has no effects on the fungus. Gram-negative bacteria have a multi-layered and very complex structure, but gram-positive bacteria have a simpler membrane. Past researches express that the antibacterial influence of essential oils have remarkable varieties mentioned to the collection area, genetics and environmental features of the herb, and extract ways, as useful as remarkable varieties in the banning of Gram-positive and Gram-negative bacteria. As we know, Gram-positive strains are more reactive and responsive, leading to that the cell wall contained of a thick layer of peptidoglycan surrounding the cytoplasmic membrane could be the microbial goal in some herbs [87]. Hence, there are many reports that ginger compounds have an effect on gram-positive bacteria and gram-negative bacteria are resistant to ginger [74,82,88].

Table 2. Antimicrobial activity of G-MMT.

Type of microorganism	G-MMT		
	MIC	MBC	DD[a]
ATCC 6633	62.50	62.50	9
ATCC 29737	250	1000	16
CIP 81.55	125	1000	19
ATCC 19615	31.25	31.25	15
ATCC 25922	62.50	250	10
ATCC 27853	< 15.63	125	24
ATCC 5702	31.25	500	10
PTCC 1188	62.50	500	17
ATCC 10231	< 15.63	< 15.63	8
ATCC 9029	-	-	-[b]

[a]DD (Disk diffusion method), inhibition zones in diameter (mm) around the impregnated disk

[b]A dash (-) depict no antimicrobial activity

Note: Concentrations of MIC and MBC as µg/mL

This may be due in part to the use of the ginger extract, which contains smaller amounts of gingerol compounds. Our results show that dried ginger has an effect on both groups of bacteria and even more on gram-negative bacteria. It can also be said that due to the dual nature of G-MMT, due to the hydrophilicity of Na^+-MMT and the hydrophobicity of ginger, G-MMT can penetrate the hydrophilic cell wall containing lipopolysaccharide and inhibit gram-negative bacteria, while ginger due to hydrophobicity cannot penetrate well into the multilayer membrane [89]. The transcriptomic analysis led to the some researches demonstrated 6-gingerol at 50 µg/mL obtained in similar differences in global gene expression as ones induced by 6-shogaol at 10 µg/mL, that shows 6-gingerol and 6-shogaol play a role at the transcriptional level. According to literature, the fundamental variety between 6-gingerol and 6-shogal effects the acts of these to effect the expressions of hyphae-regulatory genes in the hyphae signaling pathway [90]. However, as we expressed that, depending on the place, the oil tested shows a nicer influence on bactria, led to other microbial targets, such as the plasma membrane, since the constituents of essential oils of herbs especially ginger have lipophilic features that interact with membranes by variation of their fluidity and permeability [91]. Due to the antibacterial and thermal results, G-MMT can be used in coatings, nanocomposites, and sanitary ware such as toothbrushes. The unique properties of clay, such as improving its mechanical properties and increasing its thermal resistance, as well as the antibacterial properties of ginger, can create a new perspective for bacterial-resistant products. The use of antibacterial coatings in hospitals or public lavatory can greatly contribute to human health. Also, G-MMT can be used as an improver of mechanical and thermal properties and to create antibacterial properties in wood-plastic composites.

Table 3. Results of antimicrobial activity of antibiotics on microorganisms.

Type of microorganism		Rifampin		Gentamicin		Nystatin	
		MIC	DD	MIC	DD	MIC	DD
ATCC	6633	31.25	19	3.90	30	NA[a]	NA
ATCC	29737	31.25	21	1.95	27	NA	NA
CIP	81.55	1.95	27	1.95	45	NA	NA
ATCC	19615	7.81	15	3.90	31	NA	NA
ATCC	25922	3.90	11	3.90	20	NA	NA
ATCC	27853	31.25	-	7.87	20	NA	NA
ATCC	5702	15.63	8	3.90	18	NA	NA
PTCC	1188	15.36	9	3.90	17	NA	NA
ATCC	10231	NA	NA	NA	NA	125	33
ATCC	9029	NA	NA	NA	NA	31.2	27

[a] Not applicable

3. Experimental

3.1. General

The inorganic clay was Na^+-MMT were obtained from Rockwood Company (USA). Moreover, Ginger powder was purchased from local markets (Rasht city, Iran) and all other solvents and chemicals were purchased from Merck Chemicals Co. and used as received. FTIR spectra of the samples were recorded on a Bruker, Equinox 55 spectrometer. UV-VIS-NIR absorption spectra (190-1100 nm, resolution: 0.5 nm) were recorded on a Hanon double beam spectrophotometer. XRD measurements were carried out using a JEOL GSM 20A diffractometer using CuKα as the radiation source with a scan rate of 4 °C/min. EDX also was carried out on a JSM-6360LV instrument. TEM images was provided using a Philips CM10 (Netherlands) electron microscope. SEM was performed on a sigma VP-500 instrument (Germany'S ZEISS Company). In SEM test, the mentioned samples were covered with a thin layer of gold and prepared through sputtering method. TGA analysis was performed using a PL-STA-1500 thermal analysis unit equipped with differential thermal analysis. The experiments were carried out in a nitrogen atmosphere at a heating rate of 10 ºC/min.

3.2. Modification of Na+-MMT by ginger

Na^+-MMT swelling was done according to the procedure described in the literature [29,32]. Briefly, Na^+-MMT (5g) was stirred in distilled water (350 mL) at room temperature for 24 h. Then, Ginger powder (2g) was stirred in distilled water (100 mL) at room temperature for 1 h and added to the previous mixture. The mixture of Na^+-MMT and ginger was stirred at room temperature for 24 h, then the mixture was rested for 24 h. Using a centrifuge for 15 minutes at 6000 rpm, the G-MMT was removed from the solution and rinsed with distilled water. Finally, product was vacuum-dried at 30 °C for 24 h.

3.3. Antimicrobial test

The antimicrobial properties of G-MMT nanoparticles against 8 types of bacteria, 1 type of fungus and 1 type of yeast were investigated using agar diffusion method, determination of minimum growth inhibitory concentration (MIC) and determination of minimum bacterial lethal concentration (MBC) have been done. In order to reduce the error, each test was repeated 3 times and the diameter of the aura of non-growth measurement and their antimicrobial ability were compared with 3 common antibiotics such as gentamicin, rifampin and nystatin. This method has been done according to CLSI standards. For this purpose, plates containing Mueller Hinton Agar culture medium were prepared, wells with a diameter of 0.6 mm were created on the culture medium, and then 0.100 µl of bacterial suspensions with a turbidity equivalent to half a McFarland in uniform conditions at the culture medium has been followed. G-MMT nanoparticles dissolved in dimethyl sulfoxide to a concentration of 0.30 mg/ml. Microorganisms were determined by measuring the aura of non-growth. In this test, 10 microorganisms were used to evaluate the antimicrobial activity of the extract, which are shown separately in gram-positive, gram-negative, fungal and yeast bacteria in Table 1. In this method, the minimum growth inhibitory concentration for microorganisms' sensitivity to G-MMT was calculated by microdilution method. For this purpose, micro-pages of 96 sterile houses were prepared. Each page adds 95 microliters of culture medium, 5 microliters of bacterial suspension with 0.5 mA McFarland dilution and 100 µl of different G-MMT dilutions (starting MIC dilutions from 1000 micrograms per liter 7 and each dilution is half the previous competition). Then, the plate was heated in an incubator at 37 °C for 24 h. To determine the minimum bacterial lethality test, after 24 h of heating, 5 microliters of each microplate well in which there was no growth were inoculated into the agar neutrino medium and heated for 24 h at 37 °C.

Table 4. Microorganisms used to evaluate the antimicrobial activity of G-MMT.

Yeasts	Fungus	Gram-negative bacteria	Gram-positive bacteria
***Candida Albicans* (ATCC 10231)**	*Aspergillus niger* (ATCC 9029)	*Escherichia coli* (ATCC 25922)	*Bacillus subtilis* (ATCC 6633)
		Pseudomonas aeruginosa (ATCC 27853)	*Staphylococcus aureus* (ATCC 29737)
		Salmonella paratyphi-A serotype (ATCC 5702)	*Staphylococcus epidermidis* (CIP 81.55)
		Shigella dysenteriae (PTCC 1188)	*Streptococcus pyogenes* (ATCC 19615)

4. Conclusion

In summary, unlike the usual use of plants extract, we have been able to successfully incorporate ginger particles into the structure of Na^+-MMT. The results of XRD, TEM, and SEM showed that in addition to the fact that ginger has entered the Na^+-MMT layers, some have also been placed on the surface. The G-MMT has very good thermal stability. Antibacterial results showed that G-MMT can be very effective. Considering all the properties of G-MMT, it can be said that it is a suitable candidate for use in various fields. Using a combination of several natural material such as ginger, garlic and cinnamon can be attractive and achieve better results, which will be discussed in the following studies.

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