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Antibacterial Properties and Synthesis of Organoclay with Goji Berry

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Abstract

As it is known, organoclays are used in many fields such as cement, brick, tile, structural tile, floor tile, tiles, sanitary ware, filling, drilling, coatings, pottery, pottery, tile, glass, porcelain, electro porcelain, refractory industry, casting industry, cosmetics, bone cement and implants and food packaging. The antibacterial properties of organoclays, which can be used for multiple purposes, are of great importance.

In this study, Na^+-montmorillonite clay (Mt) with goji berry extract has been modified. Firstly, the extracted taurine was analyzed by high-performance liquid chromatography (HPLC), and the antioxidant effect was examined to antioxidant effect using the 2,2-diphenyl-1-pcyrylhydrazyl (DPPH) method. The modified organoclay (Mt_1) was analyzed by using the methods of X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The biocidal activities were analyzed by using agar well diffusion method against the bacteria *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus).*

Keywords: Organoclay; Taurine Extract; Antioxidant Effect; Antibacterial Properties; Characterization

Introduction

Clays formed by the separation of aluminum and magnesium-rich volcanic ash and lava and containing dominant Montmorillonite are called bentonite. In commercial terms, every clay with advanced liquid absorbent and colloidal feature is called bentonite. The structure of clay minerals consists of two types of atomic crystals. One of them is octahedral structure and the other is tetrahedral structure. The octahedral structure is a model of a cation (aluminum, iron or magnesium) atom, which is well-packed between two layers of three oxygen or hydroxyl. This building unit is called gibsit. The other structural unit (silica) is a tetrahedral structure and a cyclone atom is placed in the middle of a smooth quadruplet with oxygen atoms at the edges and it is expressed as SiO_2. Structures of clay minerals; It is formed by overlapping the knitting layers formed by these basic units with different combinations. In places where clays are used, ion exchange properties and exchange reactions are of great importance in clays. The growth of plants in the agricultural field, the formation of clay formations in geology, production areas where clay is used as raw material are related to this subject [1,2].

In this study, taurine extract of goji berry fruit was obtained. Then, the antioxidant effect of extracted taurine was investigated by DPPH method. The montmorillonite clay was then modified with taurine extract using the ion exchange method. The organoclays synthesized were characterized by FTIR and XRD and their antibacterial properties were examined agains bacteria *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus).*

Materials and Methods

Materials

Chemicals of high purity were obtained from various commercial sources, which consisted of methanol (Merck), trolox (Merck), 2, 2-diphenyl-1-pcyrylhydrazyl (DPPH) (Merck), ethanol (Merck). Na^+-Montmorillonite (Mt) was provided by Southern Clay Products Inc. (U.S.A.). Goji berry has been purchased from local producers in Turkey.

Materials

Synthesis of Goji Berry Extract (Taurine): Goji berry fruit was established by incubating homogeneously at room temperature (25 °C) for 24 h. It was then ground and ground into powder. The resulting powder sample (10 g) was mixed with 100 mL of methanol. The mixture was made homogeneous by stirring in a magnetic stirrer for 60 min, and the stock solution was prepared by ultrasonic bath for 15 min and then the samples were centrifuged at 3500 rpm for 7 min. Extraction was made with methanol

and taurine extract was obtained in goji berry fruit. The taurine extract obtained was combined with high-performance liquid chromatography (HPLC) analysis. Two extracts were obtained by 50% dilution before analysis [3].

Synthesis of Organoclay (Mt_1): Na^+-Mt (2. 0 g) was mechanically stirred with 200 mL of deionized water-ethanol v/v (1:1) at 25 °C for 3 h. Then, taurine extract (10 mL) was added to this solution and the mixture was stirred at 25 °C for 24 h under mechanically stirred. The mixture was filtered on filter paper after 24 hours. The final product obtained by filtration was dried at 70 °C for 8 h [2].

Characterization: Infrared spectra were recorded as KBr pellets in the range 4000 - 400 cm^{-1} on an ATI UNICAM systems 2000 Fourier transform spectrometer. The sample was analyzed by X-ray diffraction (XRD) for the crystal structure. Taurine extracts were analyzed by HPLC-DAD-UV-Vis. Acetonitrile and 0. 1% trichloroacetic acid were used as mobile phase. Flow rate was 1. 0 mL / min the temperature during the analysis was maintained at 30 °C, Injection volume was 20 microliters, PDA detector wavelength was 470 nm, Emission wavelength was 530 nm.

Determination of Antioxidant Capacity of Taurine Extract: The DPPH method was used for the antioxidant assays. The assays were made by studying two parallel tests. In this method, the solutions of DPPH and Trolox were prepared. For the DPPH solution, 0.0023 g of DPPH was weighed and completed with methanol in a volumetric flask (100 mL). For the Trolox solution, 10 µM Trolox at a weight of 0.0625 g was weighed and completed with methanol in a volumetric flask (25 mL). Besides these, a control solution was also prepared. For this, stock DPPH solution at a volume of 3.9 mL and 0.1 mL of methanol were added into a test tube. To prepare for the plot of the calibration curve, the stock Trolox solution was diluted at the concentrations of 0.2 µM, 0.25 µM, 0.4 µM, 0.5 µM and 0.7 µM. To plot a calibration curve, 100 µL of the solution was transferred from each of six test tubes in total with Trolox solutions including the stock Trolox solution into six separate test tubes. 3.9 mL of the stock DPPH solution was added into each of these new tubes, and a homogeneous mixture was obtained using Vortex. The control solution and these preparates were kept in darkness for 60 min. The absorbance values were read at 517 nm in the UV spectrophotometer, and the calibration curve was plotted. For the extracts, 1.5 mL of each extract and 3.9 mL of stock DPPH solution were added into test tubes. The preparates were kept in darkness, and their absorbance values were read at 470 and 530 nm in the UV spectrophotometer. Antioxidant values (%) were computed by applying these absorbance values to the following equation 2 [4,5].

$$\% \text{ Antioxidant activity} = \frac{\text{control - absorbance}}{\text{üüü}} \text{ x } 100 \qquad (1)$$

Antibacterial Assays

This study used the well diffusion method for antibacterial analyses. The bacteria S. Aureus and E.coli were inoculated onto Nutrient agar and incubated at 37 °C for 24h in an aerobic setting. The cells were spread in the same medium, and after 24h, whether or not the samples specimen created inhibition zones around the disk [6].

Results and Discussions

Antioxidant Assays

Trolox solution was used as a reference solution for antioxidant assays. The control absorbance value is 0.320 for measurements made at 470 nm; for measurements made at 530 nm, it is taken as 0.363 nm. The calibration curve of Trolox solution and its absorbance values and concentration levels are illustrated in Figure 1. The antioxidant activity values (%) of the specimens are presented in Table 1.

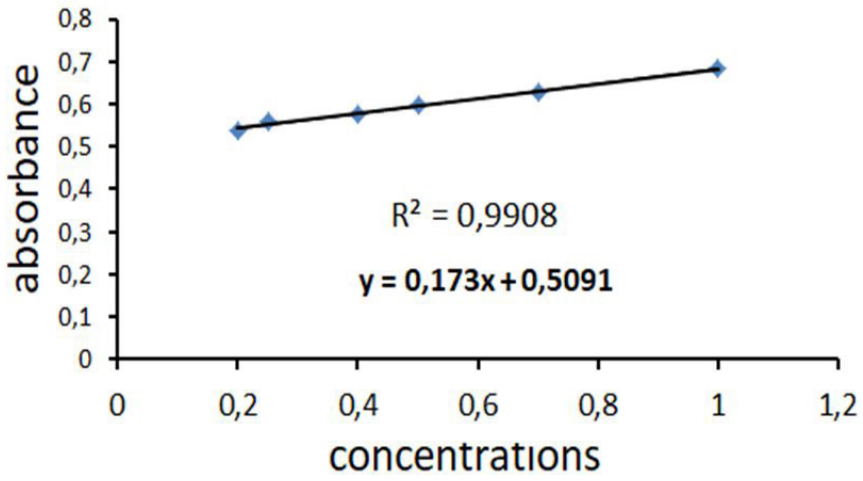

Figure 1: Trolox calibration equation curve

	470 nm	530 nm
Stock extract	%23,125	%20,3857
Diluted extract (50%)	%34,0625	%28,3747

Table 1: Antioxidant activity values of taurine extract

According to the results of the analysis, diluted extracts showed higher antioxidant capacity. In addition, the measurement results obtained at 470 nm appear to have a higher antioxidant effect.

HPLC Assays

The taurine antioxidant values obtained using the DPPH method were compared with the HPLC analysis results. According to HPLC analysis resultsTaurine supplements have also been shown to prevent oxidative stress induced by exercise (Figure 2). Therefore, the high taurine content of purified antioxidant substance is considered to contribute to the high radical scavenging activity of antioxidants. The peaks seen in the HPLC spectrum around 5.0-5.5 min and 8 min prove the presence of taurine [7].

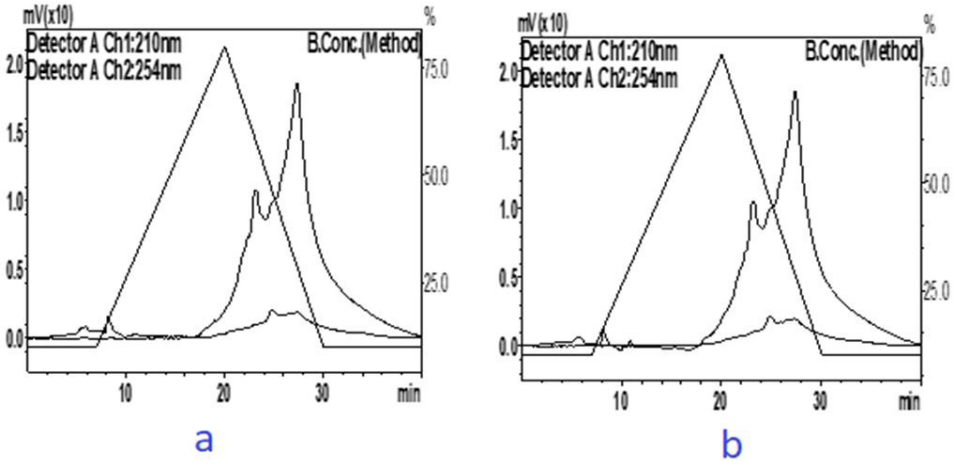

Figure 2: HPLC analysis spectra of pure taurine extract **(A)** and diluted taurine extract **(B)**

FTIR

The infrared spectra of orgaoclay (Figure 3) showed peaks at 1668 cm^{-1} assigned to carbon-carbon and carbon-nitrogen stretching vibrations in taurine extract. Silicon-oxygen and aluminum-oxygen bonds were represented at 990 and 1010 and 618 cm^{-1}, and magnesium-oxygen vibration was determined to a band between 450 and 515 cm^{-1}.

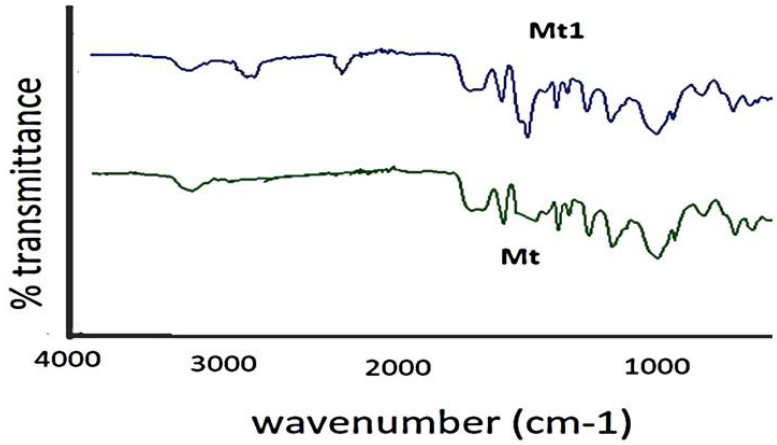

Figure 3: FTIR spectra of Na^{+}-Mt (Mt) and organoclay (Mt_1)

The band of O-H represents water adsorption on the montmorillonite at 3627 cm^{-1} (Figure 4). The stretching region of C-H is related to the surface active molecules in the region of 2850-2928 cm^{-1}. These bands are based on the C-H antisymmetric and symmetric stretching bonds [8,9]. The peaks seen in the FTIR spectrum between 2800-2900 cm^{-1} prove that taurine is bound to the clay groups and is seen as C-H tensile bands as a different from Mt spectra.

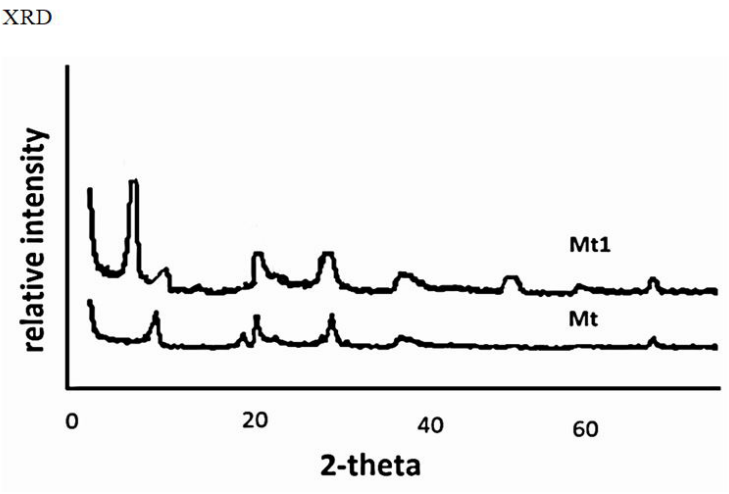

Figure 4: XRD spectra of Na^+-Mt (Mt) and organoclay (Mt_1)

Figure 2 shows the XRD spectrum of clay and organoclay. Table 2 contains the x-ray diffraction values of the samples. The d 001 spacings were calculated (Table 2) from peak positions using Bragg's law equation. XRD patterns of montmorillonite and its hybrid infi ltrated by surfactant are presented. The distance between the layers of clay was calculated according to Bragg's law. Bragg's law refers to equation $2d \sin\theta = n\lambda$, (the variable d is the distance between atomic layers in a crystal, and the variable lambda λ is the wavelength of the incident X-ray beam; n is an integer).

Sample	d-spacing (nm)
Mt	0.979
Mt_1	1.688

Table 2: X-ray diffractions data for samples

It is explained that the d-spacing for Mt (0.979 nm) increased to (1.688 nm) because the small inorganic Na^+ cation is exchanged by taurine extract group through an ion exchange process. The increase in the value of the organoclay d-spacing is proof that the modification was successful and an intercalated structure was obtained [10-12].

Antibacterial Assay

As is known, *E. coli* is a gram negative and *S. aureus* is a gram-positive bacterium. In this experimental stage, well diffusion method was used. In antibacterial analysis, 37 °C and 24 h incubation time was applied. Table 3 shows antibacterial analysis results of clay and organoclay.

Sample	Inhibition zone areas (mm^2)
E. coli	***S. aureus***
Mt 50.24	153.86
Mt_1 254.34	615.44

Table 3: Inhibition zone areas of clay and organoclay

Organoclay modified according to analysis results showed the highest antibacterial resistance against *S. aureus* bacteria. This result is because *E. coli* is a gram-negative bacterium, therefore its resistance is more difficult to break.

Conclusion

The use of organoclays in many areas reveals the importance of antibacterial resistance. In this study, taurine was extracted from goji berry fruit and its antioxidant effect was investigated by DPPH method. At the same time, the obtained extract was analyzed by HPLC and the results were compared. Then the taurine extract was used in the modification of the montmorillonite clay. The organoclay synthesized was characterized by FTIR and XRD. The results obtained proved that the modification was successful. The synthesized Mt_1 organoclay formed 153.86 and 615.44 mm^2 inhibition zones against *E. coli* and *S. aureus* bacteria, respectively. Organclay synthesized according to the results obtained showed the highest resistance against *S. aureus* bacteria. According to the results of the analysis, diluted taurine extract showed the highest antioxidant effect at 470 nm as 34%.

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