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Research Article

SYNTHESIS OF BENTONITE NANOCLAY AND INCORPORATION OF *CASSIA FISTULA* LEAF EXTRACT TO FORM ORGANOBENTONITE: CHARACTERIZATION AND ITS BIOMEDICAL APPLICATIONS

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ABSTRACT

Objective: In this work, methanolic leaf extract from *Cassia fistula* (known as aragvadha) was incorporated into bentonite nanoclay to form organobentonite. This organobentonite of nanosize was further used for its effective biomedical applications since medicinal clay finds its own advantage over decades.

Methods: The bentonite nanoclay was produced by energetic stirring followed by centrifugation and was characterized using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR). The organobentonite was produced using freeze and thaw method. Antioxidant property was studied using Molyneux method, and thrombolytic activity was analyzed using *in vitro* clot lysis method.

Results: The nanosize of bentonite nanoclay between 57 and 82 nm with irregular to spherical shape was confirmed using SEM analysis. The sharp diffraction peak in XRD analysis shows the crystalline nature of bentonite nanoclay, and FTIR results revealed the successful incorporation of the methanolic extract within the bentonite nanoclay. The organobentonite exhibits 84.5% antioxidant property as well as 31% clot lysis activity when compared to the extract and the bentonite nanoclay.

Conclusion: Thus, the non-toxic and inexpensive bentonite nanoclay have a high aspect ratio with multifarious applications in medicine, food, cosmetics, and health products. Through this study, the bentonite nanoclay modified using plant alkaloid (organobentonite) is found to possess good biomedical property.

Key words: Bentonite, Nanoclay, Organobentonite, Biomedical.

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INTRODUCTION

Nanoclay is the new class of nanomaterials belonging to clay minerals, which offers cheap and eco-friendly source for different novel applications [1,2]. In nature, the clay minerals are layered as hydrous silicate sheets, stacked one over the other [1]. Nanoclay can be obtained from clay minerals through various methods such as centrifugation, freeze drying, energetic stirring, cross-flow filtration, or through ultracentrifugation [3]. These methods can delaminate clay minerals into individual sheets which can give a high aspect ratio, at least in one dimension. The synthesis of nanoscale particles without toxic chemicals overcomes the difficulties in its use in biomedical field with enhanced properties and with lesser side effects [4,5].

Bentonite is one of the clay minerals that possess interlayer of silica and alumina. It is naturally formed from volcanic ash weathering in the presence of water. It can be used without any modification or can be modified using certain physiochemical treatments based on their applications [6-8]. It is mainly used in structural polymers, ceramic bodies, drilling fluids [9], and also in adsorption catalytic processes [10]. The incorporation of any molecules within the nanoclay system does not affect the nature of the molecules. When it is modified by the addition of any plant alkaloids, it is known as organobentonite. This incorporation occurs by different molecular interactions such as hydrogen bonding, ion exchange, and dipole interaction [11]. Furthermore, the incorporation of plant extracts into the nanoclay forms a novel nanopackaging system which helps in the storage of compounds in a highly stable form against the environment changes [12].

Cassia fistula (Golden shower tree) is a well-known flowering plant which belongs to the Fabaceae family. This medium sized (33–66 ft), fast-growing tree is widely distributed in various parts of the world such as South Africa, Asia, Mexico, Brazil, East Africa, and China. It is commonly found as an ornamental tree with compound leaves containing 4–8 leaf pairs in opposite leaflets and contains bundles of flowers in yellowish color [13]. It is used as an herbal medicine and has a high therapeutic value to treat various diseases such as diabetes, leukoderma, hematemesis, pruritus, and rheumatism. It is also found to exert analgesic, antipyretic, hypoglycemic activity, and anti-inflammatory activity [14]. For treating certain infectious diseases, it is used as a broad-spectrum antimicrobial agent [15]. Hence, in Ayurvedic medicine, it is known as disease killer. The phytochemicals of *C. fistula* are highly target specific, eco-friendly, less resistivity, and high acceptability which makes it efficient for various biomedical applications [16].

With an account of the above advantages, this work was focused on the incorporation of methanolic extract of *C. fistula* leaves into bentonite nanoclay to form organobentonite. The bentonite nanoclay was characterized before and after organobentonite formation and was utilized for its biomedical applications such as antioxidant and thrombolytic activity.

EXPERIMENTAL

Materials

Natural bentonite clay was purchased from Jain Lab Chemicals, Chennai. The other chemicals were of analytical grade purchased from Fisher Scientific. *C. fistula* leaves were collected from our campus and were washed with double-distilled water for further processes.

Characterization techniques

Ultraviolet (UV)-VIS spectroscopy (SYSTRONICS Double beam UV-VIS Spectrophotometer: 2202, scan mode from 200 nm to 700 nm), X-ray diffraction (XRD) spectroscopy (using XPERT Pro, PANalytical JDX-8030, and JEOL), scanning electron microscope (Tescan Vega 3 SBU model, 0.005 Pa to 2000 Pa), and Fourier transform-infrared (FTIR) spectroscopy (Perkin Elmer-spectrum one, range of 500–4500 cm^{-1}) were used to characterize the nanoclay and organobentonite produced.

Nanoclay preparation

The bentonite nanoclay was prepared using modified Floody *et al.* method [17]. In this method, 5 g of clay was mixed with 100 ml 1M NaCl and ultrasonicated at 4280 J g^{-1} for 5 min. It was then centrifuged at 654 g for 40 min. The first round of the supernatant contains mineral impurities; hence, it was discarded. The pellet was resuspended in 50 ml deionized water and was subjected to moderate stirring for 40 min. The supernatant was collected by repeating the process for 11 more times and was dried in hot air oven at 60°C.

Organic matter removal

The removal of organic matter was necessary to prevent the agglomeration of bentonite nanoclay. For that, 2 mg/ml suspension concentration on produced nanoclay was treated with 30% hydrogen peroxide (1:2). The method follows modified James *et al.* [18]. The above suspension was moderately stirred for 3 h to form a homogenous mixture. The pH of the homogenous mixture was neutralized using 0.5 M sodium hydroxide and was again stirred at 100 rpm for 1 h in a magnetic stirrer. The bentonite nanoclay was then obtained by drying the mixture at 60°C.

Leaf extraction

The leaves from *C. fistula* were collected and washed with double-distilled water. It was then shade dried and was powdered using a blender. 5 g of powdered leaves was mixed with 50 ml methanol (1:10) and was kept in shaker for 48 h. The extract was filtered out using Whatman filter paper No.1 [19].

Formation of organobentonite

The incorporation of the methanolic extract within the nanoclay was carried out by modified freeze and thaw method [20]. Briefly, 2 g of the nanoclay produced was mixed with 40 ml of methanolic extract to become a homogenous mixture. The mixture was then frozen at −20°C for 24 h and was thawed at room temperature.

Antioxidant property

The antioxidant property was analyzed using Molyneux method [21] using the strong antioxidant, 2,2-diphenyl-2-picrylhydrazyl (DPPH). The method follows dissolving 1.97 mg of DPPH in 50 ml of pure methanol (radical reagent). 60 µl (1 mg/ml) of extract, nanoclay and organobentonite were added to 840 µl of DPPH radical reagent and mixed together. The reaction was kept undisturbed in the dark for 15 min at room temperature. The color change was measured at 517 nm using UV-Vis spectrophotometer. The percentage of inhibition was found using: $\%inhibition = [(A_0-A_{15})/A_0]\times100$; where, A_0=OD for DPPH and A_{15}=OD for sample.

Clot lysis activity

The thrombolytic activity was studied through *in vitro* clot lysis method [22] (approved protocol by the Institutional Ethics Committee) in which venous blood was collected from a healthy volunteer with no history of oral contraceptive and anticoagulant therapy. The 500 µl of blood was evenly distributed in four pre-weighed microfuge tubes and incubated for 45 min at 37°C to form clots. The serum found above the clot was removed by pipetting, and the initial clot weight was noted. 100 µl of distilled water was added as a control, and other three tubes were added with the (0.1 mg/ ml) extract alone, nanoclay and organobentonite. The tubes were incubated at 37°C for 90 min and the final clot weight was determined using the formulae: Clot weight = $W_{clot} - W_{tube}$; W_{clot}=weight of clot containing tube and W_{tube}=weight of tube alone, by measuring the weight of the clot before and after lysis.

RESULTS AND DISCUSSION

UV-Vis spectroscopy

The organic molecules are known to have maximum absorption peak between 200 and 700 nm. The absorption of light in the UV or visible region of the electromagnetic spectrum gives the ground state to higher energy state electronic transitions due to the high degree of conjugation of molecules. In Fig. 1, the obtained peak at 252 nm denotes the presence of the conjugated system attributing to $\pi\rightarrow\pi^*$ transition of Si=O functional group. Meanwhile, the peak at 340 nm denotes the lone pairs of electron on the hydroxyl substituent and alumina conjugation attributing to $n\rightarrow\pi^*$ transitions. This result was found; moreover, similar to the results obtained by Wanyika *et al.* [23] for the montmorillonite (MMT) clay since the bentonite has MMT as its major constituent the peaks match to the earlier results.

XRD analysis

The XRD pattern in Fig. 2 reveals similar results obtained by Venkatathri [10]. The sharp peaks in the pattern infer that the clay is polycrystalline in nature. The diffraction peaks at the angles 25.28°, 27.68°, 36.94°, 37.80°, 38.57°, 55.06°, and 62.12°correspond to the crystal planes of (1 0 1), (1 1 2), (1 0 3), (0 0 4), (1 1 2), (2 1 1), and (2 1 3), respectively. Similarly, the peak at 27.68° corresponds to MMT.

Scanning electron microscopy (SEM) analysis

The SEM analysis revealed the nanosize of the produced nanoclay; however, agglomeration of the nanoclay was observed in Fig. 3 before hydrogen peroxide treatment. The agglomeration makes it difficult for the determination of the size of the nanoclay. After the treatment with 30% hydrogen peroxide, the encrusted and occluded organic matter removal prevented the agglomeration formation such that the produced nanoclay was in irregular to spherical shaped with 57–82 nm size (Fig. 4).

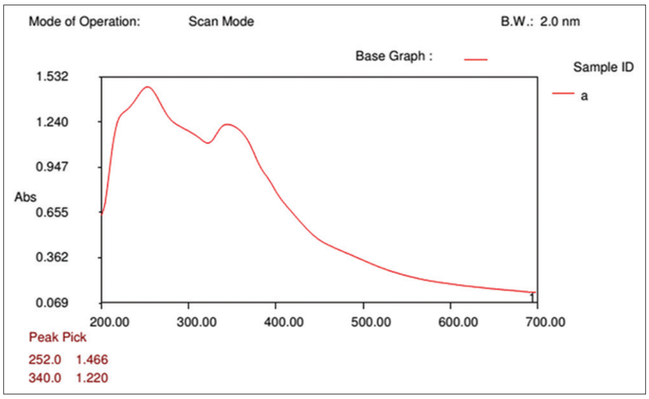

Fig. 1: Ultraviolet-visible spectroscopy analysis of nanoclay

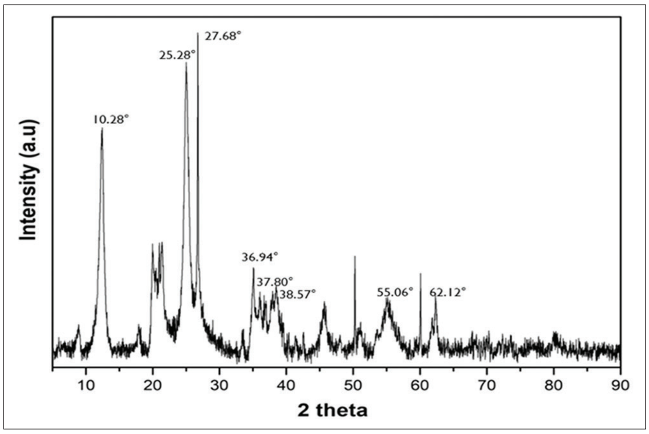

Fig. 2: X-ray diffraction analysis of nanoclay

FTIR analysis

The peaks at 466, 523, and 797 are due to the pore opening, 920 is due to the double ring formation, while 1045 and 1110 (1/cm) in Fig. 5 show the symmetric and asymmetric T-O-T vibrations (where T is Al or Si) in the bentonite nanoclay. The Si-O-Si vibrations are confirmed by the peak at 881. The silanol group (Si-OH-Al) in the crystallographic position of nanoclay was confirmed by the prominent peak at 3643 cm^{-1}.

The peaks at 675.09, 1023, 1242.16, 1361.74, 2918.30, and 3277.06 in Fig. 6 denote the presence of alkenes, alcohols, carboxylic acids, nitro compounds, hydrogen bonded alcohols, and phenols as its major phytochemical constituents.

The formation of peaks at 797, 881, 920, and 3643 in Fig. 7 along with the spectrum obtained in the pure extract (1023, 3277, and 1242.16) without nanoclay reveals that the leaf extract was successfully incorporated with the produced nanoclay.

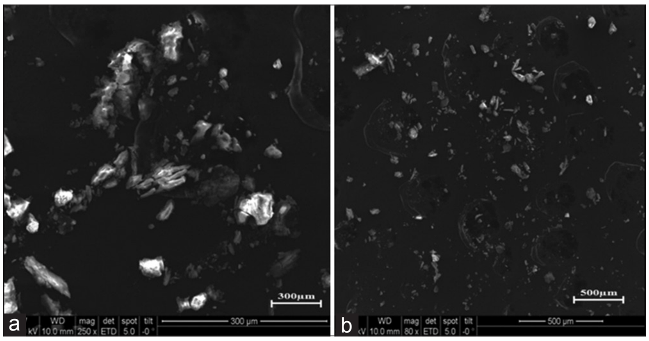

Fig. 3: (a and b) Scanning electron microscopy images of nanoclay before hydrogen peroxide treatment

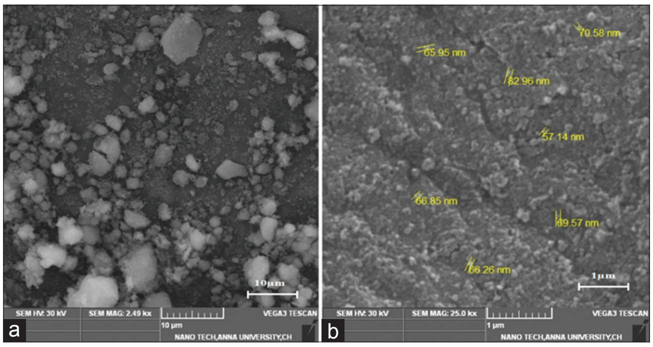

Fig. 4: (a and b) Scanning electron microscopy images of nanoclay after hydrogen peroxide treatment

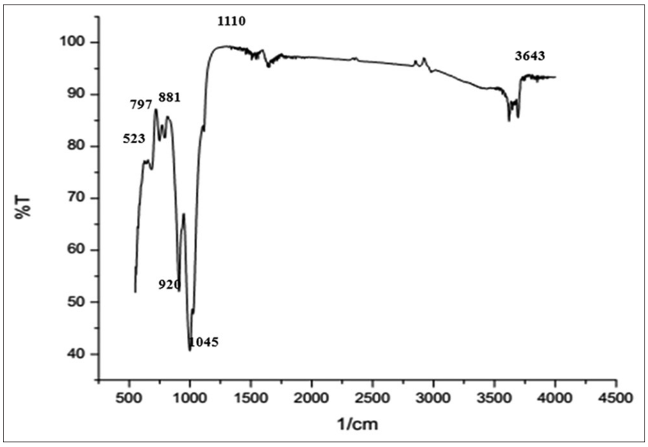

Fig. 5: Fourier transform-infrared analysis for nanoclay

Antioxidant property

The antioxidant property found using Molyneux method in Fig. 8 revealed a color change in the radical reagent from dark pink to pale brownish color in the mixture containing organobentonite as well as for the extract alone. However, the nanoclay alone (Fig. 8a) does not show any drastic change in the color. The absorbance value was also found to decrease for the three mixtures, whereas the (organobentonite) incorporated system showed highest inhibition of 84.5% than other two. The extract alone has 77% inhibition; whereas, nanoclay alone has

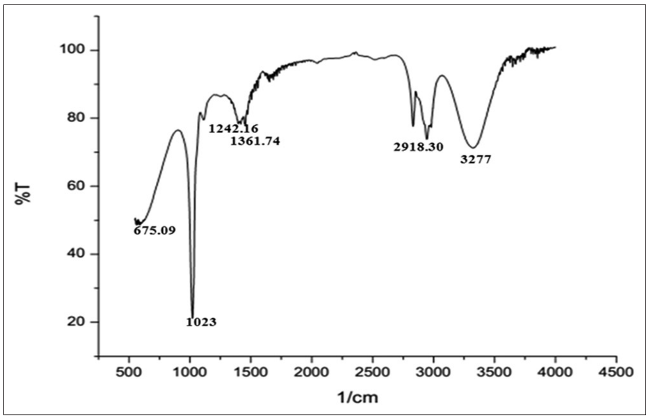

Fig. 6: Fourier transform-infrared analysis of methanolic extract of *Cassia fistula* leaf

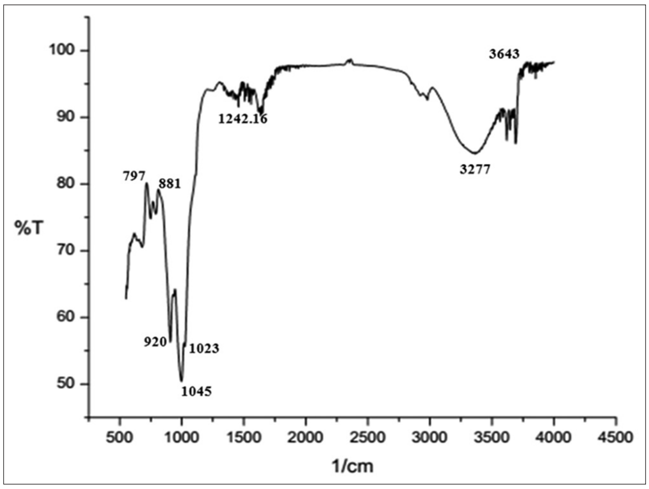

Fig. 7: Fourier transform-infrared analysis for organobentonite

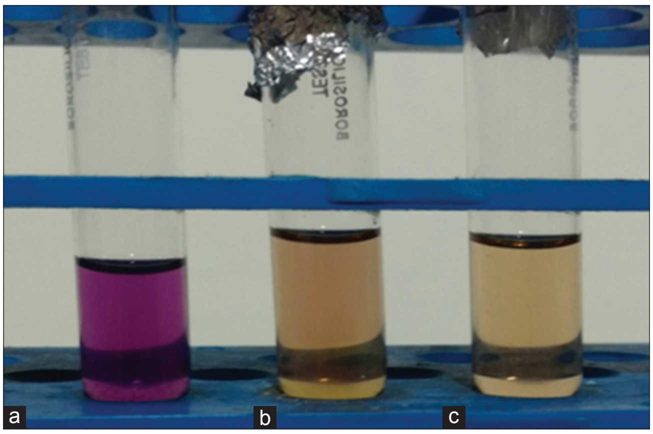

Fig. 8: Antioxidant activity (a) nanoclay, (b) extract, (c) organobentonite

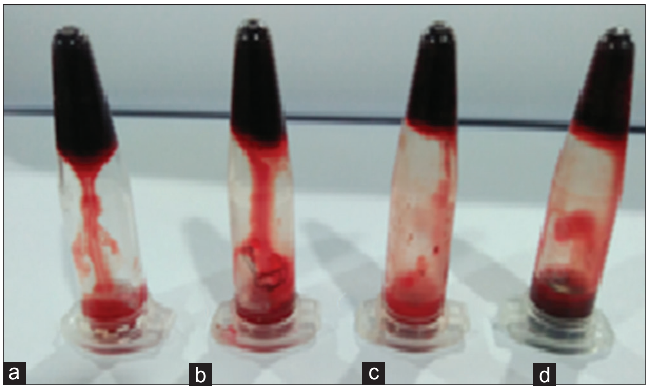

Fig. 9: Clot lysis activity (a) control, (b) nanoclay, (c) extract, (d) organobentonite

13.795% of inhibition. The result confirms the antioxidant capacity of the organobentonite.

Clot lysis analysis

The results obtained through *in vitro* method reveals about 31% of clot lysis for the organobentonite, 22% for the extract, and 16% for the nanoclay. The control with distilled water has 6% of clot lysis. Fig. 9 shows the lysis of blood clot after the addition of the samples to the pre-weighed blood clots.

CONCLUSION

Thus, it was found that the preparation of nanoclay from the natural clay minerals is a simple and inexpensive process. The nanosize of the bentonite and peroxide treatment enables easier intercalation of molecules. The FTIR analysis confirmed the successful incorporation of the methanolic extract within the bentonite nanoclay to form organobentonite. The prepared organobentonite showed good antioxidant and thrombolytic property than the nanoclay or extract.

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CONFLICTS OF INTEREST

There are no inherent conflicts of interest in the author's point of view.

AUTHOR'S CONTRIBUTION

We L.F.A. Anand Raj and J. Jeslin have designed and performed the experiments, analyzed all the data, and prepared the manuscript.

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