

# Synthesis, Characterization and Applications of Citrus Sinensis Peel-Modified Montmorillonite

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## ABSTRACT

Fruit residues represent a promising source of bioactive compounds for sustainable material development. In this study, citrus sinensis (orange) peel extract was intercalated into montmorillonite (MMT) clay through a modified freeze-thaw method to synthesize citrus sinensis peel-modified montmorillonite (OPMT). The synthesized material was characterized by UV-Vis, FTIR, XRD, and SEM analyses, confirming the successful incorporation of peel-derived functional groups into the modified montmorillonite clay. Biological assays demonstrated significant antimicrobial activity, particularly against *Candida albicans*. The antioxidant, antidiabetic, and anticancer potential were also observed. The results highlight that OPMT is a biocompatible and environmentally sustainable compound with potential applications in environmental remediation, food packaging, and biomedical fields.

**Keywords:** Citrus sinensis, organo-montmorillonite, non-toxic, biomedical fields

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## INTRODUCTION

Fruits constitute an essential component of the human diet and play a vital role in maintaining health (Sharma *et al.*, 2020). Although, large quantities of fruit residues are generated during post-harvest handling, processing, and consumption (Singh and Kumar, 2019). These residues, mainly composed of peels, cores, and injured tissues, account for approximately 10–20% of the total fruit mass and are generally discarded as waste (Li *et al.*, 2021). However, it possesses favorable physicochemical and functional properties (Patel *et al.*, 2018). Transforming fruit wastes into value-added functional composites represents a promising approach for sustainable utilization (Zhang *et al.*, 2022).

Among various fruits, *citrus sinensis* peels (orange peels) are rich in bioactive compounds such as vitamin C, carotenoids, polyphenolics, antioxidants and nutraceuticals (Martínez *et al.*, 2020, Anwar *et al.*, 2008). Orange peel residues can serve as an efficient and sustainable source for developing functional materials with potential applications in food, pharmaceutical, and environmental sectors (Ahmed *et al.*, 2021; Chen *et al.*, 2022). Citrus peel has been also

utilized in the preparation of molasses, pectin, dried pulp, and as cattle feed (Anwar *et al.*, 2008).

*Citrus sinensis* peel (orange peel) is a major by-product generated from the citrus processing industry and represents approximately 45–50% of the fresh fruit weight (Sudha *et al.*, 2016, Yangilar, 2013). The chemical composition of dried citrus peel generally contains 6.78% protein, 4.14% fat, 2.10% ash, 3.33% fiber, and 86.65% carbohydrates (Zhou *et al.*, 2020).

Montmorillonite (MMT) is a dioctahedral smectite clay mineral originating from volcanic ash altered by chemical weathering (Rajasekar *et al.*, 2016). The general chemical formula of MMT is  $(Al_2-xMg_x)Si_4O_{10}(OH)_2 \cdot nH_2O$  (Munir *et al.*, 2020). In this structure, silicon (Si) and aluminium (Al) are the major framework elements, while  $Mg^{2+}$ ,  $Fe^{2+}$  and  $Al^{3+}$  occur as exchangeable cations (Munir *et al.*, 2020).

MMT has been extensively applied in polymer nanocomposites, surface coatings, food packaging, and drug delivery systems (Belghazdis and Hachem, 2022, Edraki *et al.*, 2021). The intercalation of *citrus sinensis* peel extract into MMT occurs through ion exchange and hydrogen bonding (Karimi and

Salem, 2010). Such interactions enhance the stability of bioactive compounds against environmental degradation, supporting their use in functional materials (Gutierrez *et al.*, 2017).

In this study, *citrus sinensis* peel extract was incorporated into modified MMT to develop *citrus sinensis* peel-modified montmorillonite (OPMT), which was characterized using spectroscopic and microscopic techniques and evaluated for antimicrobial, antioxidant, antidiabetic, and anticancer properties.

## 2. MATERIALS AND METHODS

### 2.1. MATERIALS

Montmorillonite clay was obtained from India Mart (Chennai, India). Chemicals were purchased from Sigma-Aldrich (Bangalore, India). Fresh *citrus sinensis* peels were collected locally.

### 2.2. Methods

#### Preparation of modified clay

5g of montmorillonite was treated with 100 mL of 1M NaCl, ultrasonicated, centrifuged, repeatedly washed and dried at 60 °C (Floody *et al.*, 2011).

#### Removal of residues

Modified clay (2 mg) was mixed with 30% H<sub>2</sub>O<sub>2</sub> (1:2), stirred for 3 h, neutralized with 0.5 M NaOH and then stirred for 1 h and dried at 60 °C (James *et al.*, 2008).

#### Preparation of peel extract

*Citrus sinensis* peels were washed, air-dried, powdered and mixed with methanol (1:10 w/v) and subjected to shaking and filtered through Whatman No. 1 filter paper (Gowdhami *et al.*, 2014).

#### Synthesis of *citrus sinensis* peel-modified montmorillonite

2 g of modified clay was combined with 40 mL of peel extract, frozen at 20 °C for 24 h, and thawed at room temperature (Nooria *et al.*, 2015).

### 2.3. Characterization

#### • UV-Vis spectroscopy

UV-Vis spectrum of *citrus sinensis* peel-modified montmorillonite was recorded using Shimadzu UV-1800 spectrophotometer in the wavelength range of 200-800 nm.

#### • FTIR

FTIR analysis of *citrus sinensis* peel-modified montmorillonite was performed using the KBr pellet method to analyse the different functional groups of the prepared compound. The spectrum was collected in the range of 500-4500 cm<sup>-1</sup> with Shimadzu FTIR-8400s spectrometer.

### XRD

XRD patterns of *citrus sinensis* peel-modified montmorillonite was obtained over a 2θ range of 20–80°. The average crystallite size was estimated using the Debye–Scherrer equation.

### SEM

Surface morphology of *citrus sinensis* peel-modified montmorillonite was analyzed using VEGA 3 Tescan scanning electron microscope under a vacuum range of 0.005–2000 Pa.

### 2.4. Evaluation under antimicrobial studies

The performance of *citrus sinensis* peel-modified montmorillonite under microbial attack was analysed by antibacterial and antifungal studies. Agar disc diffusion (Kirby-Bauer) method was used for the determination. The microbial strains *E. coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* were used for antimicrobial assay. The ability to inhibit the bacterial growth of *citrus sinensis* peel-modified montmorillonite was identified (Bauer *et al.*, 1966).

### 2.5. Antioxidant Assays

- **DPPH assay:** Performed with different concentrations (6.25–100 µg/mL) of *citrus sinensis* peel-modified montmorillonite (OPMT) using ascorbic acid as standard. Absorbance measured at 520 nm (Blois, 1958).
- **ABTS assay:** ABTS•+ generated by potassium persulfate oxidation and *citrus sinensis* peel-modified montmorillonite (OPMT) tested at 6.25–100 µg/mL. Absorbance measured at 734 nm (Sricharoen *et al.*, 2014).

### 2.6. Antidiabetic Assays

**α-Amylase inhibition:** Enzyme and substrate incubated with *citrus sinensis* peel-modified montmorillonite (OPMT) using acarbose as standard and absorbance measured at 540 nm after DNSA reaction (Kwon *et al.*, 2006).

**α-Glucosidase inhibition:** Reaction with p-nitrophenyl-α-D-glucopyranoside and *citrus sinensis* and absorbance measured at 405 nm; acarbose used as control (Dewi *et al.*, 2007).

### 2.7. Anticancer Assays

**MTT assay:** MCF7 breast cancer cells exposed to *citrus sinensis* peel-modified montmorillonite (OPMT) at varying concentrations; cell viability assessed at 570 nm (Mosmann, 1983).

**SRB assay:** Protein binding assay performed on MCF7 cells and absorbance recorded at 510 nm (Skehan *et al.*, 1990, Vichai and Kirtikara, 2006).

## 3. RESULTS AND DISCUSSION

### 3.1. UV-Vis Spectroscopy

The *citrus sinensis* peel-modified montmorillonite (OPMT) exhibited absorption peak between 200–300 nm, corresponding to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions, attributed to phenolic compounds such as flavonoids and limonene. This confirmed the successful intercalation of peel extract into modified montmorillonite.

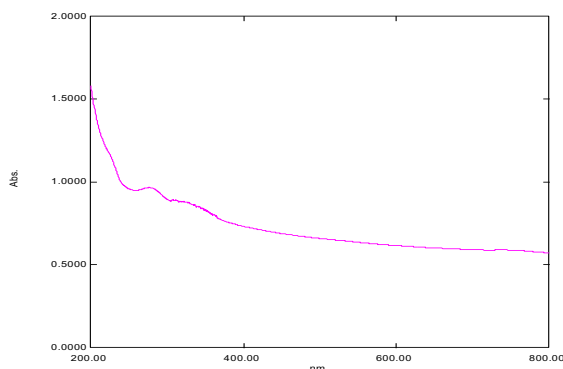


Fig. 3.1 UV-Vis spectroscopy of *citrus sinensis* peel-modified montmorillonite

### 3.2. FT-IR analysis

FTIR spectra of *citrus sinensis* peel-modified montmorillonite showed O–H stretching at  $3420\text{ cm}^{-1}$ , C–H stretching at  $2920\text{ cm}^{-1}$ , and Si–O stretching near  $1030\text{ cm}^{-1}$ , confirming incorporation of citrus peel extract into modified montmorillonite.

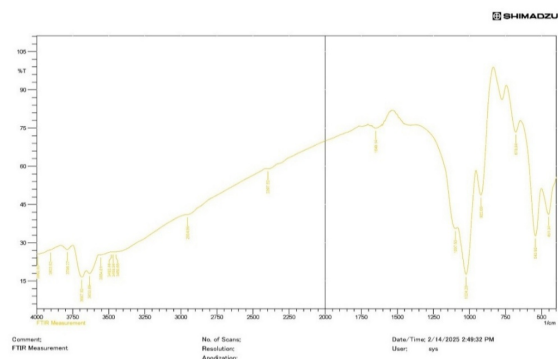


Fig. 3.2 FT-IR spectrum of *citrus sinensis* peel-modified montmorillonite

### 3.3. XRD analysis

The XRD patterns revealed the crystalline structure of *citrus sinensis* peel-modified montmorillonite. The presence of several well-defined diffraction peaks suggests the semi-crystalline structure of *citrus sinensis* peel-modified montmorillonite. The characteristic peaks at  $26.7^\circ$  corresponds to MMT, confirms the formation of *citrus sinensis* peel-modified montmorillonite.

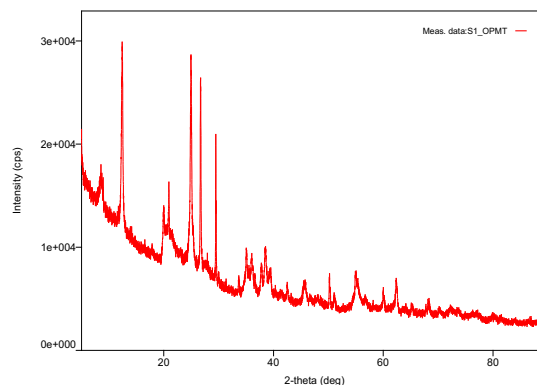
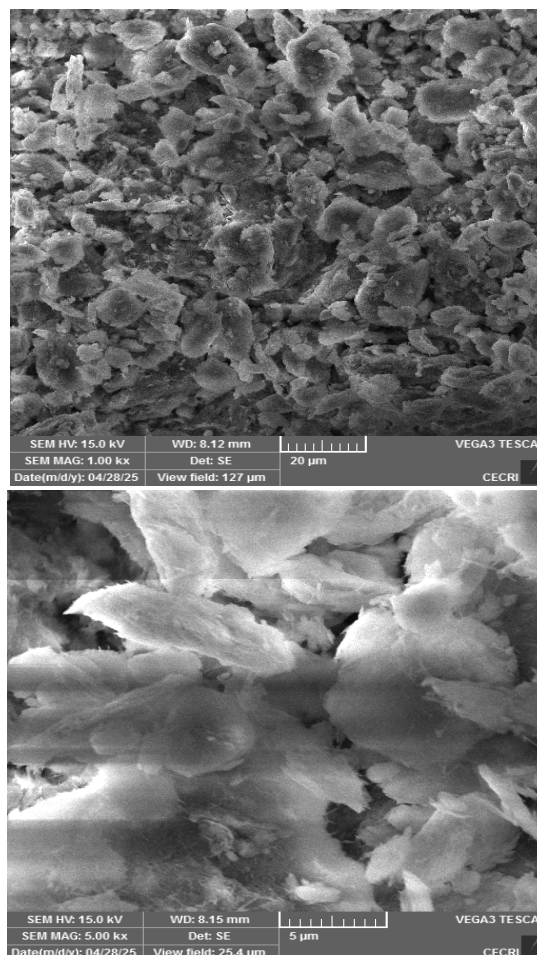


Fig. 3.3 XRD pattern of *citrus sinensis* peel-modified montmorillonite

### 3.4. SEM analysis

SEM micrographs of *citrus sinensis* peel-modified montmorillonite exhibits a layered and flaty morphology. The particles appear as irregular plate-like structures. Hence, it was suitable for bio-medical applications.



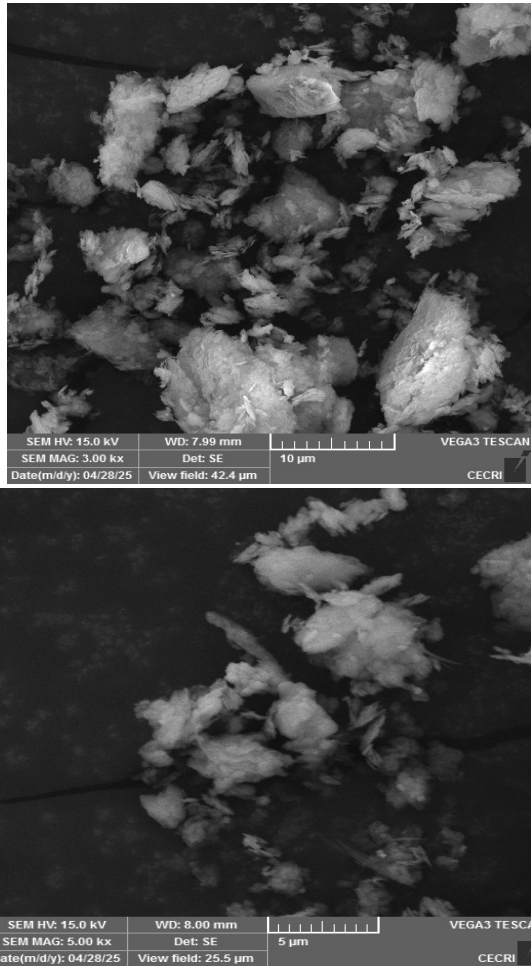
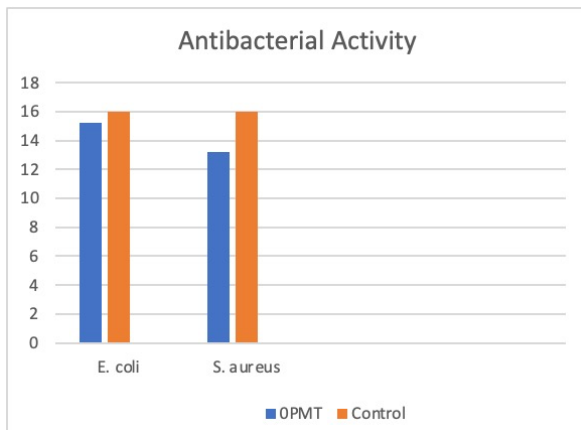


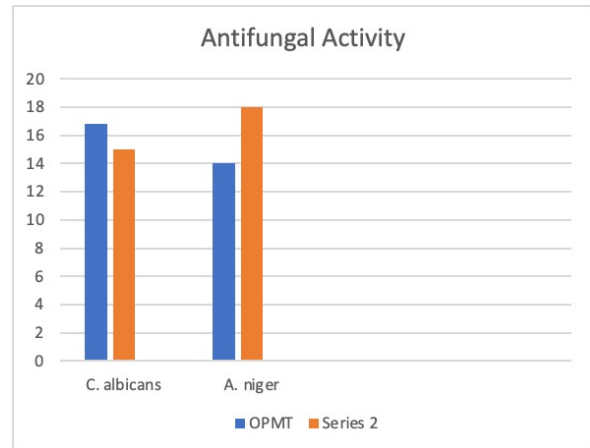
Fig. 3.4 SEM microphotographs of *citrus sinensis* peel-modified montmorillonite

3.5. ANTIMICROBIAL ACTIVITY

Disc diffusion results showed the inhibition zones against *E. coli* (15.2 mm), *S. aureus* (13.2 mm), *Candida albicans* (16.8 mm) and *Aspergillus niger* (14 mm) with stronger antifungal activity against *C. albicans* (16.8 mm) compared to control. It indicates enhanced antimicrobial efficacy of *citrus sinensis* peel-modified montmorillonite (OPMT).



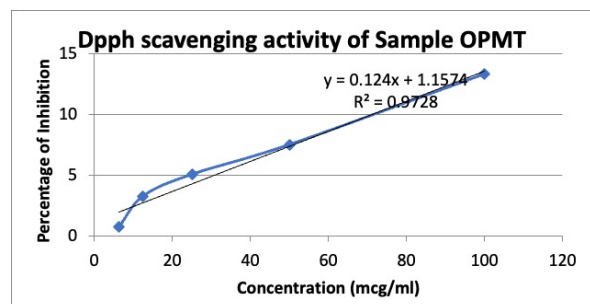
Graph 3.1. Antibacterial activity of OPMT against *E. coli* and *S. aureus*.



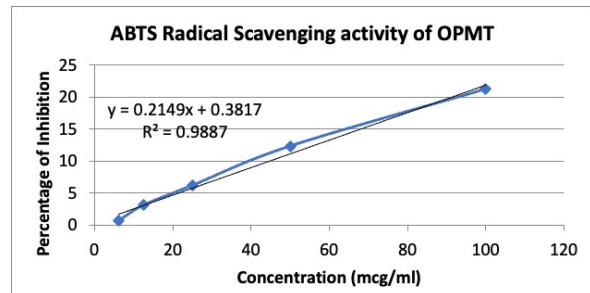
Graph 3.2. Antifungal activity of OPMT against *Candida albicans* and *Aspergillus niger*.

3.6. Antioxidant analysis

The antioxidant potential of *citrus sinensis* peel-modified montmorillonite (OPMT) was evaluated using DPPH and ABTS radical scavenging assays. A dose-dependent increase in scavenging activity was observed, confirming the contribution of phenolic compounds from *citrus sinensis* peel extract. At higher concentrations (100 µg/mL), OPMT exhibited comparable activity to the standard ascorbic acid. It demonstrates that the incorporation of peel-derived polyphenols into montmorillonite enhances free radical neutralization.



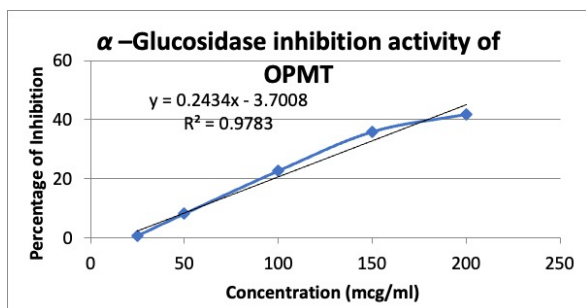
Graph 3.3. Dpph scavenging activity of OPMT



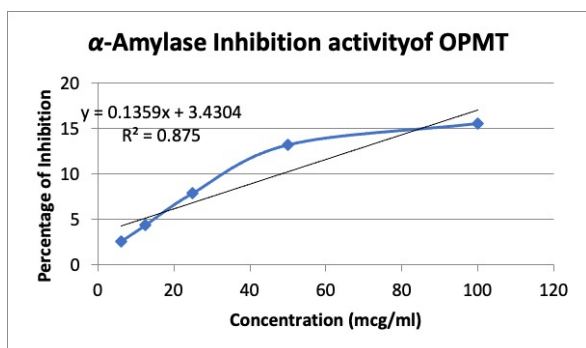
Graph 3.4. ABTS radical scavenging activity of OPMT

3.7. Antidiabetic analysis

The inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes by *citrus sinensis* peel-modified montmorillonite (OPMT) indicated its antidiabetic potential. Both enzymes play key roles in carbohydrate hydrolysis and postprandial glucose regulation. The *citrus sinensis* peel-modified montmorillonite demonstrated moderate inhibitory activity, though lower than the standard drug acarbose. It suggests that bioactive phytochemicals from the peel extract retained functional integrity after intercalation, providing glucose-lowering potential through enzyme inhibition.



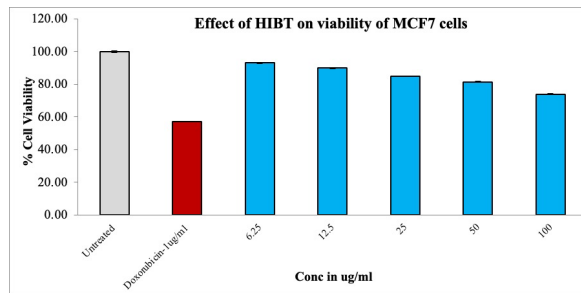
Graph 3.5.  $\alpha$ -Glucosidase inhibition activity of OPMT



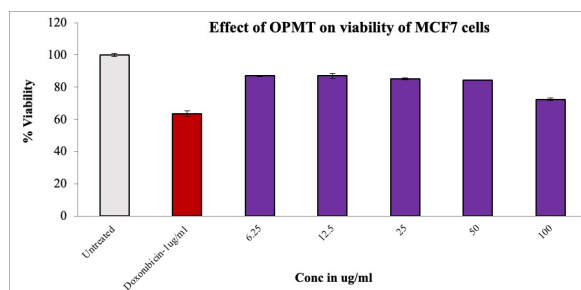
Graph 3.6.  $\alpha$ -amylase inhibition activity of OPMT

### 3.8. Anticancer analysis

The cytotoxic effect of *citrus sinensis* peel-modified montmorillonite (OPMT) was tested against MCF-7 human breast cancer cells using MTT and SRB assays. The results revealed the concentration-dependent decrease in cell viability, with significant cytotoxicity observed at higher concentrations. The activity can be attributed to phytochemicals such as flavonoids and limonoids intercalated into montmorillonite, which may induce apoptosis and disrupt cellular metabolism.



Graph 3.7. MTT assay activity of OPMT



Graph 3.8. SRB assay activity of OPMT

## 4. CONCLUSION

A green synthesis approach was employed to produce organo-montmorillonite using *citrus sinensis* peel extract. The characterization techniques confirmed the successful intercalation and enhanced surface chemistry. The organo-montmorillonite exhibited significant antimicrobial, antioxidant, antidiabetic and anticancer activity, demonstrating its potential as a nature-compatible for biomedical and environmental applications.

## 5. REFERENCES

- Ahmed, S., Rahman, M., & Khan, T. (2021). Synthesis and antimicrobial evaluation of plant-extract modified clay nanocomposites. *Journal of Cleaner Production*, 315, 128195.
- Anwar, F., Naseer, R., Bhangar, M.I., Ashraf, S., Talpur, F.N., & Aladedunye, F.A., 2008. Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. *Journal of the American Oil Chemists' Society*, 85(4), 321–330. <https://doi.org/10.1007/s11746-008-1192-6>
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 4(5), 493–496.
- Belghazdis, M., Hachem, E., 2022. Clay and clay minerals: A detailed review. *International Journal of Recent Technology and Applied Science* 4(2), 54-75. <https://doi.org/10.36079/lamintang.ijortas-0402.367>
- Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199–1200.

- Chen, X., Wu, D., & Liu, F. (2022). Enhanced adsorption and photocatalytic degradation using organo-montmorillonite composites. *Applied Surface Science*, 578, 152062.
- Dewi, R.T., Iskandar, Y.M., Hanafi, M., Kardono, L.B.S., Angelina, M., Dewijanti, I.D., Banjarnahor, S.D.S., 2007. Inhibitory effect of koji *Aspergillus terreus* on  $\alpha$ -glucosidase activity and postprandial hyperglycemia. *Pakistan Journal of Biological Sciences*, 10(8), 3131–3135.
- Edraki, M., Zaarei, D., 2019. Azole derivatives embedded in montmorillonite clay nanocarriers as corrosion inhibitors of mild steel. *Int. J. Miner. Metall. Mater.*, 26, 86-97.
- Floody, M.C., Bendall, J.S., Jara, A.A., Welland, M.E., Theng, B.K., Rumpel, C., 2011. Nanoclays from an Andisol: extraction, properties and carbon stabilization. *Geoderma*, 161, 159–167.
- Gowdhami, M., Sarkar, B.L., Ayyasamy, P.M., 2014. Screening of phytochemicals and antibacterial activity of *Annona squamosa* extracts. *International Journal of Pharmaceutical Science Inventions*, 3, 30–39.
- Gutierrez TJ, Ponce AG, Alvarez VA., 2017. Nanoclays from natural and modified montmorillonite with and without added blueberry extract for active and intelligent food nanopackaging materials. *Mater Chem Phys.*, 194, 283-92.
- James, O., Adedirin, M., Adekola, F., Odebumni, E., Adekeye, J., 2008. Beneficiation and characterisation of a bentonite from north-eastern Nigeria. *Journal of the North Carolina Academy of Science*, 124, 154–158.
- Karimi L, Salem A., 2010. The role of bentonite particle size distribution on kinetic of cation exchange capacity. *J Ind Eng Chem.*, 17, 90- 5.
- Kwon, Y., Apostolidis, E., Shetty, K., 2006. Inhibitory potential of wine and tea against  $\alpha$ -amylase and  $\alpha$ -glucosidase for management of hyperglycemia. *Journal of Food Biochemistry*, 32, 15–31.
- Li, Y., Zhang, J., Chen, H., & Wang, X. (2021). Green synthesis and antibacterial performance of modified bentonite composites. *Materials Today Chemistry*, 21, 100556.
- Martinez, L., Torres, M., & Garcia, P. (2020). Functionalized zeolite and bentonite materials for removal of organic contaminants from water. *Environmental Science and Pollution Research*, 27(10), 11543–11555.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65, 55–63.
- Munir, M., Ahmad, M., Rehan, M., Saeed, M., Lam, S. S., Nizami, A. S., Waseem, A., Sultana, S., Zafar, M., 2020. Production of high quality biodiesel from novel non-edible *Raphanus raphanistrum* L. seed oil using copper modified montmorillonite clay catalyst. *Environmental Research*. <https://doi.org/10.1016/j.envres.2020.110398>
- Nooria, S., Kokabia, M., Hassan, Z.M., 2015. Nanoclay-enhanced mechanical properties of poly(vinyl alcohol)/chitosan/montmorillonite nanocomposite hydrogel as wound dressing. *Procedia Materials Science*, 11, 152–156.
- Patel, M., Desai, P., & Shah, D. (2018). Characterization and catalytic properties of acid-activated clays. *Applied Surface Science*, 450, 355–364.
- Rajasekar A, Janakiraman V, Govindarajan K., 2016. *In vitro* cytotoxic study of green synthesized gold and silver nanoparticles using *Eclipta prostrata* (L.) against Ht-29 cell line. *Asian J Pharm Clin Res.*, 9, 189-93.
- Sharma, R., Gupta, P., & Mehta, S. (2020). Modification and characterization of natural clay minerals for environmental remediation applications. *Applied Clay Science*, 190, 105–118.
- Singh, A., & Kumar, R. (2019). A review on structural, thermal and surface modification of montmorillonite clay for nanocomposite applications. *Journal of Environmental Chemical Engineering*, 7(3), 1031–1043.
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, 82(13), 1107–1112.
- Sricharoen, P., Techawongstein, S., Luthria, D., Chanthai, S., 2014. Standardization of DPPH, ABTS and FRAP assays with reference compounds for estimating antioxidant capacity of tomato extracts. *Food Chemistry*, 178, 271–277.
- Sudha, M.L., Baskaran, V., & Leelavathi, K., 2016. Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry*, 191, 66–73. <https://doi.org/10.1016/j.foodchem.2015.01.041>
- Vichai, V., Kirtikara, K., 2006. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols*, 1(3), 1112–1116.
- Yangilar, F., 2013. The usage of dried apple, orange and carrot pomaces as fiber source in cookie production. *Academic Food Journal*, 11(1), 63–69.

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Zhang, Q., Li, X., Zhou, J., & Chen, Y. (2022). Recent advances in clay-based nanomaterials for biomedical and environmental applications. *Colloids and Surfaces B: Biointerfaces*, 217, 112680.

Zhou, Y., Ma, Y., Zeng, J., Du, X., & Wang, L., 2020. Nutritional composition and antioxidant properties of citrus peel extracts. *Food Bioscience*, 35, 100576. <https://doi.org/10.1016/j.fbio.2020.100576>