International Journal of Early Childhood Special Education (INT-JECSE)
DOI:10.9756/INTJECSE/V14I5.367 ISSN: 1308-5581 Vol 14, Issue 05 2022

PREPARATION AND BIOMEDICAL POTENTIAL OF PAPAIN LOADED HALLOSITE NANOCOMPOSITES

- **Sarojini Ramya Pillay**

Saveetha Dental College and HospitalsSaveetha Institute of Medical and Technical Sciences Saveetha UniversityChennai, India

- **Dhanraj Ganapathy**

Professor and Head,Department of Prosthodontics Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences Saveetha University Chennai, India

- **Rajeshkumar Shanmugam**

Professor Department of PharmacologySaveetha Dental College and Hospitals Saveetha Institute of Medical and Technical SciencesSaveetha UniversityChennai, India

Abstract
Biomedical science has long been on the lookout for new diagnostic and therapeutic mediums. The most recent development is the use of nanomaterials in such applications, which has given rise to the field of nanomedicine. Halloysite nanotubes (HNTs) are tubular clay nanomaterials that are formed by rolling aluminum silicate kaolin sheets many times. The aluminol and siloxane groups on the surface of HNT help to generate hydrogen bonds with the biomaterials that adhere to it. These qualities make HNT useful in a wide range of fields, including environmental sciences, waste-water treatment, dye removal, nanoelectronics and nanocomposites fabrication, catalytic research, glass coatings or anticorrosive coatings, cosmetics, stimuli response, and forensic sciences. Drug delivery, gene delivery, tissue engineering, cancer and stem cell separation, and bioimaging are just some of the few applications of HNT's unique features in biomedicine and nanomedicine.Papain is an enzyme found in the white fluid (latex) that occurs in raw papaya fruit. It is a protease,which breaks down proteins.Papain contains substances that might help fight infection and heal wounds.The efficacy of papain-halloysite nanotubes for wound healing for fighting infection and was studied in this study in-vitro.

Keywords: Halloysite, Halloysite nanotube, Halloysitenanocomposite, papain, wound pathogens

INTRODUCTION

Nanotechnology is an emerging field with numerous applications in innovation and technology, industry, the environment, energy, and other fields. Because this domain has bright future prospects, substantial research is being done to further its capabilities (1). Halloysite nanotubes (HNTs) are a resourceful nanomaterial that can be used in a wide range of biomedical applications (2–4). Halloysite is a commercially available, highly efficient clay nanomaterial derived from naturally available sources. HNTs are Halloysite tubular structures that chemically mimic kaolin. They come in a variety of shapes and sizes where short tubular and spheroidal halloysite particles with elongated tubes are the most commonly seen HNTs (5). They are layered aluminosilicates ($Al_2Si_2O_5$ $(OH)_4nH_2O$) having a hollow tubular geometry. The external diameter is projected to be between 40 and 70 nm, the internal diameter to be between 10 and 20 nm, and the length to be between 500 and 1500 nm. They have become a promising material for a spectrum of uses because of their lumens, high aspect length–diameter ratio, and low hydroxyl density on their surface. HNTs can also interact with a wide range of synthetic and biological components due to their enhanced surface area, positively entrusted interior surfaces with Al–OH groups, and negatively entrusted external surfaces with Si-OH and Si–O–Si groups (6).

HNT is commonly characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy, and X-ray diffraction (XRD). The multifunctional groups on the surface of HNTs have shown to aid in the loading of negatively charged macromolecules into the positive inner lumen of the nanotube, such as DNA encapsulation. The interactions of DNA and the HNT have been used to evaluate DNA damage using HNT gold and silver nanoparticle composites (7). HNT covered with polyethylene glycol seemed to show increased biocompatibility, lengthen circulation time, and prevent protein adsorption and accumulation in biological environments (8). This makes them perfect for modern biomedical applications such as the development of innovative medicine and gene delivery vehicles, tissue engineering, wound dressings, the isolation of malignant cells, and superior human cell adhesion (9). A nanopore serves as the active core for drug entrapment in the HNT. To improve its persistence, various studies have been done where the HNT drug has been coated with different polymers (10). This study focuses on the release of an active medicinal compound, Bromelain, from HNT.

Papain (EC 3.4.22.2) is a cysteine protease extracted from the latex of the papaya plant (Carica papaya) that has been used to protect plants from insects (11) It has been reported that the enzyme has a high optimal temperature (65°C) and a

wide pH range (12) for its activity (13) Commercial enzymes used in various industries have a different ratio of papain, chymopapain, and papaya peptidase A, resulting in distinct physical, chemical, and biological properties that lead to variations in performance (14)According to in vitro and in vivo research, it has a significant analgesic and antiinflammatory role for allergies of pain in head and tooth pain with no side effects(15). However, clinical evidence to support this claim is still insufficient. These findings may imply that papain is a good model drug for future scientific research into the proteinase class to which it belongs (16).

The goal of this study is to synthesize and test the anti-inflammatory and antimicrobial activity of a halloysite nanotube-bromelainnanocomposite against wound pathogens and infection.

MATERIALS AND METHODS

Preparation of Extract

A clean beaker was taken and 0.294 g of halloysite clay was added and dissolved in 100 mL of distilled water. The solution was filtered by using Whatman no. 1 filter paper. The filtered extract was collected and stored at 4°C for further use.

Synthesis of HNT

100 mg of papain dissolved in 2 ml of distilled water was added to 30 ml of the prepared halloysite extract and kept in a magnetic stirrer for nanoparticle synthesis. The color change was observed visually and photographs were recorded (Figure 1). The solution was centrifuged using lark refrigerated centrifuge at 8000 rpm for 10 minutes and the pellet was collected and washed with distilled water twice. The final purified pellet was collected and dried at 60°C for 2 hours and stored in an airtight eppendorf tube.

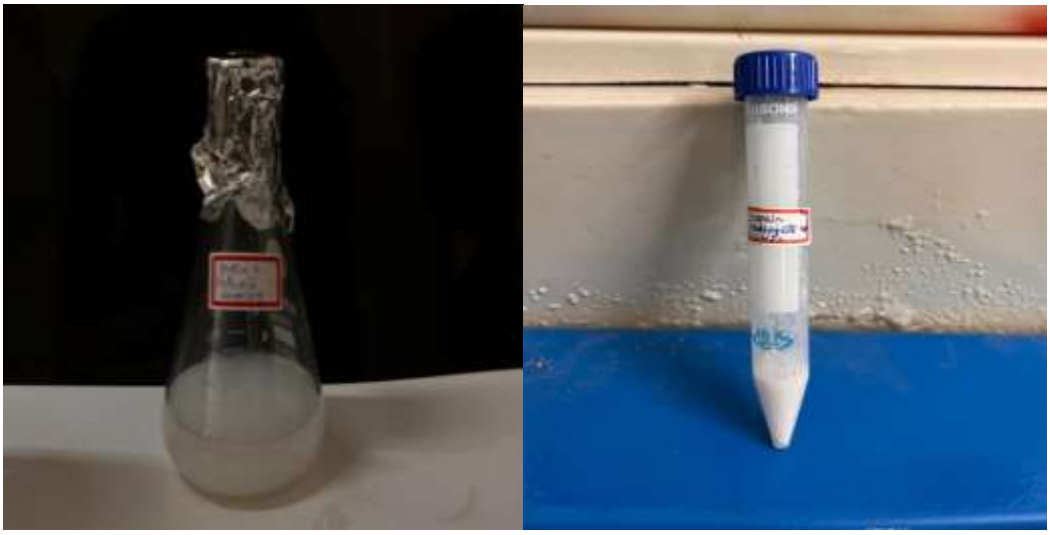

Figure 1: Visual observation of formation of SeNPs

Characterisation of HNT

The synthesized solution was preliminarily confirmed by using UV-visible-spectroscopy. 3 mL of the solution was taken in a cuvette and scanned in double beam UV-vis-spectrophotometry from 300 nm to 700 nm wavelength. The results were recorded for graphical analysis (Figure 2).

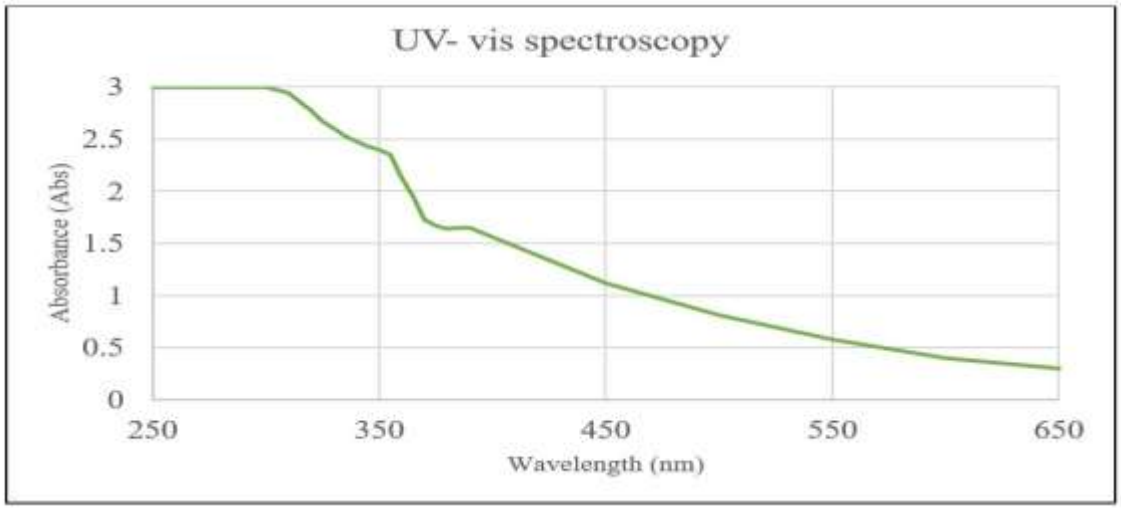

Figure 2: UV-vis spectroscopy. X-axis shows the different wavelength (in nm) and Y-axis shows the absorbance (in Abs). UV-vis spectroscopy revealed a peak at 392 nm.

Cytotoxicity Analysis:

BRINESHRIMP LETHALITY ASSAY

Salt water preparation:

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL.80 µL,100 µL). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours (Figure 3). After 24 hours, the ELISA plates were observed and noted for number of live nauplii's present and calculated by using following formula,

% death = Number of dead nauplii / Number of dead nauplii + number of live nauplii × 100

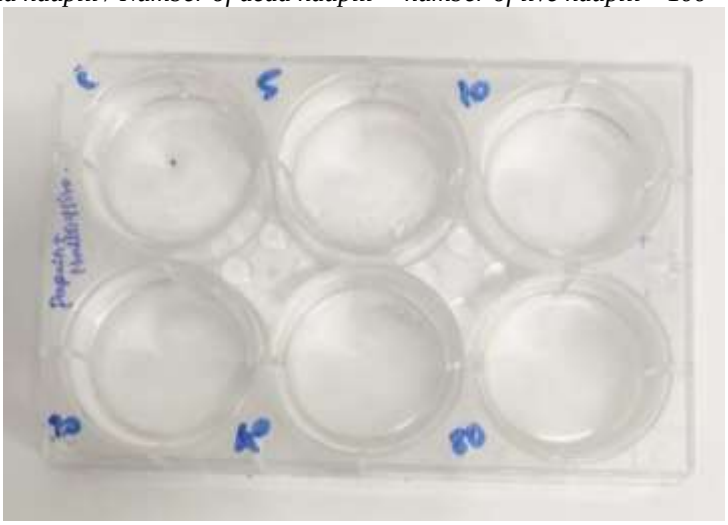

Figure 3: Brine Shrimp Lethality Assay

Antibacterial Activity

Antibacterial activity of respective nanoparticles against the strain staphylococcus aureus, Pseudomonas, and E.faecalis,C.albicans. MHA agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media was poured into the sterilized plates and let to stabilize for solidification. The wells were cut using well cutter and the test organisms were swabbed. The nanoparticles with different concentrations were loaded and the plates were incubated for 24 hours at 37°C. After the incubation time the zone of inhibition was measured (Figure 4).

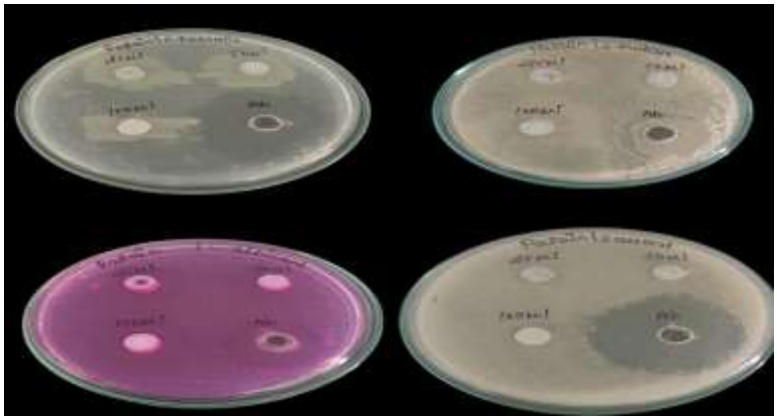

Figure 4: Anti-bacterial activity of papain incorporated HNT

Antioxidant activity

The antioxidant activity of biogenic synthesised zinc oxide nanoparticles was evaluated using the DPPH assay. Diverse concentrations of papain extract (2-10 g/ml) interceded zinc oxide nanoparticles were mixed with 1 ml of 0.1 mM DPPH in methanol and 450 l of 50 mM. Incubated for 30 minutes in TrisHCl buffer (pH 7.4). Later, the absorbance at 517 nm was used to calculate the reduction in DPPH free radicals. BHT was employed as control. The percentage of inhibition was determined from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

RESULTS

Table 1 depicts the cytotoxicity of Halloysite Nanotubes reinforced papain extract. At 5 µL concentration there was a death of 30% of nauplii and at 10µL it showed 40% death followed by 50% death of nauplii at 20µL and 40µL.The highest recorded was at 80µL where it showed 60% of naupliideath.It was seen that as the concentration increased, the cytotoxicity of the nanoparticles increased .

Concentration (µL)	*Viable Nauplii*	*% Death*
5 µL	*7*	*30*
10 µL	*6*	*40*
20 µL	*5*	*50*
40 µL	*5*	*50*
80 µL	*4*	*60*

Table 1: Cytotoxicity of papain infused HNT

Table 2 and Figure 5 describe the anti-microbial activity of the papain incorporated HNTs against wound pathogens. It was found that the HNTs did not show antimicrobial activity against *C.albicans*when compared to the control antibiotic concentration. However, the zone of inhibition obtained against *S.mutants* was constant in all three concentrations (25µL, 50µL and 100µL) and similar to that shown by the control antibiotic (9 mm).*S.aureus*in the concentration of (25µL, 50µL) had a constant zone of inhibition (9mm) and increased to 12mm at 100µL. HNTs didn't show comparable antimicrobial activity against *E.faecalis*where its *showed a constant zone of inhibition of (9mm) at concentration of* (25µL, 50µL and 100µL) respectively.

Concentration of NP solution (µL)	*S. aureus*	*S.mutans*	*C.albicans*	*E.faecalis*
25 µL	9	9	14	9
50 µL	9	9	15	9
100 µL	12	9	16	9
Control	30	9	20	30

Table 2: Antimicrobial Activity

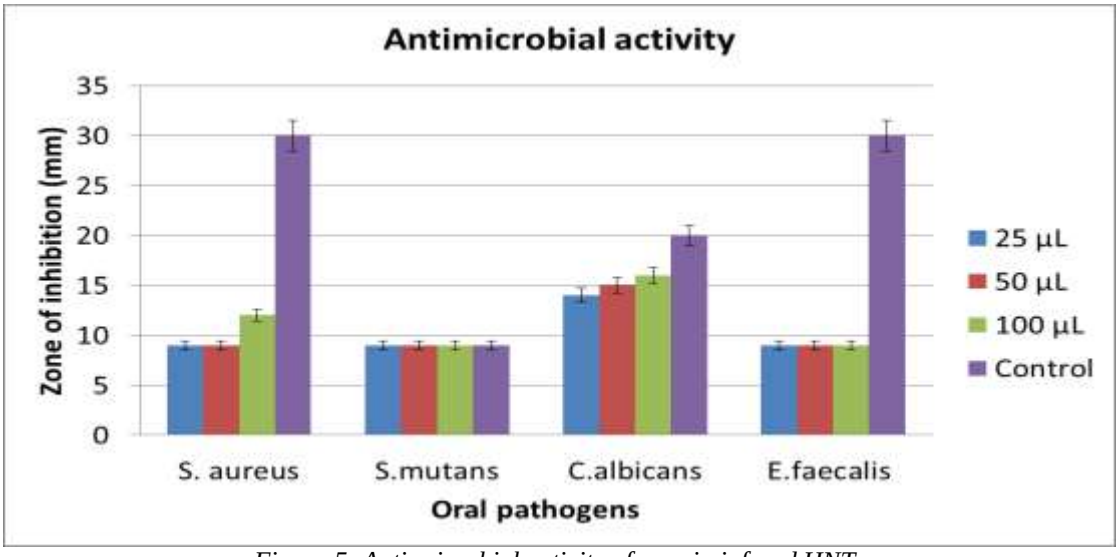

Figure 5: Anti-microbial activity of papain infused HNTs

Figure 6 describes the antioxidant activity of papain infused HNTs which was evaluated by DPPH assay with BHT as the standard (517nm).The synthesized halloysite nanotubes of papain extract showed comparable free radical scavenging activity to the of the standard.

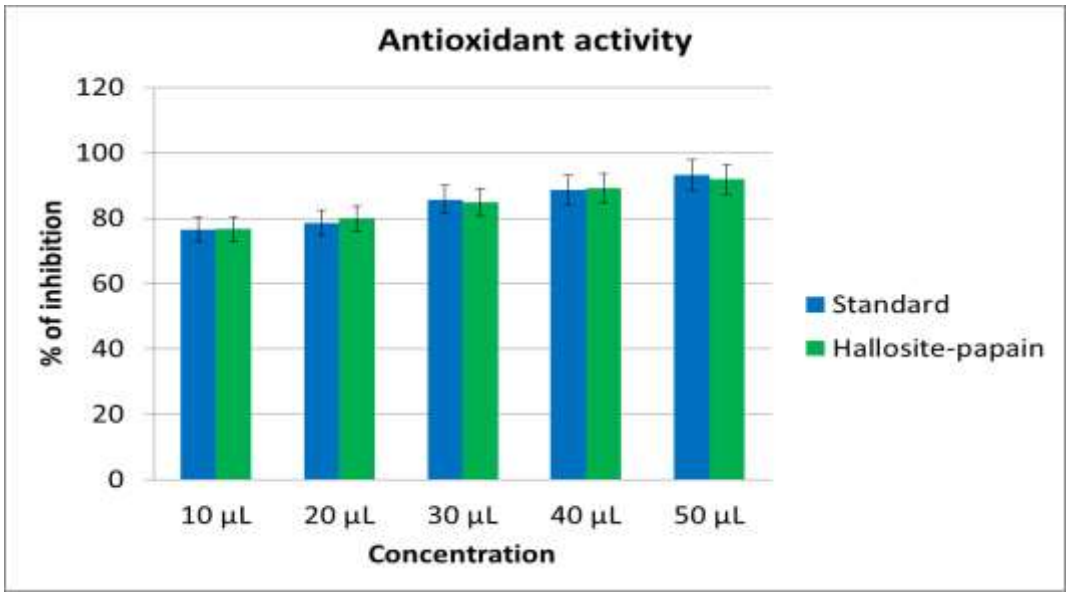

Figure 6 shows antioxidant of Papain infused HNTs

DISCUSSION

Nanoparticle production has been advancing at a rapid pace recently. Nanoparticle synthesis used to be done via physio-chemical approaches earlier. Despite the fact that traditional physical and chemical processes take less time to synthesize vast quantities of nanoparticles, hazardous compounds are necessary as capping agents to maintain stability, resulting in environmental toxicity (11,12). Thus, incorporating plant derived extracts into nanodrug synthesis has been an upcoming trend in recent years.

A study done by Suriyakalaperumalchandran et al. on the cytotoxicity of papain extract showed that the maximum percentage of nauplii death was 30% noted at both 30 µl and 40 µl respectively, which was poor (13). In this study, we observed the efficacy of bromelainwhen incorporated with HNTs was better in showing cytotoxic effects. As the concentration increased, the cytotoxicity activity of the papain incorporated HNTs increased. The highest percentage of death of nauplii was 60% in 80µL concentration of HNTs reinforced with papain extract. Papoin's efficacy as an anticancer drug, either alone or in combination with other medicines, has been limited to a few anecdotal evidence (14). Papain has inhibited humancholangiocarcinoma cell lines in a study by Taussing et al. (15). (16). In Papain-immunized mice the growth rate, invasion and metastasis of both the B16 melanoma and the Lewis lung carcinoma was inhibited.(17).In a study by Akila et al papain has maximum (85%) cytotoxicity effect against liver cancer cell line hepG2(18).papain is a complex mixture of proteolytic enzymes. Its therapeutic value may be attributed to glycoprotein, the potent ingredient in papain (19).

Anti-microbial activity of the papain incorporated HNTs in the current study didn't show any significant zone of inhibition when compared to the control. However, the zone of inhibition obtained against*S.mutants* was constant in all three concentrations (25µL, 50µL and 100µL) and similar to that shown by the control antibiotic (9 mm). papain antimicrobial function is not well understood, although it is thought that it inhibits bacterial growth by hydrolyzing certain peptide bonds in the bacterial cell wall (20) The cell wall is destroyed when papain digests the surface proteins, allowing the cell to leak, enlarge, and open (20,21). papain also prevents bacteria from adhering to certain glycoprotein receptors on the surface, which restricts their growth (22). papain has antibacterial activity against *Alicyclobacillusspp*, and *Pseudomonas aeruginosa*,*Enterococcusfaecalis, P. aeruginosa, S. aureus, Klebsiellapneumoniae, and Salmonella typhi*(23). Furthermore, when papain and antibiotics are used together, the antibacterial activity is enhanced due to higher antibiotic absorption induced by papain, resulting in improved drug distribution in microorganisms (23)

In the current study, the mechanism by which the papain incorporated HNTs may be explained by the higher total phenolics content present in it. papain is a strong antioxidants with high reducing capacity (24)which can be used. Antioxidant activities protect biological systems against damage which are related to oxidative stress in human related disease conditions(25). It was well established that the phenolic compounds may contribute directly to anti-oxidative action.(26) This antioxidant activity is attributed to the phenolic contents in plants probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.(27).Based on the findings of the current study, we can state that Bromelain incorporated HalloysiteNanotubes may be used as an effective alternative to commercially available anti-inflammatory agents.

CONCLUSION

The present study revealed that Halloysite nanotubes can be synthesized in a simple, eco-friendly method using papain extract. These papain loaded HNTs have the potential to be used as an effective antibacterial and antioxidant agent for wound pathogens. Hence, it can be employed in large scale production and used for targeted drug delivery to accelerate wound healing.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Saveetha Dental College and Hospitals, Chennai for their support in enabling us to carry out this study.

CONFLICT OF INTEREST

There exists no conflicts of interest as defined by the authors.

REFERENCES

1. Ganapathy D, Shanmugam R, Pitchiah S, Murugan P, Chinnathambi A, Alharbi SA, et al. Potential Applications of Halloysite Nanotubes as Drug Carriers: A Review. J Nanomater [Internet]. 2022 Apr 20 [cited 2022 May 24];2022. Available from: https://www.hindawi.com/journals/jnm/2022/1068536/
2. Danyliuk N, Tomaszewska J, Tatarchuk T. Halloysite nanotubes and halloysite-based composites for environmental and biomedical applications. J Mol Liq. 2020 Jul 1;309:113077.
3. Rawtani D, Khatri N, Tyagi S, Pandey G. Nanotechnology-based recent approaches for sensing and remediation of pesticides. J Environ Manage. 2017 Nov 18;206:749–62.
4. Barman M, Mahmood S, Augustine R, Hasan A, Thomas S, Ghosal K. Natural halloysite nanotubes /chitosan based bio-nanocomposite for delivering norfloxacin, an anti-microbial agent in sustained release manner. Int J BiolMacromol. 2020 Nov 1;162:1849–61.
5. Preethika M, Sundramoorthy AK. Humic acid/halloysite nanotube/flavin adenine dinucleotide nanocomposite based selective electrochemical biosensor for hydrogen peroxide. Appl Surf Sci. 2019 Sep 15;488:503–11.
6. Lvov Y, Abdullayev E. Functional polymer–clay nanotube composites with sustained release of chemical agents [Internet]. Vol. 38, Progress in Polymer Science. 2013. p. 1690–719. Available from: http://dx.doi.org/10.1016/j.progpolymsci.2013.05.009
7. Santos AC, Ferreira C, Veiga F, Ribeiro AJ, Panchal A, Lvov Y, et al.Halloysite clay nanotubes for life sciences applications: From drug encapsulation to bioscaffold. Adv Colloid Interface Sci. 2018 Jul;257:58–70.
8. Paola MD, Di Paola M, Quarta A, Pisani P, Conversano F, Sbenaglia EA, et al. Surface Coating Highly Improves Cytocompatibility of Halloysite Nanotubes: A Metabolic and Ultrastructural Study [Internet]. Vol. 15, IEEE Transactions on Nanotechnology. 2016. p. 770–4. Available from: http://dx.doi.org/10.1109/tnano.2016.2546955
9. Cavallaro G, Milioto S, Lazzara G. Halloysite Nanotubes: Interfacial Properties and Applications in Cultural Heritage. Langmuir. 2020 Apr 14;36(14):3677–89.
10. Fizir M, Dramou P, Dahiru NS, Ruya W, Huang T, He H. Halloysite nanotubes in analytical sciences and in drug delivery: A review. MikrochimActa. 2018 Jul 25;185(8):389.
11. Andersson M, Pedersen JS, Palmqvist AEC. Silver Nanoparticle Formation in Microemulsions Acting Both as Template and Reducing Agent [Internet]. Vol. 21, Langmuir. 2005. p. 11387–96. Available from: http://dx.doi.org/10.1021/la050937j
12. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of Gold Nanotrianglesand Silver Nanoparticles Using Aloe vera Plant Extract [Internet]. Vol. 22, Biotechnology Progress. 2006. p. 577–83. Available from: http://dx.doi.org/10.1021/bp0501423
13. Chandran SP, Nachimuthu K. Formulation and characterization of papain loaded solid lipid nanoparticles against

human colorectal adenocarcinoma cell line. Asian J Pharm Clin Res. 2018 Oct 7;11(10):393.
14. Chobotova K, Vernallis AB, Majid FAA. Bromelain's activity and potential as an anti-cancer agent: Current evidence and perspectives. Cancer Lett. 2010 Apr 28;290(2):148–56.
15. Taussig S, Szekerczes J, Batkin S. Inhibition of Tumour Growth*in vitro*by Bromelain, an Extract of the Pineapple Plant (*Ananas comosus*) [Internet]. Vol. 51, Planta Medica. 1985. p. 538–9. Available from: http://dx.doi.org/10.1055/s-2007-969596
16. Tysnes BB, Maurer HR, Porwol T, Probst B, Bjerkvig R, Hoover F. Bromelain reversibly inhibits invasive properties of glioma cells. Neoplasia. 2001 Nov;3(6):469–79.
17. Bellelli A, Mattioni M, Rusconi V, Sezzi ML, Bellelli L. Inhibition of tumor growth, invasion and metastasis in papain-immunized mice. Invasion Metastasis. 1990;10(3):142–69.
18. Akila, Sushama, Ramanathan. Study on in vitro cytotoxicity of papain against liver cancer cell line Hep G2. Cell [Internet]. Available from: https://www.researchgate.net/profile/Ramanathan-Kumaresan/publication/286492394_Study_on_in_vitro_cytotoxicity_of_papain_against_liver_cancer_cell_line_HEP_G2/links/56e8f19f08ae9bcb3e1cebf1/Study-on-in-vitro-cytotoxicity-of-papain-against-liver-cancer-cell-line-HEP-G2.pdf
19. Marinescu SA. STUDY ON THE CONCENTRATE OF PROTEOLYTIC ENZYMES ENRICHED IN BROMELAIN AND ITS EFFECTS ON INTERMEDIATE AND EXTENSIVE BURNS [Internet]. Vol. 67, FARMACIA. 2019. p. 522–30. Available from: http://dx.doi.org/10.31925/farmacia.2019.3.22
20. George S, Bhasker S, Madhav H, Nair A, Chinnamma M. Functional Characterization of Recombinant Bromelain of Ananas comosus Expressed in a Prokaryotic System [Internet]. Vol. 56, Molecular Biotechnology. 2014. p. 166–74. Available from: http://dx.doi.org/10.1007/s12033-013-9692-2
21. Mamo J. Antibacterial and Anticancer Property of Bromelain: A Plant Protease Enzyme from Pineapples (Ananas comosus) [Internet]. Vol. 19, Current Trends in Biomedical Engineering & Biosciences. 2019. Available from:http://dx.doi.org/10.19080/ctbeb.2019.19.556009
22. Bromelain: an Overview of Applications in Medicine and Dentistry [Internet]. Vol. 11, Biointerface Research in Applied Chemistry. 2020. p. 8165–70. Available from: http://dx.doi.org/10.33263/briac111.81658170
23. Oliveira HL da CD de, Fleming MECK, Silva PV, Paula GR de, Futuro DO, Velarde GC, et al. Influence of papain in biofilm formed by methicillin-resistant Staphylococcus epidermidis and methicillin-resistant Staphylococcus haemolyticus isolates. Braz J Pharm Sci. 2014;50(2):261–7.
24. Osman A, El-Hadary A, Korish AA, AlNafea HM, Alhakbany MA, Awad AA, et al. Angiotensin-I Converting Enzyme Inhibition and Antioxidant Activity of Papain-Hydrolyzed Camel Whey Protein and Its Hepato-Renal Protective Effects in Thioacetamide-Induced Toxicity. Foods [Internet]. 2021 Feb 20;10(2). Available from: http://dx.doi.org/10.3390/foods10020468
25. López-Pedrouso M, Borrajo P, Pateiro M, Lorenzo JM, Franco D. Antioxidant activity and peptidomic analysis of porcine liver hydrolysates using alcalase, bromelain, flavourzyme and papain enzymes. Food Res Int. 2020 Nov;137:109389.
26. Sarmadi BH, Ismail A. Antioxidative peptides from food proteins: a review. Peptides. 2010 Oct;31(10):1949–56.
27. Mahmoodani F, Ghassem M, Babji AS, Yusop SM, Khosrokhavar R. ACE inhibitory activity of pangasius catfish (Pangasius sutchi) skin and bone gelatin hydrolysate [Internet]. Vol. 51, Journal of Food Science and Technology. 2014. p. 1847–56. Available from: http://dx.doi.org/10.1007/s13197-012-0742-8