Development of Chitosan-based Films Containing *Hypericum perforatum* L. and *Citrus limon* L. as Potential Wound Dressing Materials

DENIZ AKIN SAHBAZ[1]* AND BETUL TURP KAYA[2]

[1]*Department of Chemical Engineering, Faculty of Engineering, Pamukkale University, 20070, Turkey.*

[2]*Department of Chemical Engineering, Faculty of Engineering, Afyon Kocatepe University, 03200, Turkey.*

ABSTRACT

The objective of this study is to develop chitosan-based films containing Hypericum perforatum L. and/or Citrus limon L. oils and to investigate their suitability as novel wound dressing materials. The morphology of the chitosan-based films were observed by means of employing the scanning electron microscopy (SEM) and the chemical structure characterization was performed via Fourier Transform Infrared Spectroscopy (FTIR). Hypericum perforatum L. and/or Citrus limon L. were successfully incorporated to the chitosan films. Antibacterial, swelling, and mechanical properties of these films were investigated. The antibacterial property was enhanced by incorporating Hypericum perforatum L. and Citrus limon L. oils in the chitosan films. The outcome of the study reveals that the Hypericum perforatum L. and Citrus limon L. incorporated inside chitosan-based films are of great potential wound dressing in the future, because of their good biocompatible, antimicrobial, and physical properties.

KEYWORDS: *Chitosan films, Swelling degree, Tensile strength, Wound dressing materials*

INTRODUCTION

Wound dressing materials protect the wound areas against infection and microorganisms. An ideal wound dressing has the ability to maintain moisture, promote wound healing, be nontoxic, non-adherent, applicable, flexible, biodegradable to accelerate the healing rate, and decrease the infection chance[1]. In this case, the choice of appropriate materials is the most important point. Alginate-based

J. Polym. Mater. Vol. **38,** No. 3-4, 2021, 247-256

Correspondence author e-mail: dsahbaz@pau.edu.tr
DOI : https://doi.org/10.32381/JPM.2021.38.3-4.6

composite[2], carbohydrate polymer-based silver nanocomposites[3], silk fibroin[4], collagen, gelatin and plant bioactive compounds[5], are commonly used wound dressing materials.

Chitosan is a polycationic biopolymer found in the crustaceans and exoskeleton of insects as well as in the fungi, precisely located inside its cell walls. Chitosan exhibits a lot of antimicrobial activity, and this is one of its most important properties, which attracts a lot of attention for application in drug delivery carrier[6], and other similar biomedical fields including surgical thread[7], and wound healing materials[8]. Furthermore, chitosan shows good biocompatibility, non-toxicity, stability, biodegradability, and positive effects on wound healing. Also, it posses good oxygen permeability, which prevents the oxygen deprivation on tissues around the wound[9]. Due to these properties, chitosan is an excellent candidate for the treatment of wounds as a wound dressing material[10].

In recent years, to minimize wound infection risk and provide excellent wound repair, antimicrobial agents such as sodium fusidate[11], *Hypericum perforatum* L.[12], curcumin[13],Allium sativum, Cleome droserifolia[14], silver[15], are included in the wound dressing. Among the agents, *Hypericum perforatum* L. and its derivatives have a growing interest. It is traditionally used as a herbal tea and food supplement and it has become one of the medicinal plants that are consumed the most in the world. *Hypericum perforatum* L. extract has been found to be antidepressant, antioxidant, anxiolytic, anticancer, anti-inflammatory, antimicrobial, and antiviral[16]. Therefore, *Hypericum perforatum* L. oil incorporated materials can be used to treat wounds, burns, and inflammation of the skin[12].

Citrus limon, commonly known as lemon, is a potential source of vitamin C and used for many applications such as medicines[17], cosmetics[18], aromatherapy[19], food[20], and skin diseases[21]. *Citrus limon* has antioxidative, anti-inflammatory, antiviral, antiallergic, antimutagenic, antiproliferative, and anticarcinogenic biological functions among others[17].

Although both *Citrus limon* and *Hypericum perforatum* L. oils are put into good use in the field of traditional medicine, wound dressing applications of the oils have very limited amounts of literature research. To our knowledge, no previous studies have been carried out on the use of *Citrus limon* and *Hypericum perforatum* L. oils together for wound dressing. This study aims to synthesize, for the first time, chitosan-based films containing *Citrus limon* besides *Hypericum perforatum* L. as novel wound dressing materials.

EXPERIMENTAL

Materials

A medium level of molecular weight of chitosan was purchased from Acros Organics. Glycerol (> 99.0%) used as a plasticizer and tablets with phosphate-buffered saline (pH: 7.4 PBS tablets) were delivered by Sigma-Aldrich. *Hypericum perforatum* L. and *Citrus limon* L. oils (Leafy) were purchased from a local pharmacy. Emulgin 286 and acetic acid (100%) were supplied by Serva and Merck, respectively.

Preparation of the Chitosan-based Films

The films with the chitosan base were obtained by solvent dropping method[22]. Components of each film are demonstrated in Table 1. 100 mL acetic acid solution (1%, w/w) was used to dissolve chitosan (1 g) at room temperature during the presence of magnetic mixing for 48/ h, for complete dissolution. Glycerol (0.35

mL) was added dropwise and then stirred for 1 hour. Then 0.1 mL of Emulgin 286 and a determined amount of *Hypericum perforatum* L. and *Citrus limon* L. oils (Table 1) were added dropwise. The chitosan suspension and the *Hypericum perforatum* L. and *Citrus limon* L. oils were emulsified (20,000/ rpm for 1/ min) using a high-stirrer homogenizer (WiseTis, HG-15D). Each obtained chitosan emulsion was poured onto a petri dish and was dried at 37°C under vacuum for 48 hours. The chitosan-based films were peeled off carefully from the petri dish. Chitosan-based films were obtained, which were respectively called chitosan film (C-F), chitosan/*Citrus limon* L. film (C/CL-F), chitosan/*Hypericum perforatum* L. film (C/HP-F), and chitosan/*Citrus limon* L./*Hypericum perforatum* L. film (C/CL/HP-F).

TABLE 1. Chitosan-based films' composition.

Ingredients	C-F	C/CL-F	C/HP-F	C/CL/HP-F
Chitosan Solution (mL)	100	100	100	100
Citrus limon L. (mL)	-	0.25	-	0.125
Hypericum perforatum L. (mL)	-	-	0.25	0.125

Characterization of the Chitosan-based Films

The chitosan-based films' chemical structure and the components' functional group interactions including *Hypericum perforatum* L. and/or *Citrus limon* L. oils were characterized by the FTIR instrument (4000-400 cm^{-1}) (Agilent Technologies, Cary 630). The morphology of chitosan-based films were observed using a scanning electron microscope (SEM, Leo 1430 VP, Germany).

Swelling Properties

The chitosan-based films' swelling ability was evaluated via placing the films in solution with phosphate buffered saline (pH: 5.5 and 7.4) at 37°C. Firstly, the films were split into 1 × 1 cm^2 pieces, they were placed inside a vacuum oven for the duration of 24h. The films were dried and then weighed, and later placed in pH 7.4 and pH 5.5 PBS solutions at 37°C. At the time when swelling equilibrium was reached, the specimens were weighed again after the excess water was removed from the surface of the films using a filter paper. The equilibrium swelling ratio was determined until no further weight change was detected in films. The degree of swelling for the films was calculated using the following equation[23]:

$$\%\ Swelling\ Degree = [(w - w_0)/w_0]\ x\ 10 \quad (1)$$

where w_0 is the dry film's weight, and w is the swollen film's weight during equilibrium.

Mechanical Tests

Mechanical properties like the elongation at break, tensile strength, and Elastic Modules, were determined by tensile tests. The tests were performed on dry rectangular specimens (10 mm x 70 mm) by a tensile testing machine (Instron, 8801, UK) at a strain rate of 10 mm/min.

In vitro Antibacterial Activity Evaluation of the Chitosan-based Films

Chitosan-based films' antibacterial activity was studied using the method that is detailed in ISO 22196. The research was based on two species of bacterial strains, the strains of *Escherichia coli* ATCC8739 and *Staphylococcus aureus* ATCC6538. Incubation was applied to the plates for 24 h at 37°C.

The antibacterial activity was calculated using the following equation[24]:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t \quad (2)$$

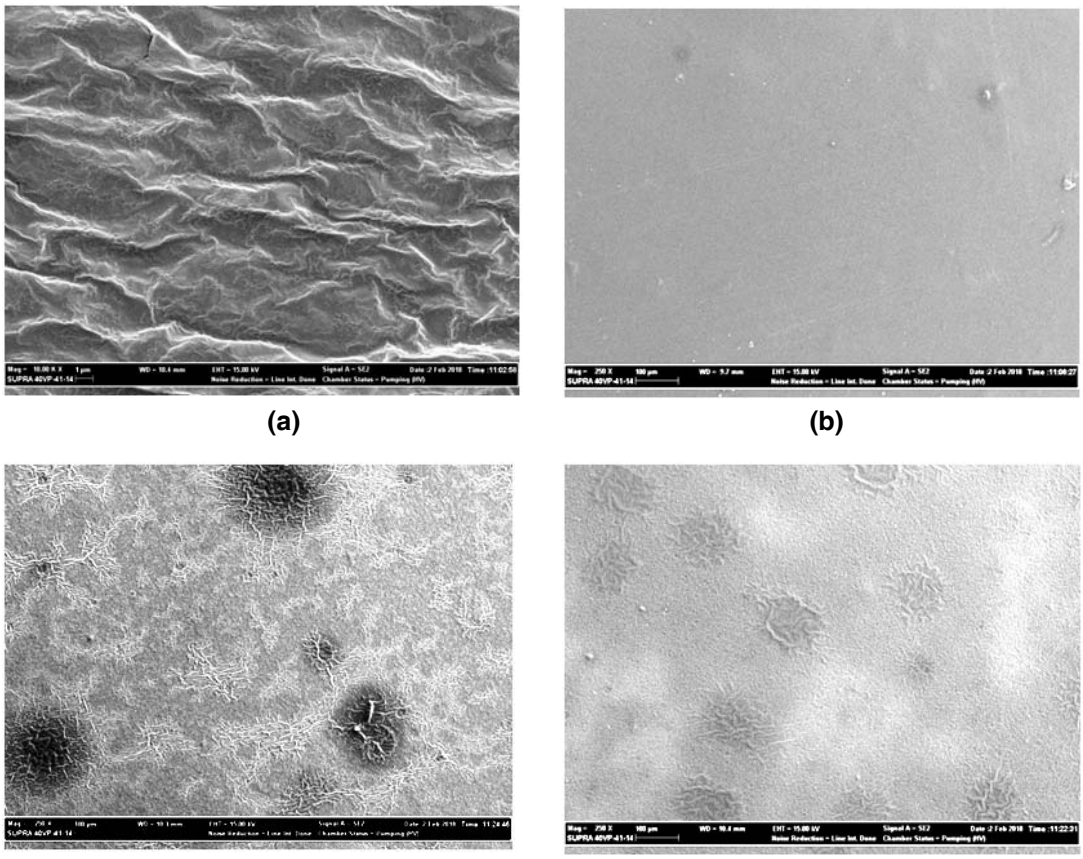
where R represents the antibacterial activity; U_0 is used for the average of the common logarithm of the number of viable bacteria (in cells/cm^2), which is recovered from the untreated test specimens right after inoculation; U_t stands for the average of the common logarithm of the number of viable bacteria, in cells/cm^2, which is recovered after 24 h from the untreated test specimens; A_t is denoting the average of the common logarithm of

the number of viable bacteria, in cells/cm^2, recovered after 24 h from the treated test specimens.

RESULTS AND DISCUSSION

SEM Analysis

The chitosan-based films' surface morphology was investigated by SEM which is shown in Figure 1. As observed in Figure 1(a), the surface of chitosan film is rough while incorporating *Hypericum perforatum* L. and *Citrus limon* L. oils in chitosan films caused an increase in the smoothness of film surface (Figure 1b to d).



(a) **(b)**

(c) **(d)**

Figure 1. SEM micrograph of the surface of the C-F (a), C/CL-F (b), C/HP-F (c), and C/CL/HP-F (d).

FTIR Analysis

FTIR spectra of C-F, C/HP-F, C/CL-F, and C/CL/HP-F are shown in Figure 2. In all chitosan-based film spectra, there are the characteristic absorbance bands that are centered at 1690 and 1650-1580 cm^{-1}, which correspond to the

C=O stretching vibration of primary amide groups and the N–H bending of $–NH_2$, respectively. The strong broadband at the wavenumber region of 3300–3500 cm^{-1} is the characteristic of the –NH– and –OH stretching vibrations[25]. The peaks under 880 cm^{-1} and at 1200 cm^{-1} show the aromatic rings and the C–O phenolic groups, respectively, 1500 and 1600 cm^{-1} are where C=C stretching appears, and that is the primary evidence for the presence of the *Hypericum perforatum* L. and *Citrus limon* L. oils in the chitosan films[26]. It is observed that there is no important change of structure in the chitosan film by incorporation of *Hypericum perforatum* L. and *Citrus limon* L. oils since there is no peak intensity change and no wavenumber shift. From the FTIR results, it can be confirmed that there is no chemical interaction between chitosan and *Hypericum perforatum* L. and *Citrus limon* L. oils[27].

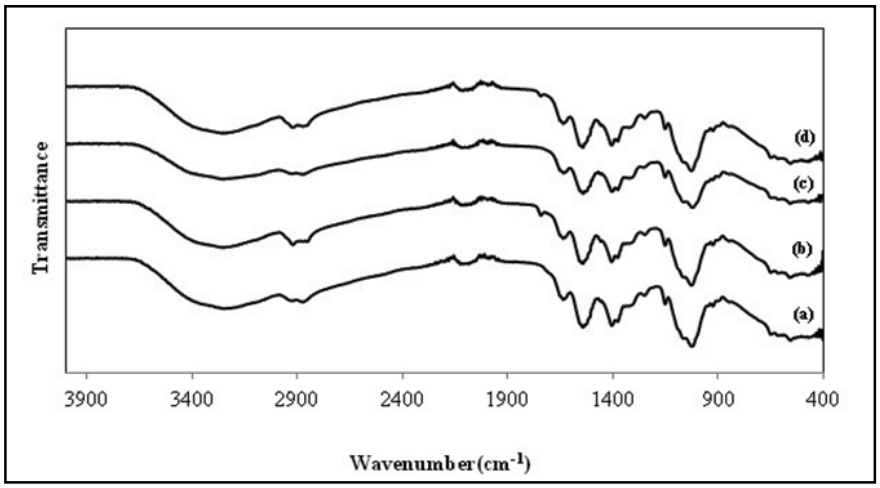


Figure 2. FTIR spectra of the C-F (a), C/HP-F (b), C/CL-F (c), and C/CL/HP-F (d).

Swelling Degree

Swelling ability is one of the most important properties of wound dressing materials. A high swelling ratio has allowed the materials to carry out the absorption of wound exudates and prevent drying of the aforementioned wound and sticking of the dressing[28]. The swelling degree values for the chitosan-based films were determined and compared with one another. As can be seen in Figure 3 and Figure 4, the swelling amount of the chitosan-based films decreases by adding *Hypericum perforatum* L. and/or *Citrus limon* L. oils in the film. C-F presents the best swelling behavior. *Citrus limon* L. oil incorporated films have a higher swelling degree in comparison with *Hypericum perforatum* L. oils incorporated films. The mean swelling degree of C-F, C/CL-F, C/HP-F and C/CL/HP-F were 170.6%, 150.0%, 98.5% and 126.7% at pH 7.4, respectively; the same values were 555.6%, 385.3%, 209.6% and 258.8% at pH 5.5, respectively. According to

these results, the synthesized chitosan-based films have a high swelling ability, this result might be related to the hydrophilic nature of chitosan (free –OH, $–NH_2$ groups), which enhance the capacity of water uptake[29,30]. By adding *Hypericum perforatum* L. and *Citrus limon* L. oils in the structure of chitosan, the swelling ratio values decreased due to the hydrophobicity of these essential oils and decline of porosity of the films[31].

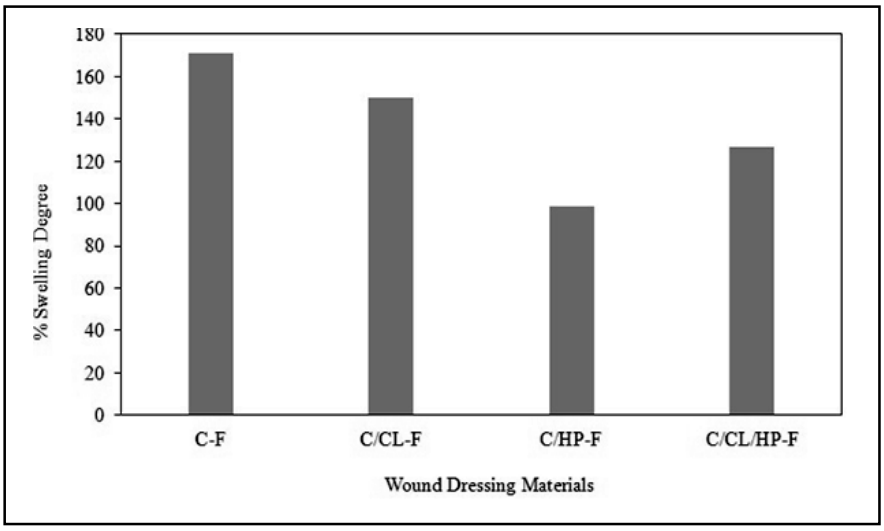


Figure 3. Swelling degree of the chitosan-based films at pH 7.4.

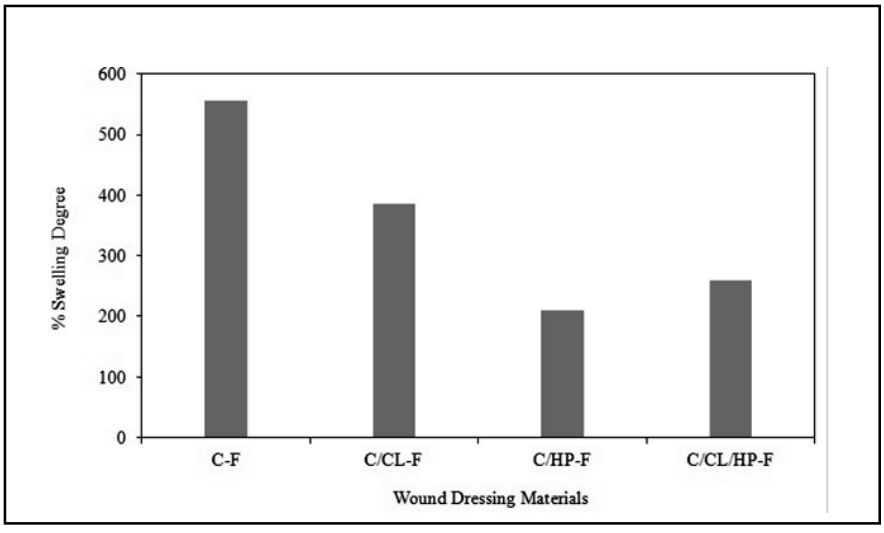


Figure 4. Swelling degree of the chitosan-based films at pH 5.5.

Mechanical Properties

The mechanical strength of wound dressing materials is crucial for easy handling and application[32]. The wound dressing materials' results of their mechanical properties are given in Table 2. As can be seen from Table 2, while the tensile strength of C-F is higher than that of the C/CL-F, C/HP-F, and C/CL/HP-F, break strain (%) of C-F is lower than that of the films. According to these results, by adding *Hypericum perforatum* L. and/or *Citrus limon* L. oils to polymer structures, elastic modules decrease too, while break strain (%) point slightly increases. The chitosan film alone has high tensile strength due to the good film forming ability[33]. With the addition of *Hypericum perforatum* L. and/or *Citrus limon* L. oils to chitosan films, elastic modules are observed to decrease by 57.1% and 75.0%, respectively. Previous studies also showed that incorporation of essential oils is resulted in a decrease in tensile strength and an increase in flexibility because of the plasticizing and interrupting impact of essential oils extracts on the hydrogen-bonding network of the polymeric matrix[33,34].

TABLE 2. Chitosan-based films' mechanical parameters.

Wound Dressing Materials	Break Strain (%)	Elastic Modules (N/mm^2)	Tensile Strength (Mpa)
C-F	6.22	2402.8	0.1178
C/CL-F	7.21	1030.8	0.1107
C/HP-F	6.96	600.4	0.0844
C/CL/HP-F	8.01	860.8	0.0824

Antibacterial Property

According to the data that was gathered from the literature, wound dressing loaded with antimicrobial agents like essential oils is regarded as a promising alternative to reduce any bacterial colonization on the wound and infection. In addition to essential oils, chitosan displays a high level of antimicrobial activity, which results from the positively charged groups of chitosan that interact in an electrostatic manner with the negatively charged groups that are situated on the cell wall of the bacteria[35]. The chitosan-based films' antimicrobial activity containing *Hypericum perforatum* L. and/or *Citrus limon* L. was investigated against *E. coli* as a Gram-negative bacterium and *S. aureus* as a Gram-positive bacterium. Table 3 and Table 4 show results that have been gathered for the chitosan-based films versus the bacterial strains that were tested. The value of the antibacterial activity is used to characterize the effectiveness of an antibacterial agent. According to standard ISO 22196, a material's surface is antibacterial when reduction becomes $R \geq 2$[36]. All chitosan-based films synthesized as wound dressing materials show antibacterial effects against *E. coli* and *S. aureus* bacterial strains. Among the films, especially the C/CL-F has an antibacterial effect that is higher on both gram-positive and gram-negative bacteria compared to other films. Antibacterial activity of the C/CL-F directly

concerned with the components such as polyphenols, ascorbic acid, protopine, and corydaline alkaloids, lactones, polyacetylene, acyclic sesquiterpenes, hypericin, and pseudohypericin in *Citrus limon* oils that they contain[37].

TABLE 3. The antibacterial activity of the chitosan-based films against *E. coli*.

Wound Dressing Material	Inoculum Density (cfu.mL^{-1})	U_0 (cfu.cm^{-2})	U_t (cfu.cm^{-2})	A_t (cfu.cm^{-2})	Antibacterial activity (R)
C-F	7.8×10^5	4×10^4	4.3×10^4	100	2.63
C/CL-F				10	3.63
C/HP-F				40	3.03
C/CL/HP-F				30	3.15

TABLE 4. The antibacterial activity of the chitosan based-films against *S. Aureus*.

Wound Dressing Material	Inoculum Density (cfu.mL^{-1})	U_0 (cfu.cm^{-2})	U_t (cfu.cm^{-2})	A_t (cfu.cm^{-2})	Antibacterial activity (R)
C-F	3×10^5	6×10^4	6.1×10^4	100	2.78
C/CL-F				10	3.78
C/HP-F				60	3.08
C/CL/HP-F				50	3.00

CONCLUSIONS

The present study demonstrates a simple method in producing novel chitosan-based films containing *Hypericum perforatum* L. and/or *Citrus limon* L. for wound dressing applications. The effect of *Hypericum perforatum* L. and/or *Citrus limon* L. addition into chitosan films, which are used to treat wounds was investigated on the antimicrobial, swelling, morphological and mechanical film properties. All chitosan-based films are found to be showing antibacterial activity. In addition, C/CL-F demonstrated the highest antibacterial activity because of primarily the components that *Citrus limon* L. oils contain. Besides, the chitosan-based films demonstrated acceptable mechanical properties and had good water absorption properties, providing a moist environment for wounds. These materials are excellent biocompatible. *Hypericum perforatum* L. and *Citrus limon* L. oils included chitosan films might be employed as possible wound dressing materials.

ACKNOWLEDGMENTS

Authors would like to kindly thank The Scientific and Technological Research Council of Turkey (TUBITAK) for providing financial support via Project 1919B011702009.

REFERENCES

1. S. Tort, F.T. Demiröz, ª. C. Cevher, S.Sarıbaþ, C. Özoðul and F. Acartürk, *Burns*. 46 (2020): 143.
2. K. Varaprasad, T.Jayaramudu, V. Kanikireddy, C. Toro and E. R. Sadiku, *Carbohydr. Polym.* 235 (2020): 116025.
3. M. Rahimi, E. B. Noruzi, E. Sheykhsaran, B. Ebadi, Z. Kariminezhad, M. Molaparast, M. G. Mehrabani, B. Mehramouz, M. Yousefi, R. Ahmadi, B. Yousefi, K. Ganbarov, F. S. Kamounah, V. Shafiei-Irannejad and H. S. Kafil, *Carbohydr. Polym.* 231 (2019): 115696.
4. M. Farokhi, F.Mottaghitalab, Y.Fatahi, A. Khademhosseini and D. L. Kaplan, *Trends Biotechnol.* 36 (2018): 907.
5. A. Gaspar-Pintiliescu, A. M.Stanciuc and O. Craciunescu, *Int. J. Biol. Macromol.*138 (2019): 854.
6. E. Kenawy, A. M. Omer, T. M. Tamer, M. A. Elmeligy and M. S. Mohy Eldin, *Int. J. Biol. Macromol.* 139 (2019): 440.
7. M. M. Islam, M. Shahruzzaman, S. Biswas, M. N. Sakib and T. U. Rashid, *Bioact. Mater.* 5 (2020): 164.
8. A. Moeini, P. Pedram, P. Makvandi, M. Malinconico and G. G. d'Ayala, *Carbohydr. Polym.* 233 (2020): 115839.
9. B. Evranos, D. Aycan and N. Alemdar, *Carbohydr. Polym.* 222 (2019): 115007.
10. H. Hamedi, S. Moradi, S. M. Hudson and A. E. Tonelli, *Carbohydr. Polym.*199 (2018): 445.
11. S. G. Jin, K. S. Kim, D. W. Kim, D. S. Kim, Y. G. Seo, T. G. Go, Y. S.Youn, J. O. Kim, C. S. Yong and H. G. Choi, *Int. J. Pharm*. 497 (2016): 114.
12. S. Günes and F. Týhmýnlýoglu, *Int. J. Biol. Macromol.*102 (2017): 933.
13. G. D. Venkatasubbu and T. Anusuya, *Int. J. Biol. Macromol.* 98 (2017): 366.
14. W. A. Sarhan, H. M. Azzazy andI. M. El-Sherbiny, *ACS Appl. Mater. Interfaces.* 8 (2016): 6379.
15. G. Man, R. Lei, W. Ting, S. Li-Ping, L. Ling-Rong and Z. Qi-Qing, *J. Appl. Polym. Sci.* 105 (2007): 1679.
16. M. Jarzebski, W.Smulek, H. M. Baranowska, L. Masewicz, J. Kobus-Cisowska, M. Ligaj and E. Kaczorek, *Food Hydrocoll.* 104 (2020): 105748.
17. J. A. Del Rio, M. D. Fuster, P. Gomez, I. Porras, A.Garcýa-Lidon and A. Ortuno, *Food Chem.* 2004, 84, 457.
18. N. Mahato, K. Sharma, M. Sinha and M. H. Cho, *J. Funct. Foods.* 40 (2018): 307.
19. B. Ali, N. A. Al-Wabel, S. Shams, A. Ahamad, S. A. Khan and F. Anwar, *Asian Pac. J. Trop. Biomed.* 5 (2015): 601.
20. E. Gonzalez-Molina, R. Dominguez-Perles, D. A. Moreno and C. Garcia-Viguera, *Natural J. Pharm. Biomed. Anal.* 51 (2010): 327.
21. W. M. Otang and A. J. S. Afolayan, *Afr. J. Bot.* 102 (2016): 46.
22. A. D. Sezer, F. Hatipoglu, E. Cevher, Z. Ogurtan, A. L. Bas and J. Akbugu, *AAPS Pharm Sci Tech.* 8 (2007): E94.
23. S. Koosehgol, M. Ebrahimian-Hosseinabadi, M. Alizadeh and A. Zamanian, *Mater. Sci. Eng. C.* 79 (2017) 66.
24. ISO 22196: Plastics — Measurement of antibacterial activity on plastics surfaces.
25. F. Lv, C. Wang, P. Zhu and C. Zhang, *Cellulose.* 21 (2014): 4405.
26. F. Pourhojat, M. Sohrabi, S. Shariati, H. Mahdavi and L. Asadpour, *Res. Chem. Intermed.* 43 (2017): 297.
27. D. Altiok, E. Altiok and F. J. Tihminlioglu, *Mater. Sci. Mater. Med.* 21 (2010): 2227.
28. K. Nesovic, A. Jankovic, T. Radetic, M. Vukasinovic-Sekulic, V. Kojic, L. Zivkovic,

A.Peric-Grujic, K. Y. Rhee and V. Miskovic-Stankovic, *Eur. Polym. J.* 121 (2019): 109257.

29. N. T. Ardekani, M. Khorram, K. Zomorodian, S. Yazdanpanah, H. Veisi and H. Veisi, *Int. J. Biol. Macromol.* 125 (2019): 743.
30. V. M. Correlo, E. D. Pinho, I. Pashkuleva, M. Bhattacharya, N. M. Neves and R. L. Reis, *Macromol. Biosci.* 7 (2007): 354.
31. Bölgen, N., Demir, D., Yalçın, M. S., Özdemir, S. *Int. J. Biol. Macromol.* 161 (2020): 1581.
32. A. Ehterami, M. Salehi, S. Farzamfar, A. Vaez, H. Samadian, H. Sahrapeyma, M. Mirzaii, S. Ghorbani and A. Goodarzi, *Int. J. Biol. Macromol.* 117 (2018): 601.
33. Amalraj, A., Haponiuk, J. T., Thomas, S., Gopi, S. *Int. J. Biol. Macromol.* 151 (2020): 366.
34. Barzegar, S., Zare, M. R., Shojaei, F., Zareshahrabadi, Z., Koohi-Hosseinabadi, O., Saharkhiz, M. J., Iraji, A., Zomorodian, K., Khorram, M. *Int. J. Pharm.* 597 (2021) 120288.
35. D. Simões, S. P. Miguel, M. P. Ribeiro, P. Coutinho, A. G. Mendonça and I. J. Correia, *Eur. J. Pharm. Biopharm.* 127 (2018): 130.
36. M. Michalska-Sionkowska, M. Walczak and A. Sionkowska, *Polym. Test.*63 (2017): 360.
37. M. J. Dhanavade, C. B. Jalkute, J. S. Ghosh and K. D. Sonawane, *Br. J. Pharmacol. Toxicol.* 2 (2011): 9.

Received: 01-08-2021

Accepted: 09-08-2021