Bioorganic Chemistry 82 (2019) 224–228

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Antimicrobial and antibiofilm activity of polyurethane/*Hypericum perforatum* extract (PHPE) composite

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ARTICLE INFO

Keywords:
Antimicrobial
Antibiofilm
Hypericum perforatum

ABSTRACT

Microbial accumulation in materials used in sectors such as medical, textile and food can lead to serious diseases, infections and uncontrollable problems. Many of the materials used in the above-mentioned industries have highly sensitive surfaces for microorganisms and cause colonization and biofilm formation. Colonization and biofilm formation threaten human health and they cause many diseases that result in death every year. Antimicrobial materials have an important role in combating pathogens. This article is about a new material with antibiofilm and antimicrobial properties combining polyurethane and *Hypericum perforatum* extract (PHPE) together. Antimicrobial effect of *H. perforatum* extract was determined against three clinical pathogens; *C. albicans*, *E. coli* and *S. aureus*. The highest antimicrobial activity of *H. perforatum* extract was found against *S. aureus* strain. Antibiofilm analysis results revealed that *H. perforatu*m was also inhibited by the biofilm formation of *S. aureus* by 56.85%. The combination of polyurethane material and *H. perforatum* extract (PHPE) resulted in 92.85% decrease in *S. aureus* biofilm compared to control group. The reduction of *S. aureus* after *H. perforatum* incorporation was revealed by Scanning Electron Microscopy (SEM) study. The results show that the polyurethane material combined with *H. perforatum* extract inhibits the formation of *S. aureus* biofilm.

1. Introduction

Microorganisms are vital organisms such as bacteria, fungi and parasites which are critical sources of infections [1]. An antimicrobial is a substance used to kill microbes or prevent their growth. Antimicrobial polymers were discovered in 1965 [2] and attracted attention both in academic and industrial research. An analysis of articles made about antimicrobial polymers, it is observed that focuses on various methods for antimicrobial polymers and antimicrobial applications of the research. Medical, food and textile industries are three important areas of applied antimicrobial polymers [3–8]. When microbes adhere to a substrate, they form biofilms to bond themselves to the substrate. In biofilms, cells grow in multicellular aggregates and are embedded in a selfgenerating matrix of extracellular polymeric material. For this reason, antimicrobial applications require strategies to prevent microbial viability or adhesion. Some of the reviews have identified antimicrobial management [9–15]. Biofilms are difficult to resist many biocides. Thus, to prevent spread of disease, inhibition of biofilm formation and reduction of microbial binding is a more promising antimicrobial strategy than the killing of microbes [16,17].

Hypericum perforatum L. (Hypericaceae), also known as St. John's Wort, has gaining interest recently and is one of the most consumed medicinal plants in the world. With a wide range of therapeutic effects such as antiinflammatory, antimicrobial, antioxidant agent, wound healing and pain relief effects, the extracts of *H. perforatum* are used as medicinal natural agents [18].

Antimicrobial polymers are frequently used in medicine, food and textile sectors. The surfaces of medical devices are sensitive to microbial infection and provide a suitable environment for microbial growth. At the same time, many of the hospital infections are caused by medical devices. In addition, antimicrobial polymeric materials are used in food packaging by the food industry, taking into account consumer health

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https://doi.org/10.1016/j.bioorg.2018.08.017
Received 25 March 2018; Received in revised form 8 August 2018; Accepted 9 August 2018
Available online 11 August 2018

and safety. Another one, textile fibers containing antimicrobial agents are used to prevent microbial growth under favourable temperature and humidity conditions in the textile industry. In summary, the surface is usually coated or impregnated with antimicrobial material to obtain the above-mentioned antimicrobial surface [19]. In this study, it is aimed to investigate antibiofilm and antimicrobial activity of a new material with combining polyurethane and *H. perforatum* extract. For the best of our knowledge, the present study is significant due to incorporation of *H. perforatum* into polyurethane and evaluation of its antibiofilm activity for the first time.

2. Materials and methods

2.1. Plant material

Hypericum perforatum L. is a member of the genus *Hypericum* (Hypericaceae) [20]. *H. perforatum* was collected from Muğla city (Turkey) in June 2016, rinsed with tap water in order to remove surface pollutions and then dried in the shade. First, dry leaves were kept in ethanol for 8 h at room temperature. Then, filtration was carried out and water was removed by vacuum evaporation. Finally, the obtained extract of *H. perforatum* was dissolved in 96% ethanol (100 mg/mL).

2.2. Microorganism

The antimicrobial activity of the *H. perforatum* extract (HPE) was tested against a group of microorganisms including *Candida albicans* (*C. albicans*) ATCC 10239, *Escherichia coli* (*E. coli*) ATCC 25922 and *Staphylococcus aureus* (*S. aureus*) ATCC 25923. *E. coli* and *S. aureus* strains were incubated at 37 ± 0.1 °C for 24–48 h while *C. albicans* was incubated at 30 ± 0.1 °C for 24–48 h. For bacteria, Nutrient Broth (NB) (Merck) and for yeast, Sabouraud Dextrose Broth (SDB) (Merck) were used as culture media. Inocula were prepared adjusting the turbidity of the medium to match the 0.5 McFarland standard dilutions. The strains were maintained in their appropriate agar slants at 4 °C throughout the study and used as stock cultures.

2.3. Antimicrobial activity of H. perforatum extract by agar well diffusion method

The antimicrobial activity of HPE was assayed by agar well diffusion method [21]. Briefly, 20 mL of Mueller-Hinton Agar (Merck) sterilized and cooled to 45–50 °C. After injecting 1000 µL microorganism cultures to sterile plates, media was distributed and mixed homogeneously. Wells of 6-mm diameter were made on agar plates using a cork borer. 20 µL 100 mg/mL of HPE was injected to the wells. Before injecting HPE, a small amount of sterile agar poured onto each well to prevent the spreading of the HPE from the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition around the wells. Studies were performed in triplicate. Discs of ampicillin (10 µg), gentamicin (10 µg) and nystatin (30 µg) were used as positive controls.

2.4. Antibiofilm activity of H. perforatum extract against S. aureus

The effect of HPE on biofilm-forming ability of *S. aureus* was tested by a microplate biofilm assay [22]. Bacterial strains were prepared as mentioned above but Trypticase Soy Broth (TSB) supplemented with 5% D-glucose was used as culture media. Cultures diluted to 1:100 in fresh TSB with 5% D-glucose. Totally 200 µL bacteria suspension and 20 µL HPE (100 mg/mL) were incubated in sterile microplate at 37 °C for 48 h. After incubation, the wells were washed with distilled water twice to remove the planktonic bacteria. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 min. Wells were washed again to remove the excess crystal violet solution. After air-drying, 200 µL glacial acetic acid were added to each well and incubated at room temperature for 10 min. 125 µL solution from each well transferred to another sterile tube and the final volume was adjusted to 1 mL with distilled water. Optical density (OD) of the solutions was measured at 550 nm (Multiskan GO UV/Vis Microplate Spectrophotometer, Thermo-Fisher Scientific, USA). The effect of *H. perforatum* extract on biofilm formation of *S. aureus* was calculated with the following equation: % Antibiofilm Effect = $[(Control_{OD} - Sample_{OD})/Control_{OD}] \times 100$, where control is the cell suspensions of bacteria without HPE.

2.5. Preparation of the polyurethane discs combined with H. perforatum extract

The commercial polyurethane foam was melted at 120 °C and the extract was added to the mass at 20%. Melting and mixing was carried out in inert atmosphere. The mixture was cast into molds and disc-shaped material was obtained.

2.6. Biofilm inhibition on polyurethane discs incorporated with H. perforatum extract

The effect of HPE on biofilm-forming ability of *S. aureus* growing on polyurethane polymer surface was determined by staining with crystal violet [23]. *S. aureus* strain was prepared as mentioned above. Trypticase Soy Broth (TSB) supplemented with 5% D-glucose was used as culture media. Cultures inoculated into the 24 wells plate containing cm square polymer discs prepared with HPE. Plates were incubated at 37 °C for 48 h After incubation, the discs were washed with sterile PBS twice to remove the planktonic bacteria. Discs were put into new wells and stained with 1 mL of PBS containing 100 µL of 1% (w:v) crystal violet (Sigma-Aldrich, St. Louis, MO) and incubated for 15 min at room temperature. The discs in the wells were washed 3 times with PBS and crystal violet then solubilized by addition of 2 mL of 95% EtOH and shaked for 15 min. Optical density of the solutions was measured at 550 nm (Multiskan GO UV/Vis Microplate Spectrophotometer, Thermo-Fisher Scientific, USA).

2.7. Colony counting

Colony counting method was also applied to determine the biofilm inhibition quantitatively [24,25]. The discs (each square disc had 1 cm × 1 cm dimensions) were incubated with *S. aureus* as mentioned above. After 24 h, the discs were removed from the tubes and placed in sterile PBS (phosphate buffered saline) solution. Tubes were sonicated for 15 min to disaggregate the biofilm layers using a sonicator (Elma Hans Schmidbauer GmbH & Co., Singen, Germany). Serial 10-fold dilutions were prepared using sterile PBS and the dilutions were plated on TSA (trypticase soy agar) supplemented with 5% D-glucose. The number of colony forming units (CFU) were calculated after counting the plated incubated at 37 °C for 24 h. The discs without HPE was used as control group. Three samples from each group (the test group and control group) were used to quantify the Colony Forming Units (CFU).

2.8. Scanning Electron Microscopy

Biofilm inhibition of the HPE was also observed by Scanning Electron Microscopy (SEM). Polyurethane discs were placed in biofilm assay tubes which prepared as mentioned above. Tubes containing discs were incubated at 37 °C for 48 h. Then, the discs were gently rinsed with PBS (pH 7.4) and fixed with 2.5% glutaraldehyde at 4 °C for 2 h. After glutaraldehyde fixation, the discs were washed again with PBS for 1 h and dehydrated by increasing concentrations of ethanol. Specimens were air-dried and coated by gold (Emmitech K550, UK) before examining with a SEM (JEOL JSM-7600F; JEOL Ltd., Tokyo, Japan). To compare the biofilm inhibition, discs without *H. perforatum* extract were used as control.

3. Results and discussion

The present study was conducted to investigate the antimicrobial activity of the HPE and its potential to inhibit microorganisms when combined with polyurethane material. For this purpose, the study was designed to detect the highest antimicrobial activity of HPE against pathogenic microorganisms.

HPE showed no inhibition activity against *C. albicans*. Similarly, a Gram-negative bacteria *E. coli* was also not affected by HPE. On the other hand, a Gram-positive bacteria S. aureus was strongly affected by HPE (Table 1). The zone of inhibition measurement clearly showed that HPE was much more effective on *S. aureus* than antibiotic disc (Fig. 1). To measure the diameter of inhibition zones; the plates were turned back, clear zones were signed with a marker pen and the diameter of the clear circle around the extract was measured using a ruler (without paying attention to the HPE channels formed on to the agar).

There are some studies available in the literature about the in vitro antibacterial and antifungal activity of *H. perforatum* extracts [26–30]. Guneş ve Tıhmınlıoğlu [18] combined the *H. perforatum* extract into chitosan films and resulted that *H. perforatum* oil incorporated films had antimicrobial effect on both *E. coli* and *S. aureus*. Similar to our results, *H. perforatum* extracts are reported to exhibit more pronounced activity against Gram-positive bacteria than Gram-negative bacteria [31,32]. In a study of Mazandari et al [33], the higher antibacterial activity of ethanolic extracts of aerial parts of *H. perforatum* against Gram-positive bacteria (*Enterococcus faecalis* and *S. aureus*, with growth-inhibition zones in the range of 25–26 mm) than of Gram-negative bacteria (*Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *E. coli* and *Pseudomonas aeruginosa*) were reported. Peng and Li [34] resulted that the sensitivity of *S. aureus* against essential oils, when compared to *E. coli*, may be due to the relatively impermeable outer membrane that surrounds Gram-negative bacteria.

According to the antimicrobial activity analysis results of HPE obtained in the present study, it is decided to continue the antibiofilm

Table 1
Inhibition zone (mm) for *H. perforatum* extract and antibiotic discs.

Microorganism	Zone of inhibition for *H. perforatum* extract (mm)	Zone of inhibition for antibiotic discs (mm)
Candida albicans	–	Nystatin 17
Escherichia coli	–	Gentamicin 20
Staphylococcus aureus	23	Amoxicillin 22

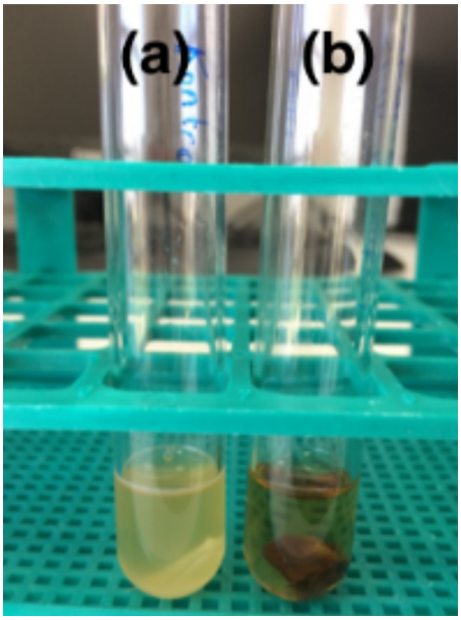

Fig. 2. Tube images of *S. aureus* cultures with polyurethane disc (a) control, (b) polyurethane disc incorporated with *H. perforatum* extract (PHPE).

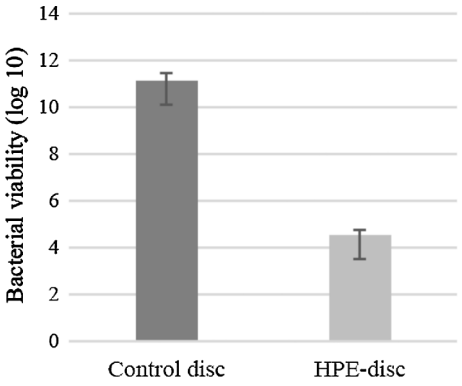

Fig. 3. Anti-biofilm activity of HPE-incorporation to polyurethane discs against biofilm formation of *S. aureus*. Shown is the mean ± SD of 3 independent experiments.

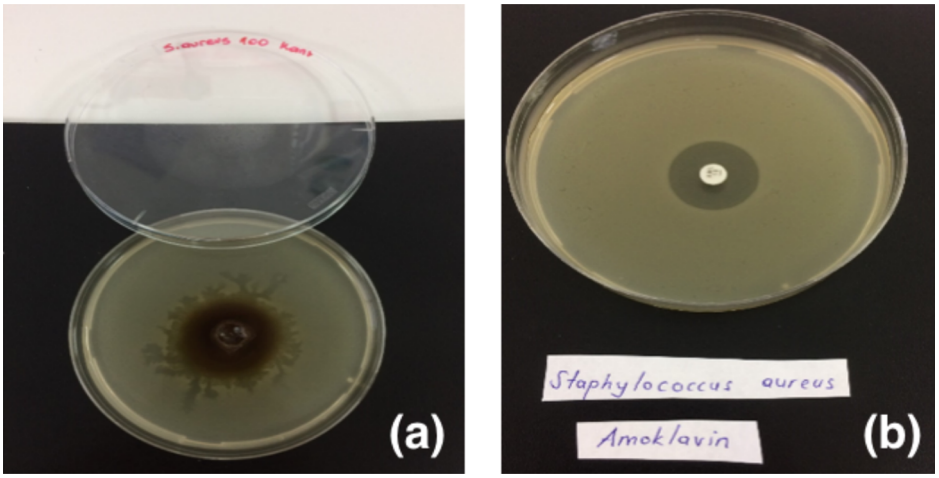

Fig. 1. Agar well diffusion assay of *H. perforatum* extract against *S. aureus* (a) and antibiogram of amoxicillin (b).

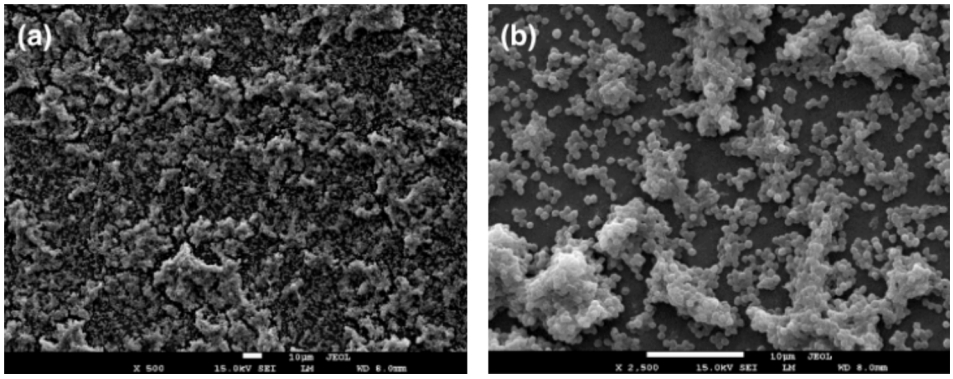

Fig. 4. SEM images of control group discs. Magnifications are: (a) ×500, (b) ×2500.

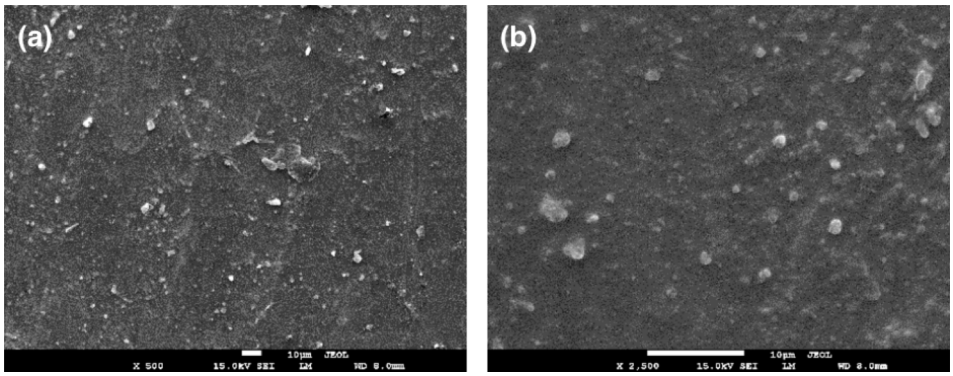

Fig. 5. SEM images of polyurethane discs incorporated with *H. perforatum* extract. Magnifications are: (a) ×500, (b) ×2500.

activity analysis against *S. aureus* bacteria. Antibiofilm activity of HPE on the biofilm forming capacity of *S. aureus* was found to be 56.85% when compared with control group. Similar to this, HPE incorporation into the polyurethane discs also decreased the S. aureus biofilm formation as 92.85%. Fig. 2 shows the bacterial inhibition in the broth tubes after incubation. The tube containing the polyurethane disc with HPE (PHPE) looks clear while the tube with control disc is blurred. This observation proves the inhibition of *S. aureus* growth in the tube containing HPE-incorporated polyurethane disc. Biofilm inhibition was also observed with Scanning Electron Microscopy (SEM). The incorporation of HPE resulted in the inhibition of *S. aureus* biofilm on the polyurethane discs. Approximately 6.6-log reduction was observed for *S. aureus* when exposed to polyurethane discs combined with HPE (Fig. 3).

Control group discs without *H. perforatum* extract were highly contaminated with *S. aureus* after 48 h incubation (Fig. 4). On the other hand, polyurethane discs with HPE (PHPE) were clear and there were individual bacterial cells without an exopolysaccharide layer (Fig. 5). *S. aureus* is known to be an important human pathogen able to adapt and evolve in terms of its resistance traits and virulence factors and it is among the most important causes of human infections in both the hospital and community settings [35]. To fight against *Staph* infections, researching new bioactive secondary metabolites with potential antibacterial activity has been gaining much importance; especially the agents that limit growth and biofilm production by pathogenic bacteria.

4. Conclusion

Antimicrobial properties of *H. perforatum* ethanolic extract were determined against three different microorganisms (*Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*) which are the members of yeast, Gram-negative bacteria and Gram-positive bacteria. Although the extract showed a strong inhibitory activity against *S. aureus*, no inhibitory activity was observed against *C. albicans* and *E. coli*. Antibiofilm analysis results revealed that *H. perforatum* was also inhibited the biofilm formation of *S. aureus* by 56.85%. The combination of polyurethane material and *H. perforatum* extract (PHPE) resulted in 92.85% decrease in *S. aureus* biofilm when compared with control group. The reduction of *S. aureus* biofilm after *H. perforatum* incorporation was revealed by Scanning Electron Microscopy (SEM) study.

The results show that the polyurethane material combined with *H. perforatum* extract inhibits the formation of *S. aureus* biofilm. Overall, *H. perforatum* extract with its potential wound healing capacity may effectively used for pharmaceutical and biomedical application, especially against resistant *S. aureus* infections.

Acknowledgements

This paper has been granted by the Mugla Sıtkı Koçman University Scientific Research Project Coordination through Project Grant Number: (15/223).

References

[1] N.R. Cooper, Complement evasion strategies of microorganisms, Immunol. Today 12 (9) (1991) 327–331, https://doi.org/10.1016/0167-5699(91)90010-Q.
[2] F. Siedenbiedel, J.C. Tiller, Antimicrobial polymers in solution and on surfaces: overview and functional principles, Polymers 4 (1) (2012) 46–71, https://doi.org/10.3390/polym4010046.
[3] M.R. Islam, Y. Gao, X. Li, Q.M. Zhang, M. Wei, M.J. Serpe, Stimuli-responsive polymeric materials for human health applications, Chin. Sci. Bull. 59 (32) (2014) 4237–4255, https://doi.org/10.1007/s11434-014-0545-6.
[4] V. Goldade, N. Vinidiktova, Antimicrobial fibers for textile clothing and medicine: current state, ISJ Theor. Appl. Sci. 3 (47) (2017) 178–194.
[5] K. Álvarez, L. Famá, T.J. Gutiérrez, Physicochemical, antimicrobial and mechanical properties of thermoplastic materials based on biopolymers with application in the food industry, Advances in Physicochemical Properties of Biopolymers (Part 1) (2017) 358.
[6] R. Asher, T. Chacartchi, M. Tandlich, L. Shapira, D. Polak, Microbial accumulation on different suture materials following oral surgery: a randomized controlled study,

Clin. Oral Invest. 1–7 (2018), https://doi.org/10.1007/s00784-018-2476-0.
[7] I. Francolini, G. Donelli, F. Crisante, V. Taresco, A. Piozzi, Antimicrobial polymers for anti-biofilm medical devices: state-of-art and perspectives, Biofilm-Based Healthcare-Associated Infections, Springer, Cham, 2015, pp. 93–117, , https://doi.org/10.1007/978-3-319-09782-4_7.
[8] N. Strempel, J. Strehmel, J. Overhage, Potential application of antimicrobial peptides in the treatment of bacterial biofilm infections, Curr. Pharm. Des. 21 (1) (2015) 67–84, https://doi.org/10.2174/1381612820666140905124312.
[9] S. Veerachamy, T. Yarlagadda, G. Manivasagam, P.K. Yarlagadda, Bacterial adherence and biofilm formation on medical implants: a review, Proc. Inst. Mech. Eng., Part H: J. Eng. Med. 228 (10) (2014) 1083–1099, https://doi.org/10.1177/0954411914556137.
[10] B.D. Brooks, A.E. Brooks, Therapeutic strategies to combat antibiotic resistance, Adv. Drug Deliv. Rev. 78 (2014) 14–27.
[11] R. Draenert, U. Seybold, E. Grützner, J.R. Bogner, Novel antibiotics: are we still in the pre–post-antibiotic era? Infection 43 (2) (2015) 145–151, https://doi.org/10.1007/s15010-015-0749-y[SPACE].
[12] M.S. Dryden, Alternative clinical indications for novel antibiotics licensed for skin and soft tissue infection? Curr. Opin. Infect. Dis. 28 (2) (2015) 117–124, https://doi.org/10.1097/QCO.0000000000000142.
[13] B. Lee, H.W. Boucher, Targeting antimicrobial-resistant bacterial respiratory tract pathogens: it is time to 'get smart', Curr. Opin. Pulm. Med. 21 (3) (2015) 293–303, https://doi.org/10.1097/MCP.0000000000000157.
[14] I. Roca, M. Akova, F. Baquero, J. Carlet, M. Cavaleri, S. Coenen, et al., The global threat of antimicrobial resistance: science for intervention, New Microbes New Infect. 6 (2015) 22–29, https://doi.org/10.1016/j.nmni.2015.02.007.
[15] Q. Yu, Z. Wu, H. Chen, Dual-function antibacterial surfaces for biomedical applications, Acta Biomater. 16 (2015) 1–13, https://doi.org/10.1016/j.actbio.2015.01.018[SPACE].
[16] R. Kargupta, S. Bok, C.M. Darr, B.D. Crist, K. Gangopadhyay, S. Gangopadhyay, et al., Coatings and surface modifications imparting antimicrobial activity to orthopedic implants, Wiley Interdisc. Rev.: Nanomed. Nanobiotechnol. 6 (5) (2014) 475–495, https://doi.org/10.1002/wnan.1273.
[17] A.M. Holban, A. Iordanskii, A.M. Grumezescu, A. Bychkova, E. Andronescu, L. Mogoanta, et al., Prosthetic devices with nanostructurated surfaces for increased resistance to microbial colonization, Curr. Pharm. Biotechnol. 16 (2) (2015) 112–120, https://doi.org/10.2174/1389201016021501121503 03.
[18] S. Güneş, F. Tıhmınlıoğlu, Hypericum perforatum incorporated chitosan films as potential bioactive wound dressing material, Int. J. Biol. Macromol. 102 (2017) 933–943, https://doi.org/10.1016/j.ijbiomac.2017.04.080.
[19] K.S. Huang, C.H. Yang, S.L. Huang, C.Y. Chen, Y.Y. Lu, Y.S. Lin, Recent advances in antimicrobial polymers: a mini-review, Int. J. Mol. Sci. 17 (9) (2016) 1578, https://doi.org/10.3390/ijms17091578[SPACE].
[20] D.J. Mabberley, The Plant-Book: A Portable Dictionary of the Vascular Plants, Cambridge University Press, 1997.
[21] Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard M07-A9, eighth ed., Clinical and Laboratory Standards Institute, Wayne, PA, 2012.
[22] J.H. Merritt, D.E. Kadouri, G.A. O'Toole, Growing and analyzing static biofilms, 1B 1B, Curr. Protocols Microbiol. 22 (1) (2011), https://doi.org/10.1002/9780471729259.mc01b01s00[SPACE].
[23] G.A. O'Toole, L.A. Pratt, P.I. Watnick, D.K. Newman, V.B. Weaver, R. Kolter, [6] Genetic approaches to study of biofilms, Methods in Enzymology, Academic Press, 1999, pp. 91–109.
[24] M. Pérez-Díaz, E. Alvarado-Gomez, M. Magaña-Aquino, R. Sánchez-Sánchez, C. Velasquillo, C. Gonzalez, et al., Anti-biofilm activity of chitosan gels formulated with silver nanoparticles and their cytotoxic effect on human fibroblasts, Mater. Sci. Eng.: C 60 (2016) 317–323, https://doi.org/10.1016/j.msec.2015.11.036.
[25] J.L. Velázquez-Velázquez, A. Santos-Flores, J. Araujo-Meléndez, R. Sanchez-Sanchez, C. Velasquillo, C. González, et al., Anti-biofilm and cytotoxicity activity of impregnated dressings with silver nanoparticles, Mater. Sci. Eng.: C 49 (2015) 604–611, https://doi.org/10.1016/j.msec.2014.12.084.
[26] O. Keleş, S. Ak, T. Bakırel, K. Alpınar, Screening of some Turkish plants for antibacterial activity, Turk. J. Vet. Anim. Sci. 25 (4) (2001) 559–565.
[27] G.E. Meral, N.U. Karabay, In vitro antibacterial activities of three Hypericum species from West Anatolia, Turk. Electron. J. Biotechnol. (2002) 6–10.
[28] F. Conforti, G.A. Statti, R. Tundis, A. Bianchi, C. Agrimonti, G. Sacchetti, et al., Comparative chemical composition and variability of biological activity of methanolic extracts from *Hypericum perforatum* L. Nat. Prod. Res. 19 (3) (2005) 295–303, https://doi.org/10.1080/14786410410001715596.
[29] T. Milosevic, S. Solujic-Sukdolak, S. Sukdolak, In vitro study of ethanolic extract of *Hypericum perforatum* L. on growth and sporulation of some bacteria and fungi, Turk. J. Biol. 31 (4) (2007) 237–241.
[30] Z. Saddiqe, I. Naeem, A. Maimoona, A review of the antibacterial activity of *Hypericum perforatum* L, J. Ethnopharmacol. 131 (3) (2010) 511–521, https://doi.org/10.1016/j.jep.2010.07.034.
[31] J. Reichling, A. Weseler, R. Saller, A current review of the antimicrobial activity of *Hypericum perforatum* L. Pharmacopsychiatry 34 (Suppl. 1) (2001) 116–118.
[32] P. Avato, F. Raffo, G. Guglielmi, C. Vitali, A. Rosato, Extracts from St John's wort and their antimicrobial activity, Phytother. Res.: Int. J. Dev. Pharmacol. Toxicol. Eval. Nat. Prod. Derivatives 18 (3) (2004) 230–232, https://doi.org/10.1002/ptr.1430.
[33] M. Mazandarani, S. Yassaghi, M.B. Rezaei, A.R. Mansourian, E.O. Ghaemi, Ethnobotany and antibacterial activities of two endemic species of Hypericum in North-East of Iran, Asian J. Plant Sci. (2007), https://doi.org/10.3923/ajps.2007.354.358.
[34] Y. Peng, Y. Li, Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films, Food Hydrocolloids 36 (2014) 287–293, https://doi.org/10.1016/j.foodhyd.2013.10.013.
[35] Y.W. Tang, C.W. Stratton, Staphylococcus aureus: an old pathogen with new weapons, Clin. Lab. Med. 30 (1) (2010) 179–208, https://doi.org/10.1016/j.cll.2010.01.005.