



Comparative Biological Activities of Free and Nanofiber-Released *Padina pavonica* Extracts from Electrospun PAN/PLA Nanofibers

Hatice Banu Keskinkaya^{1*}, Emine Sukran Okudan², Ebru Deveci³, Bahar Yilmaz Altinok⁴,
Mevlut Bayrakci⁵

¹Department of Biotechnology, Necmettin Erbakan University, Konya, Türkiye

* Corresponding Author Email: haticebanu.keskinkaya@erbakan.edu.tr-ORCID: 0000-0002-6970-6939

²Department of Basic Aquatic Sciences, Akdeniz University, Antalya, Türkiye

Email: okudanes@gmail.com-ORCID: 0000-0002-6970-6900

³Technology Department, Technical Sciences Vocational School, Konya Technical University, Konya, Türkiye

Email: edeveci@kyun.edu.tr-ORCID: 0000-0002-6970-0039

⁴Department of Bioengineering, Karamanoğlu Mehmetbey University, Karaman, Türkiye

Email: baharyilmaz@kmu.edu.tr-ORCID: 0000-0002-6970-6119

⁵Department of Bioengineering, Karamanoğlu Mehmetbey University, Karaman, Türkiye

Email: mevlutbayrak@gmail.com-ORCID: 0000-0002-6970-6009

Article Info:

DOI: 10.22399/ijcesen.5406

Received : 20 April 2024

Revised : 12 July 2024

Accepted : 14 July 2024

Keywords

Padina pavonica
electrospun nanofiber
controlled release
antibacterial activity
anticancer activity

Abstract:

Padina pavonica is a brown marine macroalga rich in bioactive compounds with antibacterial and anticancer properties. However, the instability and rapid diffusion of these compounds may reduce their biological effectiveness. In this study, *P. pavonica* extracts were incorporated into electrospun polyacrylonitrile (PAN) and polylactic acid (PLA) nanofibers to achieve sustained release and improve biological performance. The released extracts and free extracts were evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. Cytotoxic activity was assessed using MCF-7, HT-29, and L929 cell lines. The released extracts exhibited prolonged antibacterial activity and selective cytotoxicity against cancer cells while showing low toxicity toward healthy fibroblasts. These findings demonstrate that electrospun PAN and PLA nanofibers are promising delivery platforms for *P. pavonica* extracts and may contribute to future biomedical and pharmaceutical applications.

1. Introduction

Marine macroalgae are natural sources of many bioactive compounds [1]. They contain polysaccharides, proteins, phenolic compounds, pigments, vitamins, and minerals. These compounds have many biological properties [2]. They show antibacterial, antioxidant, anti-inflammatory, and anticancer activities. Therefore, marine macroalgae are widely studied for biomedical and pharmaceutical applications. *Padina pavonica* is a brown macroalga with high biological potential [3]. It contains many natural compounds that can inhibit the growth of bacteria and cancer cells. Previous studies have reported the antibacterial, antioxidant, and anticancer activities of *P. pavonica* [4]. However, the composition of the extract depends on

the extraction solvent. For this reason, different solvents such as ethanol, methanol, and water are commonly used to obtain different bioactive compounds [5]. Natural extracts are usually applied directly to cells or bacteria. However, free extracts have some disadvantages. They may degrade quickly [6]. They may also diffuse rapidly from the application area. As a result, their biological activity may decrease over time. Therefore, suitable carrier materials are needed to protect the bioactive compounds and release them gradually [7]. Electrospun nanofibers are good carrier materials for natural extracts [8]. They have a high surface area and a porous structure. These properties allow the controlled release of bioactive compounds. Polyacrylonitrile (PAN) and polylactic acid (PLA) are commonly used polymers for electrospinning [9]. They produce stable nanofiber structures and

can carry different natural products [10]. For this reason, electrospun nanofibers have been widely studied for drug delivery, wound dressing, tissue engineering, and antibacterial applications [11]. Many studies have investigated the biological activities of *P. pavonica* extracts. Other studies have focused on nanofiber-based delivery systems. However, only a few studies have compared the biological activities of free extracts with those released from electrospun nanofibers [12,13]. It is still not clear whether controlled release changes the biological activity of *P. pavonica* extracts. This comparison is important for developing effective natural biomaterials [14].

In this study, *P. pavonica* extracts were successfully incorporated into electrospun PAN and PLA nanofibers to investigate their controlled-release behavior and biological performance. The antibacterial activities of free and released extracts were evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. In addition, their cytotoxic effects were investigated using MCF-7, HT-29, and L929 cell lines. The results demonstrate that electrospun nanofibers represent promising delivery platforms for preserving and gradually releasing marine algal bioactive compounds for potential biomedical applications.

2. Material and Methods

2.1. Materials

All chemicals used in this study were of analytical grade. Polyacrylonitrile (PAN), polylactic acid (PLA), dimethylformamide (DMF), chloroform, ethanol, methanol, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Deionized water was used in all experiments. All reagents were used without further purification [15].

2.2. Collection of *P. pavonica* Samples

P. pavonica samples were collected from the Mediterranean coast of Türkiye by Emine Şukran Okudan using SCUBA and free diving techniques. The samples were taken from rocky surfaces at different depths [16]. After collection, sand, rocks, and other unwanted materials were removed. The algae were washed thoroughly with fresh water and dried in the shade at room temperature. The dried samples were ground into powder using a laboratory grinder and stored in sealed containers until extraction.

2.3. Preparation of *P. pavonica* Extracts

Powdered *P. pavonica* was extracted using ethanol, methanol, and water. Briefly, 20 g of dried algal powder was mixed with 180 mL of solvent and extracted using a Soxhlet apparatus for 6 h. Ethanol was used first, followed by methanol and water. After extraction, the solvents were removed using a rotary evaporator at 40 °C under reduced pressure. The concentrated extracts were freeze-dried to obtain dry powders [17]. The extracts were stored at -20 °C until use.

2.4. Preparation of Electrospun PAN and PLA Nanofibers

PAN and PLA solutions were prepared separately. PAN was dissolved in a mixture of DMF and chloroform (1:1, v/v) at a concentration of 10% (w/v). PLA was prepared at a concentration of 6% (w/v) using the same solvent mixture. *P. pavonica* extracts were added to each polymer solution at 50% (w/w) of the polymer weight [18]. The solutions were stirred until they became homogeneous. The polymer solutions were transferred into 10 mL syringes. Electrospinning was carried out at a flow rate of 0.7 mL/h, an applied voltage of 19 kV, and a tip-to-collector distance of 15 cm. The collected nanofibers were dried at room temperature to remove any remaining solvent. The prepared nanofibers were used in the release and biological activity studies.

2.5. Release of *P. pavonica* Extracts from Nanofibers

The release behavior of *P. pavonica* extracts from electrospun PAN and PLA nanofibers was evaluated under physiological conditions using phosphate-buffered saline (PBS, pH 7.4). Briefly, extract-loaded nanofibers were immersed in PBS and incubated at 37 °C under gentle shaking for 72 h. At predetermined time intervals, aliquots of the release medium were collected and replaced with an equal volume of fresh PBS to maintain sink conditions. The collected solutions containing the released bioactive compounds were subsequently used for antibacterial and cytotoxicity assays. Free *P. pavonica* extracts prepared in PBS were tested under the same experimental conditions and served as the control group for comparison [19–21].

2.6. Antibacterial Activity

The antibacterial activity of free and released *P. pavonica* extracts was evaluated against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*. Bacterial suspensions were prepared according to standard

microbiological methods. Different concentrations of the extracts were added to 96-well plates. The plates were incubated at 37 °C for 24 h. Bacterial growth was measured at 600 nm using a microplate reader. The antibacterial activity of free extracts and nanofiber-released extracts was compared. All experiments were performed in triplicate [23].

2.7. Cytotoxicity Assay

The cytotoxic activity of free and released *P. pavonica* extracts was evaluated using MCF-7, HT-29, and L929 cell lines. Cells were seeded into 96-well plates and allowed to attach before treatment. Free extracts and released extracts were applied at the same concentrations. After 72 h of incubation, cell viability was determined using the Alamar Blue assay. Cell viability was calculated according to the manufacturer's protocol. All experiments were carried out in triplicate [24,25].

2.8. Statistical Analysis

All experiments were performed in triplicate. The results are presented as mean $\pm$ standard deviation. Statistical analysis was performed using GraphPad Prism software. Differences between groups were analyzed by one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussions

The biological properties of *P. pavonica* extracts after incorporation into electrospun PAN and PLA nanofibers were comprehensively investigated. The release behavior of the encapsulated extracts, together with their antibacterial and cytotoxic activities, was evaluated to determine the effectiveness of the nanofiber-based delivery system. The obtained results demonstrate the potential of electrospun nanofibers to preserve and gradually release the bioactive compounds of *P. pavonica*. The release profiles of *P. pavonica* extracts from PAN and PLA nanofibers are shown in Figure 2. Both nanofiber systems showed a controlled release pattern during the 72 h study. A rapid release was observed during the first 12 h. After this period, the release rate became slower and more stable. PAN nanofibers released a higher amount of extract than PLA nanofibers at all time points. At the end of 72 h, the cumulative release reached approximately 81% for PAN and 74% for PLA. This difference may be related to the structure of the polymer matrix and the interaction between the polymer and the extract. The sustained release

observed in both nanofiber systems is beneficial for biological applications [29].



Figure 1. *P. pavonica* collected from the Mediterranean coast of Türkiye. The characteristic fan-shaped thallus and concentric calcified bands of the brown macroalga are clearly visible.

A gradual release may provide a continuous supply of bioactive compounds and help maintain antibacterial and anticancer activities over a longer period. These findings suggest that electrospun PAN and PLA nanofibers can be used as effective carriers for the controlled delivery of *P. pavonica* extracts.

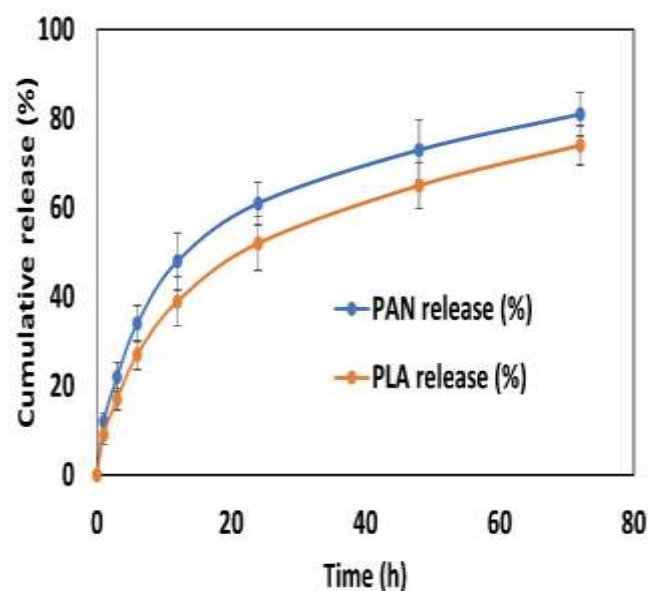


Figure 2. Cumulative release profile of *P. pavonica* extracts from electrospun PAN and PLA nanofibers in PBS (pH 7.4) over 72 h.

As shown in Figure 3, both free and nanofiber-released *P. pavonica* extracts exhibited antibacterial activity against all tested bacterial strains, although the released extracts demonstrated a more sustained

inhibitory effect due to the controlled release behavior of the nanofiber system. The antibacterial activity observed in *P. pavonica* extracts is mainly attributed to the presence of bioactive compounds such as phlorotannins, fucoidan, fucoxanthin, phenolic compounds, and flavonoids. These metabolites are known to disrupt bacterial cell membranes, interfere with essential metabolic pathways, and induce oxidative stress, thereby inhibiting bacterial growth. Similar antibacterial effects of *P. pavonica* and other brown macroalgae have been reported in previous studies [26–30].

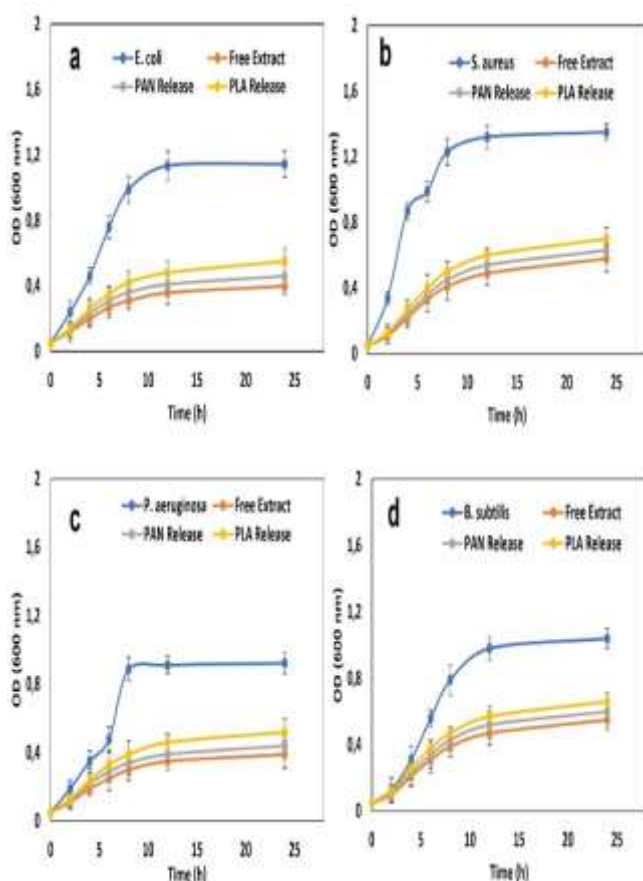


Figure 3. Growth curves of (a) *E. coli*, (b) *S. aureus*, (c) *P. aeruginosa*, and (d) *Bacillus subtilis* treated with free *P. pavonica* extract, PAN-released extract, and PLA-released extract. Untreated bacteria were used as the control. Data are presented as mean $\pm$ SD ($n = 3$).

The cytotoxic effects of *P. pavonica* extracts on HT-29, MCF-7, and L929 cells are shown in Figure 4. Cell viability decreased with increasing extract concentration in both HT-29 and MCF-7 cells, indicating a concentration-dependent anticancer effect. In HT-29 cells (Figure 4a), the methanol extract showed the strongest cytotoxic activity among the algal extracts, followed by the ethanol extract. The water extract showed the weakest effect. Cisplatin and taxol, used as positive controls, caused the greatest reduction in cell viability. A similar trend was observed in MCF-7 cells (Figure 4b). The

ethanol and methanol extracts reduced cell viability more effectively than the water extract [30]. These results suggest that the extraction solvent affects the biological activity of *P. pavonica*. In contrast, all extracts showed low cytotoxicity toward normal L929 fibroblast cells (Figure 4c). Cell viability remained above 85% even at the highest concentration. This indicates that the extracts have low toxicity toward healthy cells while maintaining cytotoxic activity against cancer cells. Overall, the results demonstrate that methanol and ethanol extracts possess stronger anticancer activity than the water extract. The selective inhibition of cancer cells suggests that *P. pavonica* extracts may be promising natural materials for future biomedical applications.

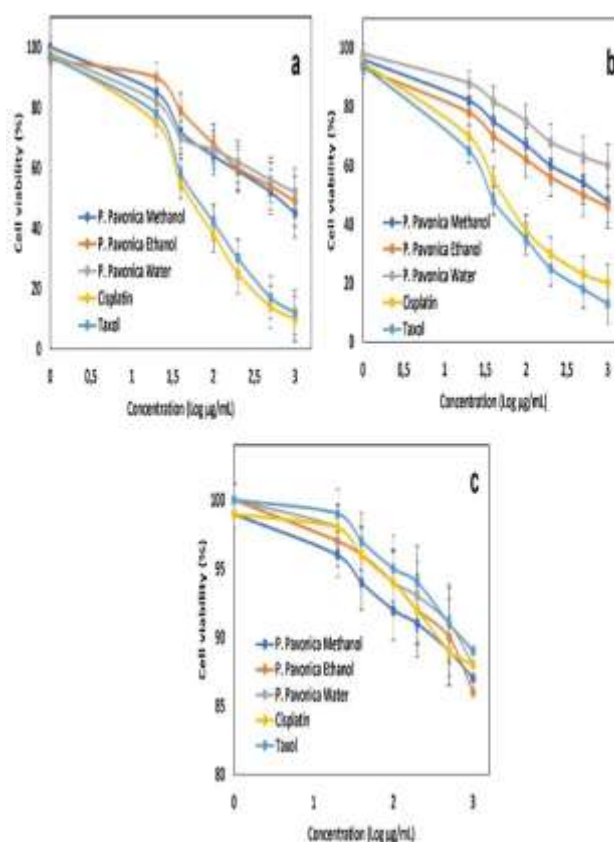


Figure 4. Concentration-dependent cytotoxic effects of *P. pavonica* methanol, ethanol, and water extracts on HT-29 (a), MCF-7 (b), and L929 (c) cell lines after 72 h of treatment. Cell viability was determined using the Alamar Blue assay. Cisplatin and taxol were used as positive controls. Data are presented as mean $\pm$ SD ($n = 3$).

The IC_{50} values (Table 1) showed that the methanol extract was more cytotoxic to HT-29 than to MCF-7 cells, while the ethanol extract exhibited similar effects on both cell lines, and the water extract showed the lowest toxicity [31]. Cisplatin and taxol displayed significantly lower IC_{50} values than the extracts, whereas no significant toxicity was observed in L929 cells for any sample, supporting

the selective cytotoxicity of *P. pavonica* extracts and highlighting the need for biodirected isolation and in vivo studies.

Table 1. IC_{50} values ($\mu\text{g/mL}$) of different *P. pavonica* extracts and reference chemotherapeutic agents on cell lines after 72 h of incubation.

Extract/Drug	HT-29 ($\mu\text{g/mL}$)	MCF-7 ($\mu\text{g/mL}$)	L929 ($\mu\text{g/mL}$)	P value
P-methanol	468.9 $\pm$ 36	843.5 $\pm$ 98.42	N.D.	0.0185
P-ethanol	488 $\pm$ 58.8	484.2 $\pm$ 75.45	N.D.	0.315
P-water	771 $\pm$ 102.3	1947 $\pm$ 124.96	N.D.	0.0002
Cisplatin	57.51 $\pm$ 21	77.52 $\pm$ 20.7	N.D.	0.30
Taxol	63.03 $\pm$ 14	60.53 $\pm$ 17.9	N.D.	0.86

The IC_{50} values were determined by cell viability tests and are presented as the mean $\pm$ standard deviation (n = 3). N.D.: Not detected. This indicates that the extracts did not exhibit significant toxic effects on the L929 normal cell line

4. Conclusions

In this study, *P. pavonica* extracts were successfully incorporated into electrospun PAN and PLA nanofibers, and their biological activities were evaluated after controlled release. The nanofibers showed a sustained release profile over 72 h, with PAN providing a slightly higher release than PLA. The released extracts retained their antibacterial activity against both Gram-positive and Gram-negative bacteria and exhibited selective cytotoxicity against HT-29 and MCF-7 cancer cells while showing low toxicity toward L929 normal fibroblast cells.

These results indicate that electrospun PAN and PLA nanofibers are suitable carriers for the controlled delivery of *P. pavonica* extracts. The combination of sustained release, antibacterial activity, and selective anticancer effects suggests that these nanofiber systems have promising potential for future biomedical and drug delivery applications.

Author Statements:

- **Ethical approval:** This study did not involve human participants or animals. Therefore, ethical approval was not required.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have

appeared to influence the work reported in this paper.

- **Acknowledgement:** Acknowledgements: The authors gratefully acknowledge the financial support provided by the Scientific Research Projects Coordination Unit (BAP) of Necmettin Erbakan University under Project No. 23GAP15003.
- **Author contributions:** Bahar Yılmaz: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – Original Draft, Writing – Review & Editing. Hatice Banu Keskinaya: Investigation, Validation, Data curation. Numan Emre Gümüş: Investigation, Formal analysis. Emine Sukran Okudan: Methodology, Investigation. Ebru Deveci: Data curation, Validation. Mevlüt Bayrakçı: Supervision, Writing – Review & Editing.
- **Funding information:** This work was supported by the Scientific Research Projects Coordination Unit (BAP) of Necmettin Erbakan University under Project No. 23GAP15003.
- **Data availability statement:** The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

- [1] Hatice Banu Keskinaya, Ebru Deveci, Bahar Yılmaz Altınok, Numan Emre Gümüş, Emine Şükran Okudan, Cengiz Akköz, Serkan Karakurt. (2023). HPLC-UV analysis of phenolic compounds and biological activities of *Padina pavonica* and *Zanardinia typus* marine macroalgae species. *Turkish Journal of Botany*. 47(3):231–243. DOI:10.55730/1300-008X.2761
- [2] Susan L. Holdt, Shaun Kraan. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology*. 23(3):543–597. DOI:10.1007/s10811-010-9632-5
- [3] Se-Kwon Kim, You-Jin Jeon. (2012). Biological activities and potential industrial applications of fucoidans isolated from brown seaweeds. *Carbohydrate Polymers*. 88(1):13–20. DOI:10.1016/j.carbpol.2011.12.029
- [4] Sinead Gupta, Nissreen Abu-Ghannam. (2011). Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science & Technology*. 22(6):315–326. DOI:10.1016/j.tifs.2011.03.011
- [5] Ellen Shannon, Nissreen Abu-Ghannam. (2016). Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications. *Marine Drugs*. 14(4):81. DOI:10.3390/md14040081

- [6] Dieter Li, Younan Xia. (2004). Electrospinning of nanofibers: Reinventing the wheel? *Advanced Materials*. 16(14):1151–1170. DOI:10.1002/adma.200400719
- [7] Andreas Greiner, Joachim H. Wendorff. (2007). Electrospinning: A fascinating method for the preparation of ultrathin fibers. *Angewandte Chemie International Edition*. 46(30):5670–5703. DOI:10.1002/anie.200604646
- [8] Armagan, E., Keskinates, M., Gumus, N. E., Aydin, Z., Yilmaz, B., & Bayrakci, M. (2024). Macroalgal (Ulva compressa) silver nanoparticles: Their characterization, cytotoxicity, and antibacterial applications. *Turkish Journal of Fisheries and Aquatic Sciences*, 24(9), TRJFAS25612. <https://doi.org/10.4194/TRJFAS25612>
- [9] Bayrakci, M., Keskinates, M., & Yilmaz, B. (2021). Antibacterial, thermal decomposition and in vitro time release studies of chloramphenicol from novel PLA and PVA nanofiber mats. *Materials Science and Engineering: C*, 122, 111895. DOI: 10.1016/j.msec.2021.111895
- [10] Jun Xue, Tuo Wu, Yijie Dai, Younan Xia. (2019). Electrospinning and electrospun nanofibers: Methods, materials, and applications. *Chemical Reviews*. 119(8):5298–5415. DOI:10.1021/acs.chemrev.8b00593
- [11] Silvia Farah, Donald G. Anderson, Robert Langer. (2016). Physical and mechanical properties of poly(lactic acid) (PLA) and their functions in widespread applications—A comprehensive review. *Advanced Drug Delivery Reviews*. 107:367–392. DOI:10.1016/j.addr.2016.06.012
- [12] Rajesh M. Rasal, Abhijit V. Janorkar, Daniel E. Hirt. (2010). Poly(lactic acid) modifications. *Progress in Polymer Science*. 35(3):338–356. DOI:10.1016/j.progpolymsci.2009.12.003
- [13] Arefeh Luraghi, Francesca Peri, Lorenzo Moroni. (2021). Electrospinning for drug delivery applications: A review. *Journal of Controlled Release*. 334:463–484. DOI:10.1016/j.jconrel.2021.03.033
- [14] Ivan Wiegand, Kai Hilpert, Robert E.W. Hancock. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*. 3(2):163–175. DOI:10.1038/nprot.2007.521
- [15] Prashant Kumar, Anil Nagarajan, Pramod D. Uchil. (2018). Analysis of cell viability by the Alamar Blue assay. *Cold Spring Harbor Protocols*. 2018(6):pdb.prot095489. DOI:10.1101/pdb.prot095489
- [16] Bayrakci, M., Ozcan, F., Yilmaz, B., & Ertul, S. (2017). Electrospun nanofibrous polyacrylonitrile/calixarene mats: An excellent adsorbent for the removal of chromate ions from aqueous solutions. *Acta Chimica Slovenica*, 64(3), 679–688. <https://doi.org/10.17344/acsi.2017.3271>
- [17] Tanja Maver, Uroš Maver, Petra Stana Kleinschek, et al. (2015). Electrospun drug-loaded nanofibers for controlled drug delivery applications. *Materials*. 8(3):1105–1119. DOI:10.3390/ma8031105
- [18] Reza Neisiany, S. Nouri Khorasani, Saeid Ramakrishna, et al. (2020). Recent advances in polyacrylonitrile-based electrospun nanofibers and their applications. *Journal of Applied Polymer Science*. 137(32):48911. DOI:10.1002/app.48911
- [19] S. Ramakrishna, K. Fujihara, W.E. Teo, T.C. Lim, Z. Ma. (2005). *An Introduction to Electrospinning and Nanofibers*. World Scientific Publishing, Singapore. ISBN:9789812564548.
- [20] A. Torres-Martínez, J.M. Cornejo-Bravo, G.L. Pérez-González, et al. (2018). Electrospun nanofibers for drug delivery systems: A review. *Drugs*. 6(4):136. DOI:10.3390/drugs6040136
- [21] Keskinates, M., Yilmaz, B., Ulusu, Y., & Bayrakci, M. (2018). Electrospinning of novel calixarene-functionalized PAN and PMMA nanofibers: Comparison of fluorescent protein adsorption performance. *Materials Chemistry and Physics*, 205, 522-529. DOI: 10.1016/j.matchemphys.2017.11.055
- [22] Hong Liu, Yan Zhang, Qian Li, et al. (2023). Recent progress of electrospun herbal medicine nanofibers for biomedical applications. *Pharmaceutics*. 15(1):184. DOI:10.3390/pharmaceutics15010184
- [23] Se-Kwon Kim, Katarzyna Chojnacka. (2015). *Marine Algae Extracts: Processes, Products, and Applications*. Wiley-VCH, Weinheim. ISBN:9783527337084.
- [24] Yilmaz, B., Bayrac, A. T., & Bayrakci, M. (2020). Evaluation of anticancer activities of novel facile synthesized calix[n]arene sulfonamide analogs. *Applied Biochemistry and Biotechnology*, 190(4), 1484–1497.
- [25] Yilmaz, B. (2022). Release of nifedipine, furosemide, and niclosamide from biocompatible poly(HEMA) hydrogel structures. *Turkish Journal of Chemistry*, 46(5), 1710–1722.
- [26] W.A.J.P. Wijesinghe, You-Jin Jeon. (2012). Exploiting biological activities of brown seaweed-derived fucoidans for potential pharmaceutical applications. *Carbohydrate Polymers*. 88(1):13–20. DOI:10.1016/j.carbpol.2011.12.029
- [27] Rina Pangestuti, Se-Kwon Kim. (2011). Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods*. 3(4):255–266. DOI:10.1016/j.jff.2011.07.001
- [28] Nissreen Abu-Ghannam, Maria Rajauria. (2013). Seaweeds as a source of bioactive compounds for health applications. *International Journal of Food Science and Technology*. 48(11):2219–2226. DOI:10.1111/ijfs.12197
- [29] Samar M. El Shafay, Salwa S. Ali, Mohamed M. El-Sheekh. (2016). Antimicrobial activity of some seaweed species from the Red Sea against multidrug-resistant bacteria. *Egyptian Journal of Aquatic Research*. 42(1):65–74. DOI:10.1016/j.ejar.2015.11.005
- [30] M. Farasat, R.A. Khavari-Nejad, F. Nabavi, A.R.B. Namjooyan. (2014). Antioxidant activity, total phenolics and flavonoid contents of selected marine macroalgae from the Persian Gulf. *Iranian Journal of Pharmaceutical Research*. 13(1):163–170.

- [31] Kenry, Chwee Teck Lim. (2017). Nanofiber technology: Current status and emerging developments in drug delivery and tissue engineering. *Progress in Polymer Science*.70:1–17. DOI:10.1016/j.progpolymsci.2017.03.002