UNIVERSITY OF MEDICINE AND PHARMACY

„CAROL DAVILA", BUCUREȘTI

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DOCTORAL SCHOOL

PHARMACY DOMAIN

Comparative physicochemical studies on new pharmaceutical formulations made from collagen extracts from Black Sea resources

DOCTORAL THESIS SUMMARY

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2026

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INTRODUCTION

The doctoral dissertation titled "*Comparative Physicochemical Studies on New Pharmaceutical Formulations Made from Collagen Extracts from Black Sea Resources*" addresses a timely issue in the medical field.

The novelty of this doctoral thesis lies in the extraction and characterization of marine collagen from the jellyfish species *Rhizostoma pulmo*, found in the Black Sea basin, and the formulation of new biocomposites derived from jellyfish collagen extracts in combination with medicinal substances, other marine compounds, or compounds derived from natural terrestrial extracts. A method for extracting collagen from the Black Sea jellyfish *R. pulmo* was established and validated using specific validation parameters; the processing of the Black Sea jellyfish *R. pulmo* represents a novelty.

The doctoral dissertation is structured in two parts: the first two chapters of the dissertation constitute the theoretical overview of the work and present the importance of marine-derived collagen as an alternative resource. *Chapter 1* covers the properties of collagen and the study of jellyfish in terms of species diversity, description, habitat, and distribution in European waters, particularly the Black Sea basin, as well as their importance in marine biodiversity. *Chapter 2* of the thesis focuses on the study of the structure and composition of marine collagen, the mechanisms of active substance penetration through the skin, the analysis of the transdermal system for the administration of medicinal substances, the analysis of the structure and rheology of topical gel-based preparations, and the study of the physicochemical and biological analytical methods used in the thesis

Chapter 3 outlines the general objective and the specific objectives of each study conducted, the thesis's working hypothesis, and the methodology employed.

The section on personal contributions begins with *Chapter 4*, which presents the extraction method used, the calculation of the yield obtained from *R. pulmo* jellyfish collagen, and the validation of the method by determining the hydroxyproline content of the extracted collagen. *Chapter 5* presents the physicochemical and microscopic analysis of *R. pulmo* jellyfish collagen.

Chapter 6 focuses on the identification of amino acids, total polyphenol content, and polyphenolic compounds using the chromatographic method (HPLC), as well as the biochemical characterization of collagen from the jellyfish species *Rhizostoma pulmo* extracted from the Black Sea basin.

Chapter 7 analyzes the biological properties of collagen from the jellyfish *R. pulmo* by determining its antioxidant activity using two methods: the DPPH method and the Reducing Power method, and by studying its antibacterial activity. *Chapter 8* covers the formulation of pharmaceutical preparations derived from *Rhizostoma pulmo* impregnated with medicinal substances and three different species of algae from the Black Sea basin. *Chapter 9* focuses on an *in vitro* study based on the scratch test, which aims to demonstrate the wound-healing and tissue re-epithelialization capabilities of a new biocomposite made from collagen derived from the jellyfish species *Rhizostoma pulmo* and the brown alga species *Cystoseira barbata*, both extracted from the Black Sea basin. *Chapter 10* presents a study of the cytotoxicity of an innovative biocomposite derived from jellyfish collagen (*R. pulmo*) and the medicinal mushroom *Ganoderma lucidum*, as well as the antibacterial activity of the new topical formulation developed for anti-tumor applications in the oral cavity.

I. GENERAL PART

CHAPTER 1. COMPARATIVE STUDIES OF MARINE COLLAGEN FROM JELLYFISH IN THE BLACK SEA AND THE MAIN EXTRACTION TECHNIQUES

The main goal of this chapter is to analyze collagen and its sources, highlight the advantages and disadvantages of using collagen from marine sources, and conduct a comparative study of extraction techniques, with a particular focus on collagen from the jellyfish species *Rhizostoma pulmo* in the Black Sea basin. The chapter presents literature data on the diversity of jellyfish species, their description, habitat, and distribution of these marine collagen sources in European waters specifically the Black Sea basin as well as their importance in the pharmaceutical, medical, nutraceutical, and even food industries.

Collagen is the most abundant protein in the body. These proteins form fibers that help build and maintain body parts; they are also present in muscles, hair, skin, bones, tendons, and ligaments. Researchers have identified at least 29 types of collagen, but those classified as types I, II, and III account for 80% to 90% of total collagen (Deyl, 2003). Collagen has a wide range of applications due to its unique properties, which include high biocompatibility, good bioactivity, and low antigenicity. Marine proteins, derived from various fish, jellyfish, sponges, or other forms of marine life, are excellent functional ingredients for the cosmetics industry, as well as for the formulation of new pharmaceutical preparations for

dermatological use (**Pesterau, 2022**). Marine collagen is extracted from both vertebrate and invertebrate marine creatures. Marine collagen extracts have been studied for applications in various fields: biomaterials, wound healing, use in diets, and cosmetics (Cadar, 2023; Gaspar-Pintilescu, 2021; Benayahu, 2020; Gallo, 2020). It has been demonstrated that jellyfish play an important role in marine ecosystems and are utilized in various fields, the most significant being: food, medical, cosmetic, and biotechnological. Although jellyfish are a rich and natural marine resource, they remain underutilized for their bioactive compounds compared to other marine animals. The advantages of marine-sourced collagen far outweigh the disadvantages, with the main benefits being safety, biocompatibility, superior absorption, the elimination of religious barriers, and the scalability of production for this type of collagen.

CHAPTER 2. MODERN METHODS FOR THE ANALYSIS AND CHARACTERIZATION OF EXTRACTS AND TOPICAL FORMULATIONS BASED ON *RHIZOSTOMA PULMO* JELLYFISH COLLAGEN

Collagen analysis is relevant to a number of fields, such as tissue engineering, regenerative medicine, drug development and delivery, and biomechanics. Currently, there are several methods for identifying and quantifying collagen, most of which are not sufficiently sensitive, specific, or cost-effective (Cadar, 2024). Chapter 2 presents data on collagen structure and its applications. The types of analyses used to characterize jellyfish collagen, as well as marine collagen-based biocomposites, are also systematized. The hydrogel pharmaceutical form, which is the most common among topical collagen-based pharmaceutical formulations, is analyzed. To assess the stability of this type of formulation, a rheological study of the formulations was conducted. Marine life forms are also a significant source of collagen, which can be extracted from sponges, fish, and jellyfish (Song, 2021). These collagens are widely used in industry but less so for research and clinical applications (Lin, 2009). In the case of jellyfish collagen, due to this genetic variation, it is unlikely that the peptide sequences of collagens derived from different species will be identical; therefore, separate characterizations of the collagens derived from each species are necessary before biomedical applications can be established and exploited for each individual jellyfish species. The triple-helix structure was also discussed, as well as the difference in molecular weight between collagen (300,000 Da), gelatin (2,000–200,000 Da),

and collagen peptides (300–8,000 Da). Ryu et al. (2021) demonstrated that proteolytic enzymes can break down proteins into hydrolysates containing small peptides consisting of 2–20 amino acids (Ryu, 2021). The molecular weight, length, and sequence of peptides, as well as their amino acid composition, influence their bioactive properties; hydrolysates produce forms of amino acids that are useful in supporting various human biological functions, as stated by Yathisha and colleagues (Yathisha, 2018). Topical applications via the transdermal route are important in the treatment of various skin diseases, cosmetic applications, and wound healing. The transdermal route of administration for many drugs is limited, as very few drugs can be delivered at a viable rate using this route (Yu, 2021). Gels have become popular in cosmetics and topical medicinal treatments, such as wound healing, due to their advantageous properties (they are non-greasy, spread easily, and are quickly absorbed). Marine life is also a significant source of collagen, which can be extracted from sponges, fish, and jellyfish (Song, 2021). These collagens are widely used in industry but less so for research and clinical applications (Lin, 2009).

CHAPTER 3. RESEARCH HYPOTHESIS AND GENERAL OBJECTIVES

This doctoral dissertation is based on several research directions, each of which had distinct hypotheses. A first working hypothesis is based on the initial aim of this thesis, namely the identification of a viable method for the extraction of marine collagen, the validation of this method through the determination of hydroxyproline content, as well as the biochemical characterization of collagen obtained from the jellyfish *Rhizostoma pulmo* found in the Black Sea basin. Another working hypothesis focuses on the physicochemical and biological characterization of collagen extracted from the jellyfish species *Rhizostoma pulmo*. The third line of research focused on the study of pharmaceutical formulations based on collagen and collagen peptides in the form of hydrogels, films, matrices, and biocomposites combined with other substances or extracts of marine (algae) or terrestrial (fungi) origin, intended for subsequent use in medical or cosmetic treatments.

The primary research directions, based on specific research methodologies, focus on: establishing an innovative biological and technological process for extracting collagen from the Black Sea jellyfish species *Rhizostoma pulmo* and validating the method for extracting collagen from this species. The physicochemical characterization of jellyfish collagen was performed using: SDS-PAGE, UV-DC dichroism spectroscopy, determination of the

denaturation temperature of jellyfish collagen, FT-IR spectroscopy analysis, thermogravimetric analysis, UV-VIS spectrophotometry, and optical and electron microscopy. Subsequently, a biochemical analysis of jellyfish collagen was performed, and the amino acids and phenolic compounds in *R. pulmo* were identified via HPLC, while the total polyphenol content was determined using the Folin-Ciocâlteu method. The biological properties of *R. pulmo* marine collagen were analyzed by determining its antioxidant and antibacterial activity. An important objective was to develop and characterize new pharmaceutical formulations based on jellyfish collagen and other marine and plant sources, as well as medicinal substances, in the form of: hydrogels, films, membranes, and matrices for topical use. The last two objectives focused on conducting *in vitro* studies of topical pharmaceutical formulations made from jellyfish collagen with algae and determining the degree of epithelialization via the scratch test, as well as determining and analyzing the healing capacity of certain biocomposites derived from collagen extracts of jellyfish and the *Ganoderma lucidum* mushroom for the purpose of preliminary testing of the *in vitro* healing capacity of certain oral squamous cell carcinomas (OSCC).

CHAPTER 4. EXTRACTION AND VALIDATION OF THE METHOD FOR OBTAINING COLLAGEN FROM THE JELLYFISH *RHIZOSTOMA PULMO*

The objective of this chapter was to describe the method for extracting collagen from jellyfish of the species *Rhizostoma pulmo* from the Black Sea. Specific objectives included: preparation of the material, pretreatment, and the actual extraction of marine collagen. The methods used for collagen extraction are: acid-solubilized collagen (ASC), pepsin-solubilized collagen (PSC), deep eutectic solvent (DES), and supercritical fluid (SF) extraction; these are the major methods reported in the literature (Addad, 2011) for collagen isolation. The materials used for extraction consist of a jellyfish from the Black Sea basin, approximately 30–40 cm in length and weighing 877–1200 g of wet tissue (see Figures 4.1 and 4.2).

Figure 4.1. *Rhizostoma pulmo,* jellyfish from the Black Sea

Figure 4.2. Length of the *Rhizostoma pulmo* jellyfish

Preparation of the jellyfish: The first step is to wash it several times with clean water, then chop it up and prepare it for the subsequent steps required for collagen extraction. Extraction of soluble collagen using the ASC method. The extraction was carried out in three distinct stages: precipitation, filtration, and then centrifugation. The jellyfish was carefully washed to remove residues (Figure 4.9.), after this stage, the material was prepared for pretreatment (Figure 4.10.), subsequently the oral arms were separated from the bell (Figure 4.11.), the jellyfish was weighed and then placed in 0.5 M acetic acid. Every 24 hours, the supernatant was removed, and the residue in the vessel was recoated with fresh 0.5 M acetic acid solution.

Figure 4.9. Rinsing the jellyfish with water

Figure 4.10. Preparation of the pretreatment tank

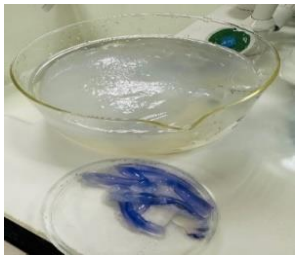

Figure 4.11. Separation of the bell from the mouth arms

Precipitation: The resulting opalescent mixture was treated with 0.9 M sodium chloride (NaCl) to obtain a homogeneous precipitate. The NaCl crystals incorporated into the structure of the collagen extracted from the *R. pulmo* jellyfish using the acid method (ASC), resulting in a homogeneous solution (Figure 4.13).

It can be observed in Figure 4.12 that supernatant 1 (F1) is slightly pink, number 2 (F2) is a pale yellow, and the third (F3) is nearly colorless. The supernatant can be described as a viscous-looking liquid, opalescent white in color.

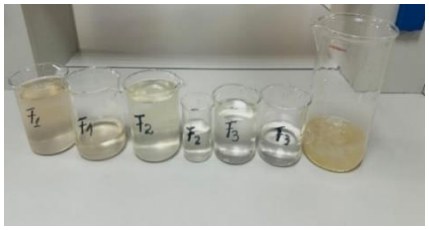

Figure 4.12. The filtrates after 72 hours were subjected to extraction with 0.5 M acetic acid

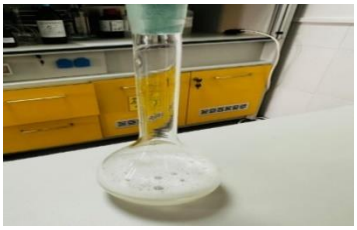

Figure 4.13. Precipitation of the filtrate combined with 0.9 M NaCl

Filtration: The mixtures were passed through a 0.5-mm mesh sieve and gauze filters, and the resulting collagen, finally, combining the precipitates yielded an opalescent mixture. **Centrifugation:** The collagen precipitate obtained from jellyfish was separated by

centrifugation (Figure 4.17). The final product (Figure 4.19) consisted of a white, gel-like collagen hydrolysate obtained following extraction with 0.5 M acetic acid.

Figure 4.17. Sample preparation

Figure 4.18. Centrifugation of collagen samples

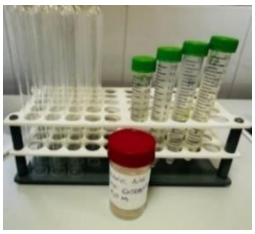

Figure 4.19. Centrifuged collagen hydrolysates

Cross-linking: This was performed using 10% tannic acid, a quantity sufficient to cross-link the collagen extracted from the jellyfish. The purpose of chemical cross-linking is to reduce the antigenicity of the biomaterials by preventing collagen degradation. Figure 4.20 shows the jellyfish collagen hydrolysate before cross-linking. Following the process, a final product with a 2% concentration was obtained; it was white, homogeneous, and had a gelatinous appearance (Figure 4.21).

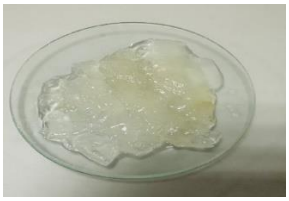

Figure 4.20. Jellyfish collagen hydrolysate prior to cross-linking

Figure 4.21. Cross-linking of jellyfish collagen hydrolysate with tannic acid

Freeze-drying: Freeze-dried collagen from the jellyfish species *Rhizostoma pulmo* appears as a white, irregular, slightly spongy film, referred to as a matrix, with properties similar to those of the extracellular matrix. Freeze-drying yielded collagen in the form of a white powder.

The collagen yield obtained from the jellyfish *Rhizostoma pulmo* using the two extraction methods (ASC) and (PSC). The highest collagen yield was obtained using primarily the oral arms of *Rhizostoma pulmo* and the pepsin extraction method (2–10 mg collagen/g wet tissue). Pepsin extraction is an improved method for extracting collagen, which allows the cleavage of the telopeptide regions of the triple helix, facilitating the release of collagen peptides into solution and increasing extraction yields.

Validation of the extraction method

The method used to determine the hydroxyproline content in jellyfish protein products was validated in accordance with existing performance criteria. The standard curve obtained

is linear, the coefficient of variation of the method is $Vx_0 = 4.03 < 10\%$, the correlation coefficient $R = 0.997 > 0.995$, and the comparative informant value $F = 4.54$; linearity was maintained across the entire established working range; in this case, the calibration curve meets the performance criteria (limit of detection and quantification, accuracy, precision, stability, robustness, repeatability, and measurement uncertainty) (**Peșterău 2025**).

The performance criteria meet the requirements, indicating that the method is viable for analyzing the hydroxyproline content in the extract obtained from the jellyfish species *Rhizostoma pulmo*.

CHAPTER 5. STUDIES ON THE PHYSICO-CHEMICAL CHARACTERIZATION OF COLLAGEN FROM THE *RHIZOSTOMA PULMO* JELLYFISH IN THE BLACK SEA BASIN

Chapter 5 aimed to conduct an advanced physicochemical and microscopic characterization study of collagen extracted from the *Rhizostoma pulmo* jellyfish found in the Black Sea basin, with the goal of evaluating its potential as a biotechnological resource that can be utilized in multiple fields.

Identification and characterization of collagen extracted from the jellyfish *Rhizostoma pulmo* using the SDS-PAGE method

The presence of α1 and α2 chains is evident in both the collagen from the *R. pulmo* jellyfish and that from *Grey mullet* skin, while the β and γ chains are identified in different regions, but are more abundant in fish collagen than in jellyfish collagen. The two extraction methods (ASC and PSC) revealed similar characteristics.

The electrophoretic pattern is characteristic of type I collagen, with distinct α1 and α2 chains of different mobility. SDS-PAGE analysis revealed several major protein bands for collagen extracted from jellyfish, which is consistent with previous publications on the molecular weight of collagens extracted from *R. pulmo* by (De Domenico, 2019; Addad, 2011; Widdowson, 2018; Paradiso, 2019).

Analysis by UV-DC dichroism spectroscopy. This analysis aims to demonstrate the helical structure of collagen from the jellyfish *R. pulmo,* and one of its main objectives is to determine the degree of denaturation and renaturation of marine collagen samples extracted from this invertebrate. By comparing the results obtained from the UV-DC spectroscopy

analysis of jellyfish collagen, we can conclude that they are consistent with the scientific literature, confirming the triple-helix structure typical of collagen.

Denaturation temperature of collagen from the jellyfish species *R. pulmo*. The melting temperature of the analyzed jellyfish collagen was approximately 28.9°C; it is worth noting that this is significantly lower than the denaturation temperature of collagen from animal sources (37°C). This is due to the more temperate habitat of the *R. pulmo* jellyfish and, consequently, the lower physiological temperature required for tissue remodeling *in vivo* (Subhan, 2015).

FT-IR spectroscopy analysis. The spectral patterns of the amide bands of collagen extracted from the jellyfish *R. pulmo* were comparable to those of mammalian collagen sources, as were the peak frequencies of amides I, II, and III. Amide I was at 1647 cm^{-1} in *R. pulmo*, and the amide II peak was at 1550 cm^{-1}. Amide III exhibited a peak frequency of 1238 cm^{-1} in *R. pulmo*. FT-IR analysis confirmed the triple-helix structure of jellyfish collagen, the high intermolecular order, and the similar secondary structure of proteins among the different collagen sources.

Thermogravimetric analysis. According to the TGA results for collagen extracted from jellyfish, water was lost at approximately 100°C and accounted for 12.87% of the total collagen weight. The thermogravimetric analysis profile of collagen from the jellyfish species *R. pulmo* shows that the mass loss of collagen occurs gradually in two stages. The components of jellyfish collagen were 24.36% (organic component) and 70.28% water; after 600°C, only the inorganic components (ash) remained, amounting to 5.36%.

UV-VIS Spectrophotometric Analysis

The ultraviolet range (200–280 nm), the developed film provides protection against UV radiation (radiation transmission approaches zero). Transmission through the sample in the 200–280 nm range is 0 indicating that ultraviolet radiation is blocked by the film, which contains collagen from the jellyfish *R. pulmo.*

Optical and electron microscopy (SEM) analysis

According to electron microscopy images jellyfish collagen has an irregular, dense fibrous structure containing pores of varying sizes and complex fibrillar forms, such as ribbon-like and branched filaments, as has also been reported in the literature. Analysis of *R. pulmo* jellyfish collagen reveals a distinct collagen microstructure characterized by a dense, irregular fibrous pattern; this unique morphology is a key feature when evaluating the suitability of jellyfish collagen as a biomaterial, for example, in tissue engineering, as previously noted in the literature (Balikci, 2024).

CHAPTER 6. STUDIES ON THE BIOCHEMICAL COMPOSITION OF COLLAGEN EXTRACTED FROM THE JELLYFISH *RHIZOSTOMA PULMO*

Biochemical analysis of collagen extracted from *Rhizostoma pulmo*

Table VI.1. presents the results of the analysis of collagen extracted from the jellyfish *R. pulmo* by determining its biochemical composition and expressing the protein, lipid, and carbohydrate content numerically as a percentage of dry weight (dry tissue) in different anatomical parts of the jellyfish: whole body, bell, oral arms, and gonads (**Pesterau, 2025**). For comparison, data from the literature regarding the composition of *R. pulmo* are also presented (Leone, 2015; D'Ambra, 2022; De Domenico, 2019).

Table VI.1. Biochemical characteristics of collagen from *R. pulmo*

Hydrogel characteristics	Collagen extract from *R. pulmo* with 10% pepsin	References
Moisture % (dry tissue)	**15,1 ± 0,1**	**-**
Ash 600–800 °C % (dry tissue)	**0,55 ± 0,1**	**-**
Protein % (dry tissue)	**60,48 ± 1,72** **23,59 ± 1,89 B; 32 ± 1,19 OA; 17,56 ± 1,98 G**	**61,8 (**Leone, 2015**)** **6 W; 8,7–13,7 B; (**D'Ambra, 2022**)** **27 OA; 18 G (**D'Ambra, 2022**)**
Collagen content % (dry tissue)	**57,1 ± 0,6**	**56,3 (**De Domenico, 2019)
Lipids % (dry tissue)	**4,9 ± 0,81 W; 1,95 ± 0,3 G**	**2,3 W; (**D'Ambra, 2022**)** **4,0 ± 0,1 W; 0,8 Brațe orale; 1.2 Gonade; (**D'Ambra, 2022**)**
Carbohydrates % (dry tissue)	0,59 ± 1,25 W; 0,25 ± 0,65 G	-

The data are expressed as percentages of dry weight (DW) of the whole body (W) or of different body parts (B, bell; OA, oral arms; G, gonads). ±SD for n = 3.

Determination of Amino Acids in the Jellyfish *R. pulmo*. The amino acid content of the jellyfish *R. pulmo* collected from the Romanian Black Sea coast is presented in Table VI.2. For reference, the values obtained by James et al. (2023) are also included, based on the analysis of amino acid content in *R. pulmo* (James, 2023). Small amounts of cysteine (*Cys*), histidine (*His*), and serine (*Ser*) were identified in the collagen extract of *R. pulmo* from the Black Sea. Following earlier research conducted by James et al. (2023) on the jellyfish *Rhizostoma pulmo*, the following amino acids were reported in the order: *Gly, Glu, Ala, Asp, Leu* (James, 2023), whereas the analysis performed on the jellyfish from Romania shows the following order: *Gly, Glu, Leu, Ala, Asp, Lys, Arg* (**Pesterau, 2025**).

Determination of the total polyphenol content in the jellyfish *R. pulmo*. The presence of polyphenols was subsequently confirmed using the Folin-Ciocâlteu colorimetric method,

and their concentration was quantified relative to a gallic acid standard. The total phenolic content of the collagen hydrolysate from the *R. pulmo* jellyfish was determined to be 1540 ± 291 μg GAE/g DW, a result comparable to those in other specialized studies (Leone, 2019). According to the analysis performed using the Folin-Ciocâlteu colorimetric method on the collagen extract from the jellyfish species *Rhizostoma pulmo*, it can be stated that polyphenols are present in the structure of this invertebrate, a fact that accounts for the antioxidant activity of the jellyfish's bioactive compounds, which is consistent with the literature (Leone, 2019). Determination of phenolic compounds in the collagen extract of the jellyfish species *Rhizostoma pulmo* using HPLC.

Given that the presence of phenolic compounds has been demonstrated, a specific objective has been set to further investigate the types of polyphenols present in the collagen extract of the jellyfish *R. pulmo*, harvested from the Black Sea, by identifying and naming each individual component using high-performance liquid chromatography (HPLC) with diode-array detection (HPLC-DAD).

Three polyphenols were identified in samples of collagen from the jellyfish species *Rhizostoma pulmo* extracted from the Black Sea: caftaric, gallic, and syringic acids. HPLC chromatographic analysis of the two samples filtrate and marine collagen extract, respectively, from the species *R. pulmo* revealed the presence of three polyphenols: caftaric acid (280 nm), gallic acid (280 nm), and syringic acid (230 nm) (**Pesterau, 2025**). The number of polyphenols identified by HPLC in the jellyfish collagen extract was not high because it is not typical for invertebrates to store polyphenolic compounds characteristic of the plant realm.

CHAPTER 7. BIOLOGICAL PROPERTIES OF COLLAGEN EXTRACTED FROM THE JELLYFISH *RHIZOSTOMA PULMO*

Chapter 7 discusses the analysis of the biological properties of collagen obtained from the jellyfish species *Rhizostoma pulmo*, extracted from the Black Sea basin. Antioxidant activity was tested using the DPPH method and the Reducing Power method, as well as antibacterial activity against several bacterial species.

Determination of antioxidant activity using the DPPH method. The antioxidant activity of jellyfish collagen extracted from *R. pulmo* was tested using two different methods: DPPH

absorption and reducing power assays. The results were obtained 30 minutes after the addition of the DPPH solution (**Pesterau, 2025**).

According to the graph, the maximum value for collagen extracted from the jellyfish *R. pulmo* is 30.62%, compared to ascorbic acid, which has a maximum of 98.50%. It can be observed that the antioxidant potency of jellyfish collagen is lower compared to the standard used, ascorbic acid.

The calculated IC_{50} value (the concentration of compounds capable of inhibiting 50% of total DPPH radicals) for ascorbic acid, used as a reference standard, was 187.82 mg/mL, while the values for the *R. pulmo* jellyfish collagen (JPC) samples were 225.74 mg/mL.

Determination of antioxidant activity by the Reducing Power method

Ascorbic acid processed under the same conditions as the *R. pulmo* jellyfish collagen solutions was used as a standard at a concentration of 200 µg/mL (**Pesterau, 2025**). The reducing power of jellyfish collagen is lower than that of the ascorbic acid standard used in the study. The maximum reducing power of the *R. pulmo* jellyfish extract was 0.552 µg/mL, while ascorbic acid had a maximum of 2.113 µg/mL.

Determination of the antibacterial activity of *R. pulmo* jellyfish collagen

This subsection focused on the antimicrobial analysis of collagen extracted from the jellyfish species *Rhizostoma pulmo*. The antimicrobial activities of collagen extracts from the jellyfish *R. pulmo* (JPC) were evaluated using the agar well diffusion method, with slight modifications.

Antimicrobial efficacy was assessed by measuring the diameter of the inhibition zones using the Kirby-Bauer zone-dichromatic method, and the results are reported as the average of three independent experiments (Hossain, 2024). Minimum inhibitory concentration (MIC) measurements were performed for collagen hydrolysates from *R. pulmo* (JPC); these were determined using the broth dilution method, as described by Tajbakhsh and colleagues (Tajbakhsh, 2011). *S. epidermidis* exhibited the lowest susceptibility to the collagen peptides from *R. pulmo*.

The microbiological study conducted on collagen peptides from *R. pulmo* demonstrates that they exhibit antimicrobial activity against the tested bacteria. According to the results obtained, it can be observed that *S. aureus*, *Streptococcus epidermidis*, Gram-positive bacteria, and Gram-negative *E. coli* developed the highest minimum inhibitory concentration, while *P. mirabilis* exhibits the lowest antibacterial activity against the analyzed *R. pulmo* collagen samples. A plausible explanation may involve differences in cell wall structure and membrane composition among microbial species.

CHAPTER 8. STUDIES ON THE FORMULATION OF TOPICAL PHARMACEUTICAL PREPARATIONS BASED ON COLLAGEN FROM THE JELLYFISH SPECIES *RHIZOSTOMA PULMO*

Chapter 8 presents and characterizes the newly developed pharmaceutical preparations based on collagen biomaterials: hydrogels, membranes, films, and porous matrices composed of jellyfish collagen combined with substances from various classes of drugs (antibacterial, antiseptic, antifungal, and wound-healing agents) and three species of Black Sea algae. Formulation of collagen hydrogels derived from *Rhizostoma pulmo* with various active substances. The collagen hydrogels (Figure 8.1.) were prepared by mixing *R. pulmo* collagen with various active substances at different concentrations, adjusted to a concentration of 0.4% and a pH of 7 using triethanolamine. The hydrogels were cross-linked with 10% tannic acid (relative to collagen) and maintained for 24 hours at 4°C.

Figure 8.1. Jellyfish hydrogel, species *Rhizostoma pulmo*

Hydrogels made from *R. pulmo* jellyfish collagen and iodoform, as shown in Figure 8.4, appear as pale yellow, gel-like structures with a strong, characteristic iodine odor; they could be successfully used in wound repair and tissue healing. Depending on the dose, tetracycline has a bactericidal or bacteriostatic effect on microorganisms such as: *Borrelia recurrentis*, *Mycobacterium marinum*, *Mycoplasma pneumoniae*, *Staphylococcus aureus* (including methicillin-resistant strains – MRSA), *Vibrio vulnificus*, and vancomycin-resistant enterococcus (VRE) (Semenova, 2025). Nystatin is used to treat *Candida albicans* infections of the digestive tract and as an adjunctive therapy for vaginal and cutaneous candidiasis.

Formulation of collagen hydrogels with various marine algae

New pharmaceutical formulations based on type I fibrillar collagen (undenatured) extracted from jellyfish were developed, into which algae extracts were subsequently incorporated. Figure 8.11 below shows the hydrogels obtained, both the simple one made from jellyfish collagen and those in which Black Sea algae species such as *Ulva lactuca*, *Ceramium rubrum*, and *Cladophora vagabunda* were incorporated.

Figure 8.11. (1) Simple jellyfish collagen hydrogel
(2) Jellyfish hydrogel with *Ulva lactuca*
(3) Jellyfish hydrogel with *Ceramium rubrum*
(4) Jellyfish hydrogel with *Cladophora vagabunda*

Formulation of collagen films derived from jellyfish with various active ingredients

Jellyfish collagen films and medicinal active substances possess different biological properties: antibacterial, antiseptic, and disinfectant in the case of iodoform and tetracycline, and antifungal in the case of nystatin. All films are yellow in color and have a characteristic odor corresponding to the substances they contain.

Formulation of collagen films from the jellyfish species *R. pulmo* with marine algae

The images below (Figures 8.21 and 8.22) show these marine jellyfish collagen hydrogels, as well as two species of green algae: *Ulva lactuca* and *Cladophora vagabunda*.

Figure 8.12. Jellyfish collagen film

Figure 8.23. Jellyfish collagen film with *Cladophora vagabunda*

Figure 8.21. Jellyfish collagen film with *Ulva lactuca*

CHAPTER 9. A STUDY OF WOUND-HEALING CAPACITY USING PREPARATIONS CONTAINING COLLAGEN EXTRACTED FROM THE JELLYFISH *RHIZOSTOMA PULMO*

The aim was to demonstrate the regenerative properties of new marine-derived bioactive compounds, particularly collagen extracted from the Black Sea jellyfish *Rhizostoma pulmo*, in synergy with the biological and pharmacological properties of bioactive compounds from hydroalcoholic extracts obtained from the brown alga *Cystoseira barbata*. The study of the effects of JPC-ALG composites and individual extracts on fibroblast and keratocyte cell lines is presented (**Pesterau, 2025**).

Biological evaluation of a new JPC-ALG composite for wound healing applications. Preliminary biological studies were conducted using BALB/3T3 fibroblasts, clone A31, in

accordance with ISO 10993-1 (Geneva, Switzerland, 2018), given the essential role of these fibroblasts in the wound healing process (ISO 10993-1, 2018). To determine the optimal concentration for the scratch test, cytotoxicity assessments were first performed.

These tests evaluated the effects of JPC (collagen peptides from *R. pulmo*), as well as the compound formulations JPC-ALG1 (collagen peptides with 10% *C. barbata* extract) and JPC-ALG2 (collagen peptides with 20% *C. barbata extract*). After 24 hours of incubation, high cell viability was maintained at lower concentrations (0.015–0.125 mg/mL), with values ranging from 87% to 96% for all samples tested. At higher concentrations (up to 2 mg/mL), viability decreased gradually but remained between 66–74% after 24 hours. At 48 hours, a further reduction in cell proliferation was observed as concentrations increased (**Pesterau, 2025**). Specifically, cell proliferation remained above 80% at 0.01 mg/mL for all samples, but decreased to 37–40% at 0.5 mg/mL and to 24–26% at 2 mg/mL. These results indicate acceptable cytocompatibility at lower concentrations, consistent with previous findings regarding jellyfish-derived collagen peptides (JSPs), as reported by Migone et al. (2022) and Li et al. (2017) (Migone, 2022; Li, 2017).

Wound Healing via the Fibroblast Scratch Assay

The wound-healing capacity of the new biocomposite, based on collagen from the jellyfish species *Rhizostoma pulmo* and extract from the brown alga *C. barbata*, was evaluated using the scratch assay. The scratch assay is a well-established *in vitro* method used to mimic wound healing by mechanically creating a gap in a confluent monolayer of cells. This assay models cell migration and proliferation toward the wound site, as demonstrated by Migone and Morgner (Migone, 2022; Morgner, 2022).

The aim was to evaluate the dermal healing potential of the new topical pharmaceutical formulation (JPC-ALG) derived from *R. pulmo* jellyfish collagen and *C. barbata* brown algae, both extracted from the Black Sea basin. The effects of JPC, JPC-ALG 4, and JPC-ALG 2 on fibroblast migration were evaluated by monitoring wound closure at 4, 24, and 48 hours after injury.

All tested samples significantly accelerated cell migration and improved the confluence rate compared to the untreated control.

At 48 hours, the control group showed only 5% confluence, while JPC-treated cells achieved 66% wound closure. It should be noted that JPC-ALG 1 and JPC-ALG 2 demonstrated even higher wound closure rates, reaching approximately 86–92% closure. These findings support the potential of various concentrations of the JPC-ALG1 and JPC-ALG2 formulations in skin tissue regeneration (**Pesterau, 2025**).

Wound healing via the keratinocyte scratch assay

The scratch assay was used to evaluate the effect of the biocomposite formulations on the migration of HaCaT keratinocytes. Using a single-chamber assay, wound closure capacity was monitored over a 24-hour period. The increased migratory response produced by JPC-ALG 1 and JPC-ALG 2, demonstrating their potential efficacy in promoting wound healing through increased keratinocyte motility (**Pesterau, 2025**). This test revealed different behaviors depending on the treatment administered (JPC-ALG1 or JPC-ALG2) and the incubation period. At both 2 hours and 24 hours of incubation, both analyzed groups, JPC-ALG1 and JPC-ALG2, showed statistically significant differences compared to the control (CTRL) (**Pesterau, 2025**). This biocompatibility was also observed in other jellyfish collagen fractions (Felician, 2019; Migone, 2022)

CHAPTER 10. STUDIES ON THE *IN VITRO* EVALUATION OF ANTITUMOR EFFECTS IN THE ORAL CAVITY OF A BIOCOMPOSITE MADE FROM *RHIZOSTOMA PULMO* JELLYFISH COLLAGEN AND THE MEDICINAL MUSHROOM *GANODERMA LUCIDUM*

The aim was to analyze a new biocomposite based on bioactive compounds from the jellyfish *Rhizostoma pulmo* and the medicinal mushroom *Ganoderma lucidum*, intended for the *in vitro* evaluation of antitumor effects in the oral cavity. An antibacterial analysis was performed on the new biocomposite obtained from the ethanolic extract of *Ganoderma lucidum*, a mushroom species from the Bistrița-Năsăud area, Romania, and the collagen extract obtained by the enzymatic method with pepsin from the jellyfish species *Rhizostoma pulmo* from the Black Sea basin, in the Constanța coastal area, Romania.

Antimicrobial activity. The antimicrobial activity of the collagen extract from *R. pulmo* (JPC), the *Ganoderma lucidum* ethanol extract (GL), and the newly developed formulation E2 (JPC–GL) was evaluated against common oral pathogens: *S. mutans*, *S. aureus*, and *C. albicans*. The results of antimicrobial activity expressed as inhibition zone diameters (mm), determined using the disk diffusion method. This method was applied to collagen peptides extracted from *R. pulmo*, the ethanolic extract of *G. lucidum*, the newly formulated E2 (JPC–GL) biocomposite, and Amoxicillin (used as a reference antibiotic). It is worth noting the enhanced inhibitory activity of the *G. lucidum* and *R. pulmo* biocomposite against

Staphylococcus aureus compared to the individual components. This selective synergistic effect was not observed for other microorganisms tested.

Determination of the antitumor activity of biocomposites derived from R. pulmo and G. lucidum

The antitumor activity of the extract derived from *R. pulmo* jellyfish collagen and the medicinal mushroom *G. lucidum* (GL), as well as that of the biocomposites E1 (JPC–GL) and E2 (JPC–GL), was evaluated using oral squamous cell carcinoma (OSCC) cell lines, namely SCC-9 and HSC-3. Cytotoxic activity was evaluated in vitro by determining cell viability in two oral squamous cell carcinoma (OSCC) cell lines: SCC-9 and HSC-3 cells.

The antitumor effects of the tested samples. Cell viability decreased progressively over time, specifically at 24 hours (from 69–71% to 32–35%), at 48 hours (from 66–68% to 26–28%), and at 72 hours (from 64–66% to 18–19%). These results demonstrate the strong, time- and dose-dependent cytotoxic effects of both the JPC–GL biocomposites and the GL extract on the cells. Both formulations, E1 and E2, exhibited similar inhibitory effects on SCC-9 cell viability; these results demonstrate the strong, time- and dose-dependent cytotoxic effects of JPC–GL biocomposites on OSCC cells. Both formulations, E1 and E2, exhibited similar inhibitory effects on the viability of SCC-9 cells. *In vitro* evaluation of oral cavity tumor cells demonstrated that formulations E1 (JPC-GL) and E2 (JPC-GL) caused a significant, dose-dependent reduction in cell viability.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

This doctoral dissertation, entitled "*Comparative physicochemical studies on new pharmaceutical formulations made from collagen extracts sourced from the Black Sea*," aimed to develop new pharmaceutical formulations based on collagen from the jellyfish species *Rhizostoma pulmo*, extracted from the Black Sea basin, their analysis, and the demonstration of the biomedical properties possessed by biocomposites made from marine collagen and other biocomposites. The objectives proposed in this doctoral thesis were achieved, namely: the extraction of jellyfish collagen from the Black Sea basin, the analysis and physicochemical characterization of the collagen extract as well as of the new pharmaceutical formulations for topical application prepared to demonstrate biological properties of tissue regeneration, wound healing, and antitumor activity in oral cancers.

Results were obtained that confirm the achievement of the objectives set for all proposed research directions, demonstrating the applicability of collagen extracts from *Rhizostoma pulmo* and the newly formulated preparations in the medical-pharmaceutical field. The experimental studies in the second part of the thesis are included in the section on personal contributions in Chapters 4–10. ***Chapter 4*** describes the extraction of jellyfish collagen using the ASC method and the PSC method. Subsequently, the extraction process used to obtain marine collagen from the jellyfish *Rhizostoma pulmo* was validated by meeting the performance criteria for validation, based on the hydroxyproline content, which is an indicator of collagen presence in the samples analyzed. In ***Chapter 5***, the triple-helix structure of jellyfish collagen was confirmed through various types of physicochemical analyses; the electrophoretic pattern is characteristic of type I collagen. According to microscopy images, jellyfish collagen has an irregular and dense fibrous structure containing pores of irregular sizes and complex fibrillar forms, such as ribbon-like and branched filaments. ***Chapter 6*** revealed that the main amino acids present in the collagen extract of *R. pulmo* are: glycine (*Gly*), which is present in the highest quantity, followed by glutamic acid (*Glu*), and tryptophan (*Trp*), which is also found in the *R. pulmo* jellyfish from the Black Sea; the amino acids identified in small quantities in the samples were: cysteine (*Cys*), histidine (*His*), and serine (*Ser*). HPLC chromatographic analysis of the two samples filtrate and marine collagen extract, respectively, from the species *R. pulmo* revealed the presence of three polyphenols: caffeic acid (280 nm), gallic acid (280 nm), and syringic acid (230 nm). ***Chapter 7*** highlights the biological properties of *R. pulmo* jellyfish collagen. Analysis of antioxidant activity using the DPPH method revealed a maximum value of 30.62% for collagen extracted from *R. pulmo*, compared to the chosen standard (ascorbic acid), which had a value of 98.50%. The antioxidant activity of the collagen extract from *R. pulmo*, as determined by the Reducing Power method, demonstrated the reducing capacity of marine jellyfish collagen. Following the antibacterial analysis, it was observed that *S. aureus*, *Streptococcus epidermidis*, Gram-positive bacteria, and Gram-negative *E. coli* developed the highest minimum inhibitory concentration, while *P. mirabilis* exhibited the lowest antibacterial activity among the analyzed *R. pulmo* collagen samples.

Chapter 8 presents new preparations derived from jellyfish collagen (the species *Rhizostoma pulmo*) that are described and analyzed macroscopically, containing active substances such as iodoform, tetracycline, and nystatin, as well as hydrogels, membranes, films, and porous matrices based on *R. pulmo* jellyfish collagen combined with various species of algae: *Cladophora vagabunda*, *Ulva lactuca*, and *Ceramium rubrum.*

Chapter 9 evaluated the *in vitro* wound-healing potential of the new formulation (JPC-ALG) derived from jellyfish collagen (*Rhizostoma pulmo*) and the ethanolic extract of *C. barbata* using the scratch assay in cell cultures. The results demonstrated synergistic effects of the new biocomposite, enhancing the migration and proliferation of fibroblasts and keratinocytes key factors in wound closure supporting the potential application of this bioactive marine-derived composite as a promising biomaterial for wound healing therapies.

Chapter 10 presents an analysis of the new formulations based on collagen extract from *R. pulmo* and the *Ganoderma lucidum* mushroom (E1 and E2), which exhibited similar inhibitory effects on SCC-9 cell viability; the results demonstrate the potent, time- and dose-dependent cytotoxic effects of JPC–GL biocomposites on OSCC cells.

SELECTED BIBLIOGRAPHY

1. Addad S., Exposito J.-Y., Faye C., Ricard-Blum S., Lethias, C. (2011). Isolation, Characterization and Biological Evaluation of Jellyfish Collagen for Use in Biomedical Applications. . *Mar. Drugs*. 9, 967–983.
2. Balikci E., Baran, E.T., Tahmasebifar A. (2024). Characterization of Collagen from Jellyfish Aurelia aurita and Investigation of Biomaterials Potentials. *Appl Biochem Biotechnol*. 196, 6200–6221.
3. Benayahu D., Pomeraniec, L., Shemesh, S., Heller S., Rosenthal Y., Rath-Wolfson L., Benayahu Y. (2020). Biocompatibility of a Marine Collagen-Based Scaffold In Vitro and In Vivo. *Mar. Drugs.* 18, 420–431.
4. Cadar E., **Pesterau A.M**., Prasacu I., Ionescu A.M., Pascale C., Dragan A.L., Sirbu R., Tomescu C.L. (2024). Marine Antioxidants from Marine Collagen and Collagen Peptides with Nutraceuticals Applications: A Review. *Antioxidants* (Basel). 13(8):919.
5. Cadar E., **Pesterau A.M**., Sirbu R., Negreanu-Pirjol B.S., Tomescu C.L. (2023). Jellyfishs - significant marine resources with potential in wound healing process. A Review. *Mar. Drugs, 21*, 201-228.
6. D'Ambra I., Merquiol L. (2022). Jellyfish from Fisheries By-Catches as a Sustainable Source of High-Value Compounds with Biotechnological Applications. *Mar. Drugs*. 266(20).
7. De Domenico, S. De Rinaldis, G. Paulmery, M. Piraino, S. Leone, A. (2019). Barrel Jellyfish (Rhizostoma pulmo) as Source of Antioxidant Peptides. *Mar. Drugs* 17, 134-155.
8. Deyl Z., Miksik I., Eckhardt A. (2003). Preparative procedures and purity assessment of collagen proteins, *J. Chromatogr*. B. 790, 245–275.
9. Felician, F.F. Yu, R.-H. Li, M.-Z. Li, C.-J., Chen H.-Q., Jiang Y Tang, T., Qi, W.-Y., Xu, H.-M. (2019). The wound healing potential of collagen peptides derived from the jellyfish Rhopilema esculentum. *Chin. J. Traumatol*. 22, 12–20.
10. Gallo N., Natali M.L., Sannino A., Salvatore L. (2020). An Overview of the Use of Equine Collagen as Emerging Material for Biomedical Applications. *J. Funct. Biomater*. 11(4), 79-106.
11. Gaspar-Pintiliescu, A. Anton, E.D. Iosageanu, A. Berger, D. Matei, C., Mitran, R.A., Negreanu-Pirjol, T. Craciunescu, O. Moldovan, L. (2021). Enhanced Wound Healing

Activity of Undenatured Type I Collagen Isolated from Discarded Skin of Black 1111 Sea Gilthead Bream (*Sparus aurata*) Conditioned as 3D Porous Dressing. *Chemistry & Biodiversit.* 18, 1–14.

12. Hossain M.J., Azad A.K., Shahid M.S.B., Shahjahan M., Ferdous J. (2024). Prevalence, antibiotic resistance pattern for bacteriuria from patients with urinary tract infections. *Health Sci. Rep*. 7(4):e2039.

13. ISO-10993-1; Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing within a Risk Management Process. International Organization for Standardisation: Geneva, Switzerland, 2018. Disponibil online la https://www.iso.org/standard/68936.html.

14. James S., Tilvi, S., Khandeparker R., Sreepada R.A., Thakur N., Gauthankar M. (2023). Jellyfish Rhizostoma pulmo collected off Goa Coast (India) as a rich source of tryptophan containing collagen and its enhanced antioxidant potential. *J. Food Sci. Technol*. 60, 2825-2834.

15. Kristoffersen K.A., Måge I., Wubshet S.G., Böcker U., Riiser Dankel K., Lislelid A., Rønningen M.A., Afseth N.K.. (2023). FTIR-based prediction of collagen content in hydrolyzed protein samples. *Spectrochim Acta A Mol Biomol Spectrosc*. 301:122919.

16. Leone A., Lecci R., Milisenda G., Piraino S. Mediterranean jellyfish as novel food: Effects of thermal processing on antioxidant, phenolic, and protein contents. (2019). *Eur. Food Res. Technol*. 245, 1611–1627.

17. Leone A., Lecci R.M., Durante M., Meli F., Piraino S. (2015). The Bright Side of Gelatinous Blooms: Nutraceutical Value and Antioxidant Properties of Three Mediterranean. *Mar. Drugs*. 13:4654–4681.

18. Li Q.M., Wang J.F., Zha X.Q. Pan L.H., Zhang H.L., Luo J.P., (2017). Structural characterization and immunomodulatory activity of a new polysaccharide from jellyfish. *Carbohydr. Polym.* 159, 188–194.

19. Lin Y., Liu D. (2006). Comparison of physical-chemical properties of type I collagen from different species. *Food Chem*. 99, 244–251.

20. Migone C., Scacciati N., Grassiri B., De Leo M., Braca,A., Puppi D., Zambito Y., Piras A.M. (2022). Jellyfish Polysaccharides for Wound Healing Applications. *Int. J. Mol. Sci*. 23, 11491.

21. Morgner B., Husmark J., Arvidsson A., Wiegand C. (2022). Effect of a DACC-coated dressing on keratinocytes and fibroblasts in wound healing using an in vitro scratch model. *J. Mater. Sci. Mater. Med*. 33, 22.

22. Paradiso, F., Fitzgerald J., Yao S., Barry F., Taraballi F., Gonzalez D., Francis L. (2019). Marine Collagen Substrates for 2D and 3D Ovarian Cancer Cell Systems. *Front. Bioeng. Biotechnol*. 7, p.343.

23. Pascale C., Burcea A., Bogdan-Andreescu C.F., Cadar E., Popescu A., Negreanu-Pirjol T., Busuricu F., Pesterau A.-M., Rosca A.C., Sirbu R. (2026). Characterization of a New Biocomposite Based on Bioactive Compounds from Ganoderma lucidum and Jellyfish Collagen Destined for In Vitro Evaluation of Antitumor Effects in the Oral Cavity. *Pharmaceuticals.* 19, 108.

24. Peşterău A.-M., R. Sîrbu, E. Cadâr. (2022). Method for Obtaining and Physico-Chemical Characterization of Collagenic Extract of Rhizostoma pulmo from the Black Sea. *EJNSM,* 5(1), 48-57.

25. Peşterău A.-M., Sîrbu R., Cadâr E. (2025). Extraction, Identication and Characterization by Sds-Page Method of Collagen Extracted from Rhizostoma Pulmo, A Jellyfish Found in the Black Sea Basin. *EJNSM*. 8(1), 53-61.

26. **Peșterău A-M**, Sîrbu R, Cadâr E. (2025). Extraction and Method Validation for Collagen Obtained from the Jellysh Rhizostoma Pulmo Found on the Romanian Black Sea Coast. *EJNSM*. 7(1), 1-18.
27. **Pesterau A.-M**., Popescu A., Sirbu R., Cadar E., Busuricu F., Dragan A.-M.L., Pascale C., Ionescu A.-M., Bogdan-Andreescu C.F., Radu M.-D. (2025). Marine Jellyfish Collagen and Other Bioactive Natural Compounds from the Sea, with Significant Potential forWound Healing and Repair Materials. *Mar. Drugs*. 23, 252.
28. Ryu B., Shin K.H., Kim S.K. (2021). Muscle Protein Hydrolysates and Amino Acid Composition in Fish. *Mar. Drugs.* 19, 377–389.
29. Semenova Y., Makalkina L., Glushkova N., Gaipov A. (2025). Tetracyclines in the Modern Era: Global Consumption, Antimicrobial Resistance, Environmental Occurrence, and Degradation Techniques. *Antibiotics.* 14, 1183.
30. Song Z., Liu H., Chen, L., Chen L., Zhou C., Hong P., Deng C. (2021). Characterization and comparison of collagen extracted from the skin of the Nile tilapia by fermentation and chemical pretreatment. *Food Chem*. 340, 128139.
31. Subhan F., Ikram M., Shehzad A., Ghafoor A. (2015). Marine Collagen: An Emerging Player in Biomedical applications. *J. Food Sci. Technol.* (8):4703-7.
32. Tajbakhsh S., Ilkhani M., Rustaiyan A., Larijani K., Sartavi K., Tahmasebi R., Asayesh, G. (2011). Antibacterial effect of the brown alga Cystoseira trinodis. *J. Med. Plants Res*. 5, 4654–4657.
33. Widdowson P., Picton J., Vinc,V., Wright C., Mearns-Spragg A. (2018). In vivo comparison of jellyfish and bovine collagen sponges as prototype medical devices. *J. Biomed. Mater. Res. B Appl. Biomater.* 106, 1524–1533.
34. Yathisha U.G., Bhat I., Karunasagar I., Mamatha B.S. (2018). Antihypertensive activity of fish protein hydrolysates and its peptides. *Crit. Rev. Food Sci. Nutr*. 59, 2363–2374.
35. Yu Y.Q., Yang X., Wu X.F., Fan Y.B. (2021). Enhancing Permeation of Drug Molecules Across the Skin via Delivery in Nanocarriers: Novel Strategies for Effective *Transdermal Applications. Front Bioeng Biotechnol.* 9:646554.

LIST OF PUBLISHED SCIENTIFIC WORKS

I. ARTICLES PUBLISHED IN ISI WEB OF SCIENCE LISTED JOURNALS WITH IF> 0.5

1. Cadar E., **Pesterau A.M.**, Sirbu R., Negreanu-Pirjol B.S., Tomescu C.L. Jellyfishes-Significant Marine Resources with Potential in the Wound-Healing Process: A Review. *Marine Drugs,* **2023,** 21(4), 201. **IF 5.4/2023 Q1**.
   https://doi.org/10.3390/md21040201
   https://pubmed.ncbi.nlm.nih.gov/37103346/
   34 citations WOS la data 01.04.2026. (Chapter 2, pg .21, 23, 27 and Chapter 6, pg. 75).
2. Cadar, E., **Pesterau, A.-M**.;Prasacu, I.; Ionescu, A.-M.; Pascale, C.; Dragan, A.-M.L.; Sirbu, R.; Tomescu,C.L. Marine Antioxidants from Marine Collagen and Collagen Peptides with Nutraceuticals Applications: A Review. *Antioxidants* **2024**, 13(8), 919. **IF 6/2024 Q1**.
   https://doi.org/10.3390/antiox13080919
   https://www.mdpi.com/2890954
   48 citations WOS la data 01.04.2026. (Chapter 1, pg.7, 8 and Chapter 2, pg. 17, 18, 22).

3. Emin Cadar; Antoanela Popescu; Ana-Maria-Laura Dragan; **Ana-Maria Pesterau**; Carolina Pascale; Valentina Anuta; Irina Prasacu; Bruno Stefan Velescu; Cezar Laurentiu Tomescu; Claudia Florina Bogdan-Andreescu; Rodica Sirbu; Ana-Maria Ionescu. Bioactive Compounds of Marine Algae and Their Potential Health and Nutraceutical Applications: A Review. *Marine Drugs* **2025**, 23(4), 152. **IF 5.4/2025 Q1**.
https://doi.org/10.3390/md23040152
https://www.mdpi.com/3249700
69 citations WOS la data 01.04.2026. (Chapter 1, pg. 7, 8 and Chapter 2, pg. 18).
4. **Pesterau, A.-M**.; Popescu, A.; Sirbu, R.; Cadar, E.; Busuricu, F.; Dragan, A.-M.L.; Pascale, C.; Ionescu, A.-M.; Bogdan-Andreescu, C.F.; Radu, M.-D.; et al. Marine Jellyfish Collagen and Other Bioactive Natural Compounds from the Sea, with Significant Potential for Wound Healing and Repair Materials. *Mar. Drugs* **2025**, 23(6), 252. **IF 5.4/2025 Q1**.
https://doi.org/10.3390/md23060252
https://www.mdpi.com/3356832
10 citations WOS la data 01.04.2026. (Chapter 2, pg. 23, 26, 27; Chapter 6, pg. 72, 74, 80; Chapter 7, pg. 88; Chapter 8, pg. 97; Chapter 9, pg. 104, 105, 106 111, 112, 113, 114 and Chapter 10, pg. 116).
5. Pascale, C.; Burcea, A.; Bogdan-Andreescu, C.F.; Cadar, E.; Popescu, A.; Negreanu-Pirjol, T.; Busuricu, F.; **Pesterau, A.-M**.; Rosca, A.C.; Sirbu, R. Characterization of a New Biocomposite Based on Bioactive Compounds from Ganoderma lucidum and Jellyfish Collagen Destined for In Vitro Evaluation of Antitumor Effects in the Oral Cavity. *Pharmaceuticals* **2026**, 19(1), 108. **IF 4.8/2026 Q1**.
https://doi.org/10.3390/ph19010108
https://www.mdpi.com/3670662
(Chapter 2, pg. 26, 28 and Chapter 10, pg. 116,118,119).

II. ARTICLES PUBLISHED IN BDI/PUBMED INDEXED JOURNALS

1. **Pesterau Ana-Maria**, Rodica Sirbu, Emin Cadar, "Method for Obtaining and Physico-Chemical Characterization of Collagenic Extract of Rhizostoma pulmo from the Black Sea". *EJNSM* **2022,** 5(1), 48-57.
DOI:10.26417/769szy13
https://revistia.com/files/articles/ejmn_v6_i1_23/Pesterau.pdf
1 citations Google Scholar la data 01.04.2026. (Chapter 1, pg. 6 and Chapter 4, pg. 35, 41).
2. **Pesterău Ana-Maria**, Rodica Sirbu, Emin Cadar. "Biomedical Applications Based on Marine Collagen Obtained from the Jellyfish Species Rhizostoma Pulmo Extracted from the Black Sea. *EJNSM* **2023,** 6(2), 89-99.
DOI: 10.2478/ejnsm-2023-0009
https://revistia.com/ejmn/article/view/3185
6 citations Google Scholar la data 01.04.2026. (Chapter 2, pg. 19 and Chapter 8, pg. 99).
3. **Pesterau Ana-Maria**, Sîrbu Rodica, Cadâr Emin. "Extraction and Method Validation for Collagen Obtained from the Jellysh Rhizostoma Pulmo Found on the Romanian Black Sea Coast". *EJNSM,* **2025**, 7(1), 1-18.
https://doi.org/10.26417/180g2280
https://revistia.com/ejmn/article/view/3169
2 citations Google Scholar la data 01.04.2026. (Chapter 4, pg. 49, 50, 52, 53).

4. **Pesterău Ana-Maria**, Sirbu Rodica, Cadar Emin "Extraction, Identication and Characterization by Sds-Page Method of Collagen Extracted from Rhizostoma Pulmo, A Jellyfish Found in the Black Sea Basin" *EJNSM,* **2025**, 8(1), 53-61. https://doi.org/10.26417/g9nnp502 https://revistia.com/ejmn/article/view/3165 1 citare Google Scholar la data 01.04.2026. (Chapter 2, pg. 27 and Chapter 5, pg. 55, 56, 57).

III. SCIENTIFIC WORKS PUBLISHED DURING THE YEARS OF STUDY

1. **Pesterau Ana-Maria**, Rodica Sirbu, Emin Cadar, "Method for Obtaining and Physico-Chemical Characterization of Collagenic Extract of Rhizostoma pulmo from the Black Sea", European Journal of Natural Science and Medicine, Volume 5, Issue 1, 2022, p. 48-57. https://revistia.com/files/articles/ejmn_v6_i1_23/Pesterau.pdf.
2. Cadar Emin, **Pesterău Ana-Maria**, Sirbu Rodica, Negreanu-Pirjol, Bogdan-Stefan, Tomescu Cezar Laurenţiu. *Jellyfishes—Significant Marine Resources with Potential in the Wound-Healing Process: A Review.* Marine Drugs, Volume 21, Issue 4, 2023, 201. https://doi.org/10.3390/md21040201.
3. **Pesterau Ana-Maria,** Rodica Sirbu, Emin Cadar. "Biomedical Applications Based on Marine Collagen Obtained from the Jellyfish Species Rhizostoma Pulmo Extracted from the Black Sea. EJNSM 2023, 6(2), 89-99. https://revistia.com/ejmn/article/view/3185.
4. **Ana-Maria Peşterău**, Cristina-L Erimia. Legislative aspects of the advertising of medicinal products for human use, Vol.3 Issue 4, 81-86 (2023), DOI: https://doi.org/10.47577/biochemmed.v3i2.6935.
5. **Pesterau Ana-Maria**, Sîrbu Rodica, Cadâr Emin. Pharmaceutical Management in the Context of Artificial Intelligence. EJMN, 2023, 6(2), 37–43. Retrieved from https://revistia.com/index.php/ejmn/article/view/6978.
6. Emin Cadâr, Laurențiu Tomescu Cezar, **Pesterau Ana-Maria**. Risk Management Considerations and Multiple Responsibilities in Medical Pharmaceutical Practice. EJMN, 2023 6(2), 148–160. https://revistia.org/index.php/ejnm/article/view/6150.
7. **Ana-Maria Peşterău,** Nicoleta Mirela Blebea, Interactions between medicinal plants used in the treatment of heart diseases and antihypertensive medication, Farmacist.ro.DOI:10.26416/Farm.217.2.2024.9504. https://www.medichub.ro/reviste-de-specialitate/farmacist-ro/interactiuni-intre-plantele-medicinale-utilizate-in-tratamentul-afectiunilor-cardiace-si-medicatia-antihipertensiva-id-9504-cmsid-62.
8. Cadar, E.; **Pesterau**, A.-M.;Prasacu, I.; Ionescu, A.-M.; Pascale, C.; Dragan, A.-M.L.; Sirbu, R.; Tomescu,C.L. Marine Antioxidants from Marine Collagen and Collagen Peptides with Nutraceuticals Applications: A Review. Antioxidants 2024, 13, 919. https://doi.org/10.3390/antiox13080919.
9. **Pesterau Ana-Maria**, Sirbu Rodica, Cadar Emin. "Extraction and Method Validation for Collagen Obtained from the Jellysh Rhizostoma Pulmo Found on the Romanian Black Sea Coast". *EJNSM* **2025**, 7(1), 1-18. https://revistia.com/ejmn/article/view/3169.
10. **Peșterău Ana-Maria**, Sîrbu Rodica, Cadâr Emin (2024) "Extraction, Identication and Characterization by Sds-Page Method of Collagen Extracted from Rhizostoma Pulmo, A Jellyfish Found in the Black Sea Basin" EJNSM 2025, 8(1), 53-61. https://revistia.com/ejmn/article/view/3165.
11. Emin Cadar; Antoanela Popescu; Ana-Maria-Laura Dragan; **Ana-Maria Pesterau**; Carolina Pascale; Valentina Anuta; Irina Prasacu; Bruno Stefan Velescu; Cezar

Laurentiu Tomescu; Claudia Florina Bogdan-Andreescu; Rodica Sirbu; Ana-Maria Ionescu. Bioactive Compounds of Marine Algae and Their Potential Health and Nutraceutical Applications: A Review. *Marine Drugs* **2025**, *23*, 152. https://doi.org/10.3390/md23040152.

12. **Pesterau, A.-M**.; Popescu, A.; Sirbu, R.; Cadar, E.; Busuricu, F.; Dragan, A.-M.L.; Pascale, C.; Ionescu, A.-M.; Bogdan-Andreescu, C.F.; Radu, M.-D.; et al. Marine Jellyfish Collagen and Other Bioactive Natural Compounds from the Sea, with Significant Potential for Wound Healing and Repair Materials. Mar. Drugs 2025, 23, 252. https://doi.org/10.3390/md23060252.
13. Pascale, C.; Burcea, A.; Bogdan-Andreescu, C.F.; Cadar, E.; Popescu, A.; Negreanu-Pirjol, T.; Busuricu, F.; **Pesterau, A.-M**.; Rosca, A.C.; Sirbu, R. Characterization of a New Biocomposite Based on Bioactive Compounds from Ganoderma lucidum and Jellyfish Collagen Destined for In Vitro Evaluation of Antitumor Effects in the Oral Cavity. Pharmaceuticals 2026, 19, 108. https://doi.org/10.3390/ph19010108.

IV. BOOKS AND BOOK CHAPTERS

1. Marine Algae Exploring Their Nutritional, Health, and Nutraceutical Potential, 2025, Edited by Leonel Pereira and Ana Marta Gonçalves, *Marine Drugs* (ISSN 1660-3397), MDPI AG, Grosspeteranlage 5, 4052 Basel, Switzerland, ISBN 978-3-7258-4943-7, pg. 135. https://mdpi-res.com/bookfiles/book/11471/Marine_Algae.pdf?v=1775265098.

V. PRESENTATIONS, CONFERENCES, CONGRESSES, SYMPOSIUMS

1. Participation in Workshop with international participation Alternative and complementary therapies Participation in the Naturist Medicine Course – Homeopathy/Phytotherapy, 26-27 March 2021, Constanta, Romania – Poster presentation –Marine collagen from jellyfish species of Rhizostoma pulmo from the Black Sea and its uses in alternative therapies.
2. Mention in Workshop with international participation Alternative and complementary therapies Participation in the Naturist Medicine Course – Homeopathy/Phytotherapy, 05-06 November 2021, Constanta, Romania – Poster presentation – The importance of collagen and its medical applications.
3. Participation in the International Conference ICMS XXVIII - 28th International Multidisciplinary Conference "Recent Research and Ideas" Amsterdam, 12-14 May 2022.
4. Mention for Poster: Interactions between medicinal plants used in the treatment of heart diseases and antihypertensive medication at the International Conference on Law in Health Services "Scientific, Educational and Social Interferences", 2nd Edition, May 5-6, 2023 – Article presentation.
5. Participation in the Symposium with international participation on Alternative and Complementary Therapies (Homeopathy/Phytotherapy), 7th Edition, October 27-28, 2023, Constanţa – Romania.
6. Symposium with international participation - Days of the Faculty of Pharmacy in Constanta, September 17-19, 2024, Constanta. Poster presentation - Study on the hypocholesterolemic actions of Inclisiran.
7. Participation in the International Symposium on Alternative and Complementary Therapies (Homeopathy/Phytotherapy), 8th Edition, November 21-22, 2025, Constanta – Romania. 1st Prize – Poster: The content of polyphenols present in collagen extracts of marine origin from the Black Sea basin.