PHARMACEUTICAL MICROBIOLOGY

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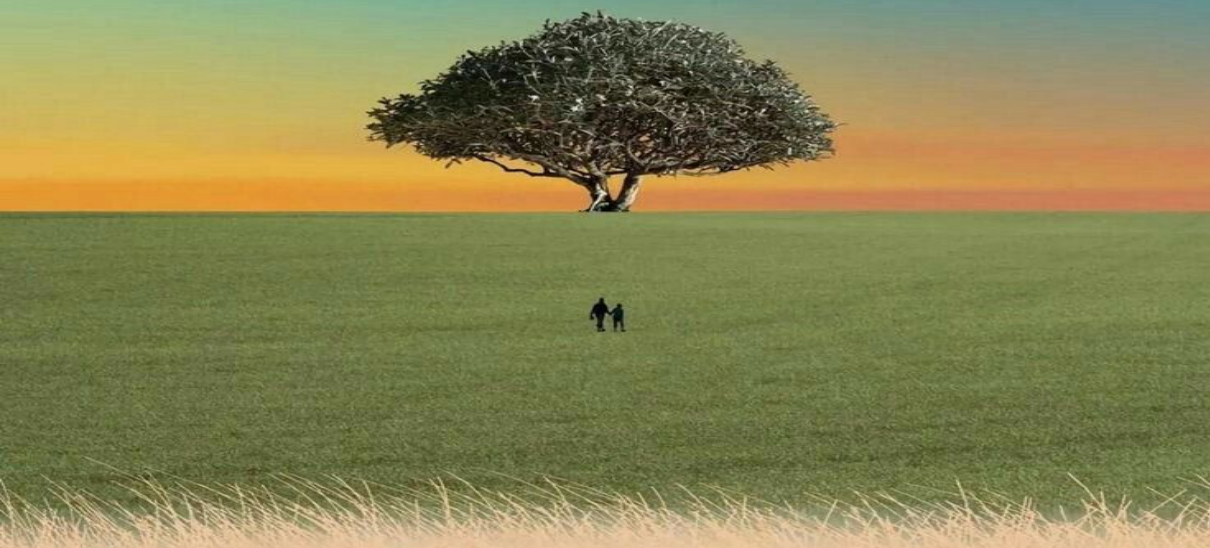

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İÇİNDEKİLER

COMPARISON OF ADVANTAGES AND DISADVANTAGES OF DRUGS OBTAINED FROM MICROBIAL SOURCES

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1. INTRODUCTION

Nature has unlimited bioactive molecules that can contribute significantly to the drug discovery process, and investigating these sources is extremely valuable (1). The bioactive molecules are natural metabolites derived from organisms such as plants, animals, and microorganisms (2). For thousands of years, natural products have been widely used due to their low cost and easy accessibility, and they serve as an important source of drugs, especially in developing countries. With their chemical diversity, natural products are one of the most valuable sources of drug discovery and development since they contain different bioactivities (3). According to the World Health Organization (WHO), approximately 60% of the world's population benefits from traditional medicine for health services (4-9). The use of various and specific plants by people to treat diseases is the oldest prehistoric knowledge that natural products were used as medical agents (10, 11).

A wide structural diversity of secondary metabolites from natural sources serves humans (12, 13). This diversity includes

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bioactive antimicrobial agents but also consists of various compounds, such as enzyme inhibitors and antitumor agents, which are biological antitumor agents (14). Antibiotics are clinically important as bioactive products such as antitumor and immunosuppressive agents (15). In 1928, Alexander Fleming discovered that a compound that killed *Staphylococcus aureus* was produced by a mold fungus, heralding the beginning of the era of microbial medicine (16). The yeast, known as *Penicillium notatum*, produces the active ingredient penicillin (17). Penicillin was later isolated and used as a powerful antibacterial agent (17). Natural products obtained from microorganisms have a wide therapeutic application area, frequently produced by primary or secondary metabolism, and thanks to the developments in separation and isolation techniques (18). Plants produce 50-60% of these, and 5% of these plant products are of microbial origin (19). Approximately 20-25% of all reported natural products show biological activity, and approximately 10% are obtained from microorganisms. Microorganisms produce many compounds with biological activity (19, 20, 21, 22). Approximately 40% of the 22,500 biologically active compounds obtained from microorganisms so far have been produced by fungi (22). The contribution of fungi to the production of antibiotics and other drugs used to treat non-communicable diseases is of great importance (23). This study aims to contribute to the literature by comparing the advantages and disadvantages of drugs obtained from microbial sources.

1.1. Natural Products

The traditional definition of natural products emphasizes that these products are generally chemical (carbon) compounds isolated from various living things (22). These compounds can be obtained by primary or, more specifically, secondary metabolism processes of living organisms (22).

1.2. Metabolic Products

1.2.1. Primary Metabolism

Metabolic activity fundamental to all living organisms is the biosynthesis and degradation of proteins, fats, nucleic acids, and carbohydrates. The compounds involved in these metabolic pathways are known as primary metabolites and are called primary metabolism (24).

1.2.2. Secondary Metabolism

The compounds of an organism, called secondary metabolites, are generally known as a unique characteristic of the organism or an expression of the individuality of a species and are called secondary metabolites (24, 25). The precise definition, real location, and function of secondary metabolites are among the long-standing and most debated issues in microbiology (26).

1.3. Bioactivity

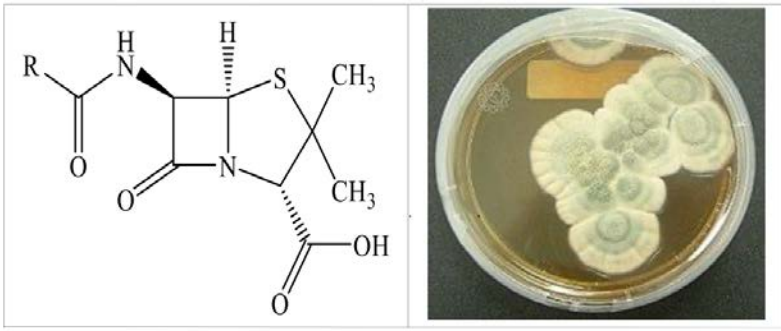
The term bioactivity or biological activity indicates the possible differences between the two. This activity can occur at the molecular level in vitro or be studied on the whole organism at the in vivo level. This term expresses the interaction between chemicals and any molecular target or living organism when specific characteristics are considered (22).

1.4. Antibiotics

The term antibiotic originates from the word ‘antibiosis,’ which means against life (27). In the past, antibiotics were considered organic compounds produced by a microorganism and harmful to other microorganisms (Figure 1) (27). As a result of this concept, an antibiotic is generally defined as a substance of biological origin produced by a microorganism or that, at low concentrations, can inhibit the growth of other microorganisms or is lethal to them (28, 29). However, in modern times, this definition has been changed to include antimicrobials that are

partially or wholly synthetically produced (30). While some antibiotics can completely kill bacteria, others can only prevent the growth of bacteria. Antimicrobials that are lethal to bacteria or fungi are called bactericidal or fungicidal, respectively, and those that inhibit the growth of these microorganisms are called bacteriostatic or fungistatic, respectively (31). Although the term antibiotic generally means antimicrobial, antibiotic compounds can be defined as antibacterial, antifungal and antiviral depending on the group of microorganisms they affect (32).

Figure 1. (A) Penicillin, the first antibiotic obtained from a fungus; (B) *Penicillium notatum* (33).



Recently, there has been increasing interest in natural products derived from unexplored microbial sources, especially actinomycetes (34), marine ecosystems (35), plant-associated microorganisms (36), mammals and invertebrates (37), and marine and terrestrial habitats (38). Although most of the most important antibiotics currently used have been obtained from cultivable microorganisms, only a small fraction of microorganisms can be grown in routine laboratory cultures (39). Most microorganisms in biological samples cannot be grown and cultured under standard laboratory conditions. Instead, they can be grown using synthetic media and other special cultivation strategies that mimic biosystem conditions (40).

1.4.1. Microbial Antibiotic Sources

Natural products and synthetic analogs of these products originating from microorganisms are one of the most effective defense systems we have developed against infectious diseases, with the sensitive structures of organisms have evolved over centuries to have potent and selective biological activities (41, 42). More than 5000 antibiotics have been identified, especially from members of the Actinomycetales order, and more than 90% of these antibiotics are specific to the Streptomyces genus (43, 44). Large-scale screening programs have identified many antimicrobial agents after the discovery of streptomycin and streptothricin in the 1940s (43, 45). The genus *Bacillus* has made significant contributions to antibiotic discovery and, similar to species of the order Myxococcales, produces lanthionine-containing antibiotics, gramicidins, and bacteriocins (46, 47, 48, 49). *Myxobacteria* (Myxococcales), a mostly soil-dwelling bacteria characterized by gliding or crawling motility and often social behavior, are also known as efficient antibiotic producers (50, 51, 52, 53).

1.5. Distribution of Microbial Products According to Source

1.5.1. Products Obtained from Fungal Sources

Fungi are eukaryotic, heterotrophic microorganisms that are widespread and generally live symbiotically. Humans have used them for many years, such as beer, wine, yeast bread, and soy products. In addition, metabolites produced by yeasts were used in the treatment of intestinal diseases thousands of years ago. Fungi have provided the source for discovering many therapeutic agents, such as penicillin obtained from the fungus *Penicillium notatum* (54).

Various therapeutic agents, such as those with immunosuppressive effects, such as cyclosporine and

mycophenolic acid, and those with antimicrobial effects, such as fusidic acid and griseofulvin, have been derived from fungal metabolites (Figure 2). In addition, new semi-synthetic antifungal drugs such as anidulafungin and caspofungin are also obtained from this source (54). Recently, cyclosporine has been used to develop Debio 025 (a non-immunosuppressive analog of cyclosporine), which has been clinically proven to have potent antiviral activity (55).

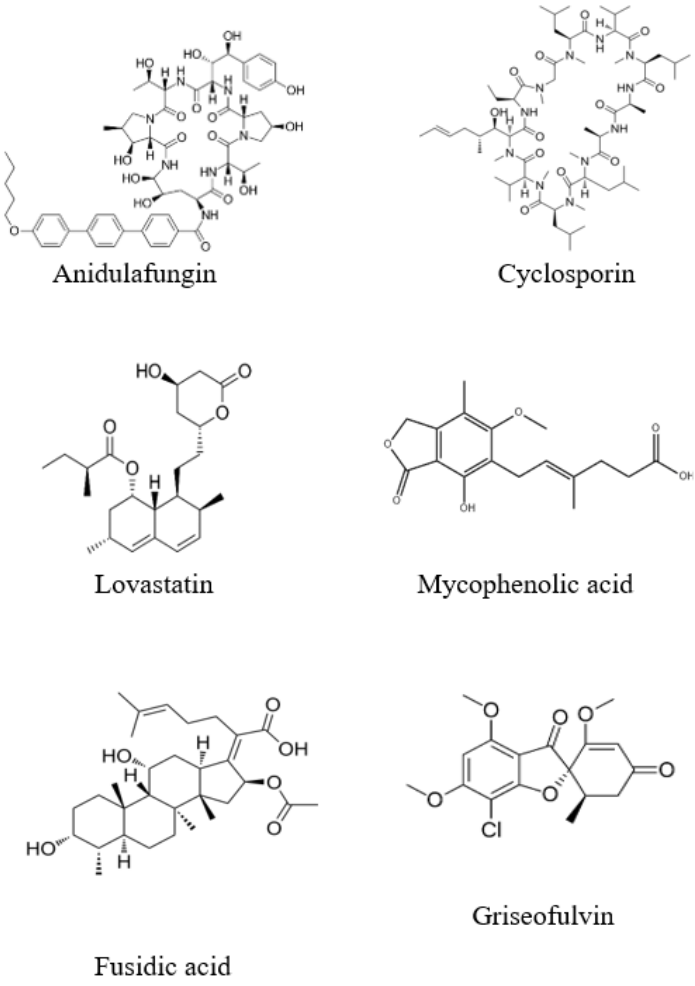


Figure 2. Some important natural compounds obtained from yeast

1.5.2. Products Obtained from Bacterial Sources

Almost three-quarters of bioactive compounds of microbial origin come from Actinomycetes bacteria. Streptomycetes are the most widely known group of gram-positive aerobic filamentous bacteria commonly found in soil and produce a variety of biologically active compounds (56).

Streptomycetes are good soil dwellers and important sources of many enzymes, such as cellulases and lipases (57). By transferring some genes obtained from these bacteria to plants, genetically modified plants have been produced, and thus, plants with improved characteristics can be obtained (58). Streptomycetes is an important source of many bioactive compounds and host most of the macrolide polyene antifungals, such as nystatin, which is commonly produced by *Streptomyces noursei* (59, 60). *Streptomyces nodosus* produces amphotericin B and *Streptomyces natalensis* produces natamycin (61). Starting with aminoglycosides, many *Streptomyces*-derived compounds have been used as antibacterial agents, and many antibiotics with antibacterial activity, such as streptomycin, are produced by *Streptomyces griseus* (Figure 3) (62).

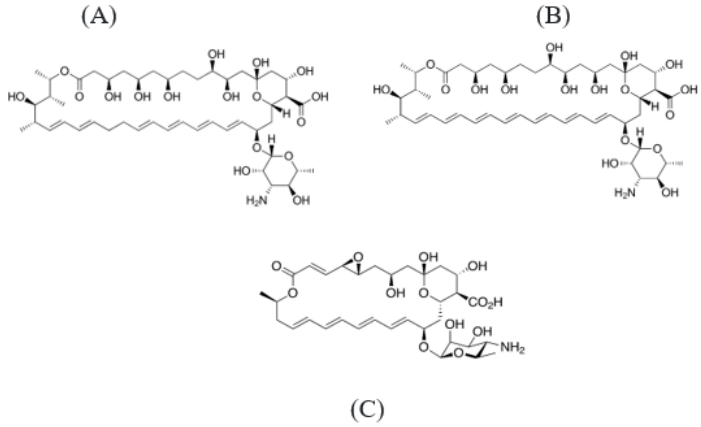
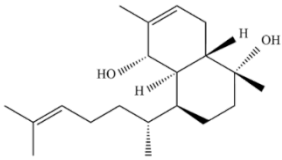


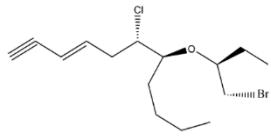
Figure 3. Chemical structures of (A) Nystatin, (B) Amphotericin B, (C) Natamycin.

1.5.3. Products Obtained from Algae

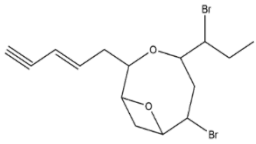
Algae are an effective source in natural product chemistry, including prokaryotic and eukaryotic species. Approximately 30,000 species in the biosphere represent algae and fulfill the function of providing oxygen (63). Algae are an important food source for fish and humans and are also used in the production of medicines and fertilizers. Algae produce the most important compounds, including terpenoids belonging to many classes, such as brominated and phenazine derivatives, nitrogen and oxygen heterocycles, guanidine and amino acids derivatives (64). The research on natural products obtained from algae began in 1970 (65). Among the important compounds produced by algae is polycavernoside A obtained from the red alga *Polycaverosa tsudai* (66).



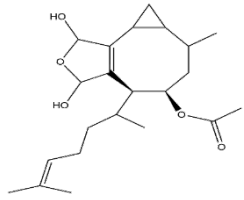
Dictyolide a



Laurepinnacin



Laureatin



Crenuladia

Figure 4. Some new bioactive compounds from marine algae

1.6. Biotechnological Products Produced From Microbial Sources

1.6.1. Glycosaminoglycans

Glycosaminoglycans (GAGs) belongs to a class of polysaccharides composed of alternating disaccharide units (67). GAGs are classified as hyaluronic acid (HA), chondroitin sulfate (CS), heparan sulfate (HS), dermatan sulfate (DS), keratan sulfate (KS) and heparin (HP) based on their monosaccharide components, glycosidic bonds, and sulfation patterns (68). GAGs (except HA) are covalently bound to proteins during their synthesis in the human body and play important roles in various physiological and pathological processes (69, 70). In recent years, significant progress has been made in using GAGs in cosmetics, health products, and pharmaceuticals (71, 72). In addition, researchs have shown that the functions of GAGs are related to their molecular weights (73).

Hyaluronic Acid

Hyaluronic acid is the only non-sulfated glycosaminoglycan. HA consists of disaccharide units of glucuronic acid (GlcA) and *N*-acetyl glucosamine (GlcNAc) linked by β-1,3- and β-1,4-glycosidic bonds. Negatively charged GlcA moieties, evenly spaced along the linear chains, form a loose network structure that facilitates intermolecular interactions and confers strong hydration and viscoelastic properties to HA (74). The most commonly used microbial hosts include naturally HA-producing strains, particularly *Streptococcus zooepidemicus* (75, 76).

Chondroitin Sulfate

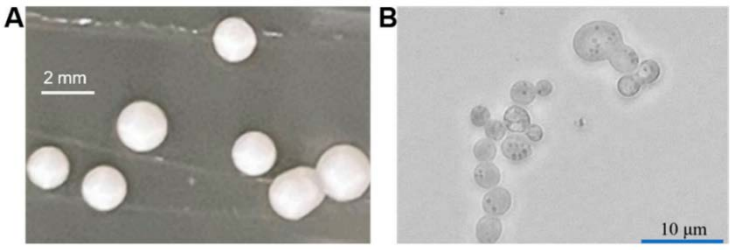
Chondroitin Sulfate (CS) is one of the most abundant GAGs in the human body. CS is widely found in cartilage tissues. CS increase water viscosity and provide lubrication and

protective properties to cartilage tissues. This allows various applications in the treatment of osteoarthritis (77). Commercial CS production is generally based on extractions from animal tissues (78). However, in addition to commercial production of CS, microbial biotechnological output has also attracted widespread attention (20, 21, 22). At the same time, specific sulfation modification systems have been developed, including various microbial/enzymatic strategies (79, 80). Three essential components, namely chondroitin, sulfotransferase enzymes, and 3'-phosphoadenosine-5'-phosphosulfate (PAPS), are required for CS synthesis (81).

Heparin

Heparin sulfate is considered one of the most complex polysaccharides known in animals, and heparin is a highly sulfated form of HS. Due to the density and arrangement of sulfated groups, HS interacts with many proteins that perform various biological functions (82). Heparin and HS are primarily obtained from animal tissues like pig intestines. However, using animal-derived products is significantly restricted by difficulties such as lack of quality control, structural variations, and raw material shortages (83). Synthetic production of heparin is harrowing due to low product yield and high cost, and the synthesis of large oligosaccharides and polysaccharides by chemical methods is impossible (84).

Figure 5. (A) Colony morphology and (B) microscopic image of *P. pastoris* (85).



1.6.2. Microbial Enzymes

Enzymes have many vital roles in the pharmaceutical and diagnostic industries. They are widely used as therapeutic drugs in health problems associated with enzymatic deficiencies and digestive disorders (86). Enzyme applications in medicine are as widespread and rapidly developing as industrial uses. For example, some of the medical uses of microbial enzymes are observed in areas such as removing dead skin and treating burns through proteolytic enzymes. In addition, nattokinase, a potent fibrinolytic enzyme, has attracted attention as a promising agent in treating thrombosis (87). Microbial lipases (from *Candida* sp., *Aspergillus* sp., *Penicillium* sp., Rhizopus, Mucor) and polyphenol oxidases play a significant role in the synthesis of some medically essential compounds, such as 3,4-dihydroxyphenylalanine (DOPA), which is used in the treatment of Parkinson's disease (88). Proteases are generally synthesized by bacteria such as *Pseudomonas, Bacillus, Clostridium*. In addition, some yeasts are known to produce these enzymes (89). Xylanases are produced by fungi from the *Trichoderma, Penicillium,* and *Aspergillus* (90). In light of all this information, the usability of microbially produced biochemical products in medicine and pharmacy is one of the groundbreaking discoveries of the last century.

2. CONCLUSION

Natural products play a critical role in human life. Microbial natural products have attracted significant attention due to their unique structures and functions, which play a fundamental role in drug discovery. Although using microorganisms as drug sources is promising in discovering new drugs, many commercially available antibiotics and anti-infective agents are widely used as antimicrobial sources. Recent research has

focused on endophytic microorganisms, which are important as sources of new compounds and are of great interest.

In addition, microorganisms and marine macroalgae provide numerous resources as they cover approximately 70% of the world, providing potential for discovering new bioactive compounds. Culture production methods are included in the literature as a factor used and diversified in drug discovery from microorganisms. Microorganism species are produced by bringing two or more microorganisms from different species together and using various combinations. This allows microorganisms to physiologically produce bioactive compounds that are not obtainable in typical growth environments.

Among the advantages of drugs obtained from microbial sources, the diversity and potential of microorganisms in the natural environment, and access to high biodiversity are important. This diversity is considered an important resource in discovering and developing new drug candidates. In addition, drugs obtained from microbial sources generally have environmental advantages, such as being of natural origin and causing less environmental damage.

According to the information obtained from the literature, microbial sources are mostly biocompatible, non-toxic, anti-allergic. Therefore, using microbial sources to discover new drugs is one of the best discoveries that will contribute to science in the last century. Using antimicrobials obtained from microbial sources as natural antimicrobial agents will open new horizons and shed light on future advanced scientific studies. In the last quarter century, antimicrobial resistance developed by microorganisms has led scientists to research the discovery of new antibiotics. Drugs obtained from microbial sources are likely to fill this gap.

However, the disadvantages of drugs obtained from microbial sources should not be ignored. The discovery and development of these drugs can often require long periods and high costs. In addition, it can be challenging to culture some microorganisms and produce them on a large scale. In addition, it should not be forgotten that drugs obtained from microbial sources may cause unwanted side effects or resistance development in some cases, although it is unlikely.

As a result, by evaluating the advantages and disadvantages of drugs obtained from microbial sources, we can make suggestions to understand better and use the potential in this area. First, more investments should be made in research and technology development studies to use microbial sources more effectively in drug discovery and development processes. In addition, developing and optimizing production and formulation techniques of drugs obtained from microbial sources are important issues. Finally, a careful monitoring and management strategy should be established to minimize resistance and side effect problems that may arise during the clinical use of these drugs. These suggestions will ensure that drugs obtained from microbial sources are used more effectively and safely.

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ANTI-CANCER AND ANTIMICROBIAL ACTIVITIES OF GALLERIA MELLONELLA PEPTIDES[1]

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1. INTRODUCTION

Galleria mellonella (G. mellonella), as a wax moth, is a species of the order Lepidopetra, family Pyralidae, and subfamily Galleriinae (Ménard et. al., 2021; Kwadha et. al., 2017). At first described in *Apis cerana* (oriental or Asian honey bee) colonies, i.e., wild honeybees found in South and East Asia (Wojda et. al., 2020). It is a pest of *Apis mellifera* Linnaeus and *Apis cerana* Fabricius (Kwadha et. al., 2017). The *G. mellonella* life cycle includes four developmental stages: egg, larva, pupa, and adult (Desalermos et al., 2012). *G. mellonella* has developed a very effective humoral and cellular immune system based on innate mechanisms.

Antimicrobial Peptides (AMPs) and proteins are important components of the humoral immune response, and hemocytes mediate cellular reactions such as phagocytosis, nodulation, and encapsulation. AMPs are synthesized in the fat body during the systemic response to pathogens and then secreted into the hemolymph (Mak, et al. 2010). Two types of AMPs have been identified, anionic and cationic antimicrobials. Cationic peptides are the most important AMPs found in *G. mellonella*

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(Lange, et al. 2018). The presence of a wide repertoire of antimicrobial peptides effective against bacteria has been demonstrated in the hemolymph of *G. mellonella* larvae. To date, at least eighteen *G. mellonella* defense peptides with different biochemical and antimicrobial properties have been identified. These include linear α-helical peptides (cecropins and moricin-like peptides), cysteine-stabilized peptides (defensins), proline-rich peptides and glycine-rich peptides (gloverin) (Wojda, I. (2017). In this section, *G. mellonella,* its peptides, the use of peptides, especially the antimicrobial activity of *G. mellonella* peptides, their place in cancer treatment and their future importance will be discussed in the light of the literature.

1.1. Morphology

Egg: Average egg length and width were determined as 0.478 mm and 0.394 mm, respectively.

Larvae: In the larval stage, sex is not distinct. After hatching, they are approximately 1–3 mm long and 0.12–0.15 mm in diameter. Before the pupal stage, late-stage larvae are approximately 25–30 mm long and 5–7 mm in diameter. The larvae are off-white in color, consisting of sclerotized body segments. There are three well-developed apical teeth in the head region. Retractile antennae are present and can be seen under a light microscope (Ellis et al., 2013).

Pupa: Average 12–20 mm long and 5–7 mm in diameter. Female pupae are normally longer than males. The pupa is of the obtect type, with all limbs attached to the body by a secretion produced during ecdysis. Initially, it is white to yellow in color, gradually turning brown and then dark brown with age and development. Sexual dimorphism is present in the pupal stage, as in the adult stage (Hosamani, et al. 2017).

Adult: Marked sexual dimorphism is observed. The average body length of female wax moths is 15–20 mm, the

wingspan is 31 mm, and the weight is 169 mg. Males are significantly smaller and lighter in color than females. After emerging from the cocoon, adults remain immobile until their wings are fully extended and hardened (Fasasi, et.all, 2006; Wojda, et. al. 2020).

1.2. *Galleria mellonella* Defense System and Immunity

The cellular immune mechanism of *G. mellonella* is formed by hemocytes, which are found in the digestive tract and fat body and circulate freely in the hemolymph. The hemocyte concentration may change during the infection. Six types of hemocytes have been identified; the most common types are prohemocytes, plasmatocytes, granulocytes, spherulocytes and eunutocytes (Pereira, et al. 2018). (Taszłow, et al. 2017). The hemolymph of *G. mellonella* is rich in many proteins. Some are constantly present in the hemolymph, but their amounts may vary depending on the immune status and in response to infection (Dubovskiĭ, et al. 2010).

1.3. Immunity proteins of *Galleria mellonella*

Studies on the identification of immune peptides and proteins of *G. mellonella* are increasing (Junqueira, J., 2012).

1.3.1. *Apolipophorin III (apoLp-III)*

Apolipophorin III is a modifiable component of the lipophorin complex, an 18 kDa protein structure that also includes apolipophorins I and II, and has been identified in *G. mellonella* at both the gene and protein levels. The complex is responsible for lipid transport, which provides energy to the flight muscles. It may act in synergy with proteins that are constantly present in the hemolymph and secreted in response to infection. In addition to stimulating the activity of antimicrobial peptides, it has been shown to increase in the presence of Muramidase activity (Dettloff, et al. 2001). It binds to bacterial cell wall components

such as lipopolysaccharides (LPS) of Gram-negative bacteria, lipoteichoic acids of Gram-positive bacteria, and fungal β-1,3-glucan. This property may indicate that it participates in the opsonization and detoxification of non-self components (Kordaczuk, et al. 2022; Zdybicka-Barabas, et al. 2013).

1.3.2. *Insect metalloproteinase inhibitor (IMPI)*

G. mellonella produces metalloproteinase inhibitor (IMPI) in response to infection. This is a peptide with antimicrobial activity and is the first and only animal-specific inhibitor of microbial metalloproteinases found so far (Wedde, et al. 2007). IMPI-1, isolated from the hemolymph of *G. mellonella*, is a glycosylated, heat-stable peptide with a molecular weight of 8.6 kDa containing 5 intermolecular disulfide bonds. This peptide, which has the ability to inhibit zinc-containing metalloproteinases, appears in the hemolymph in response to bacterial or fungal stimuli (Wojda, et al. 2020). IMPI regulation is a good example of the interaction between the host and the invading pathogen. Thermolysin-like proteases secreted by the invader in the body of the infected insect can degrade most of the host's hemolymph proteins, forming peptide fragments called profragments that stimulate the insect's immune response (Seitz et al., 2003; Altınçiçek et al., 2007). In 2018, Eisenhardt and colleagues tested the ability of the fusion protein IMPI-GST (glutathione-S-transferase) produced by fermentation in *Escherichia coli* to inhibit the proteolytic activity of the M4 metalloproteinases thermolysin and *Pseudomonas elastase*. The results suggest that IMPI is a promising drug candidate for the treatment of *P. aeruginosa* infections (Cutuli et al., 2019).

1.4. AMPs and Mechanism of Action

AMPs are small, cationic peptides produced by various tissues and cell types of human, plant and animal cells, part of innate immunity and encoded by genes (Akar, S. and Uyanıkgil,

E., 2020). AMPs are evolutionarily conserved in the genome and are produced by all life forms from prokaryotes to humans (Mahlapuu et al., 2016). The discovery of AMPs is based on Dubois V's isolation of an antimicrobial substance from soil bacillus in 1939. This substance has been shown to protect against pneumococcal infection in mice (Dubos, R. J. and Cattaneo, C. 1939). In 1940, Dubois and Hotchkiss discovered the existence of an AMP, which they named Gramicidin, in their work on the same substance. Studies have shown that some doses are associated with toxicity, but have positive effects in the topical treatment of wounds and ulcers. In general, AMPs offer promising alternatives to standard treatments as anti-infectives and immunomodulatory agents with mechanisms of action that are less prone to resistance formation than traditional antibiotics (Fjell, et al. 2012; Umerska, et al. 2016).

AMPs have been shown to have a completely different mechanism of action than antibiotics on infectious microorganisms. The interaction and structure of these peptides with biological membranes depend on the lipids present in the cell membrane. Individual AMPs interact with the bacterial cell membrane and thus interfere with the structure of the inner or outer bacterial membrane, causing cell death. It has been shown that impaired membrane integrity occurs as a result of the interaction of AMP with a negatively charged cell membrane. It has been reported that this mechanism may occur due to inhibition of DNA and RNA synthesis or interaction with specific intracellular targets (Talapko, et al. 2022). Electrostatic forces between cationic AMPs and the negatively charged bacterial surface are critical points of interaction between the peptide and the bacterial membrane (Baharin, et al. 2021). The cytoplasmic membranes of Gram-positive and Gram-negative bacteria are rich in phospholipids containing negatively charged major groups, phosphatidylglycerol and cardiolipin. This situation provides the

strong effect of positively charged AMPs (Baharin, et al. 2021; Talapko, et al. 2022).

1.5. Antimicrobial effects of AMPs

1.5.1. *Antibacterial activity of AMPs*

It has been determined that the main purpose of research on AMPs is to combat antibiotic-resistant bacteria. The peptides produced for this purpose are called antibacterial peptides (ABPs). They can destroy bacteria by breaking down the bacterial cell wall and membrane, by intracellular action, by a combination of dual destruction mechanisms and by acting on the bacterial biofilm. ABPs can prevent and control cell wall formation by binding to lipid II, which is a part of the peptidoglycan molecule and an important factor in cell wall synthesis, and can also destroy previously formed cell walls. Their effects on the membrane can occur by creating a detergent-like model that causes the integrity of the bacterial membrane to be lost and opened. Many peptides work by disrupting intracellular functions and can thus kill bacteria. Some of them inhibit DNA, RNA, and protein synthesis (Moravej, et al., 2018; Luo, Y., & Song, Y. 2021). The search and discovery of peptides with antimicrobial effects indicate that certain peptides do not have only one mode of action. On the contrary, many share a range of mechanisms. After crossing the tolerance threshold, AMPs can act at all stages of biofilm development. They can prevent biofilm formation by disrupting the signaling pathways of bacterial cells. They limit nutrients in the biofilm by inducing bacteria to produce guanosine tetraphosphate (ppGpp) and pentaphosphate (pppGpp), thereby inhibiting nucleic acid synthesis. Another effect is the reduction in the expression of genes that bind protein transporters necessary for bacterial biofilm formation. In addition, ABP can destroy previously formed biofilms by acting on the membrane potential of bacteria (Di Somma et al., 2020; Zhang et al. 2021).

1.5.2. *Antiviral activity of AMPs*

Due to the increasing resistance of viruses to therapeutics and the limited efficacy of common drugs, antiviral peptides have acquired important roles as treatment options (Di Somma, et al., 2020). AMPs with antiviral activity are called antiviral peptides (AVPs). They have several different mechanisms that can inhibit the virus cycle at various stages (Moravej, et al., 2018). Some AVPs can destabilise the viral membrane or neutralise the virus by integrating it into the viral envelope and cell membrane, thus preventing infection. some can prevent the virus from binding to its target receptor (Herpes simplex virus - Heparan). Some can inhibit viral binding and prevent the formation of membrane fusion in the virus cell (Influenza virus). The main mechanism of action of AVP on influenza virus is the regulation of the human immune system through increased expression of cytokines and chemokines in the antigen complex, activation of immune system cells and inactivation of viral pathogens (e.g. H1N1 virus). In addition, AVP can inhibit virus activity by acting on the replication cycle. AVP has effects similar to those described for influenza virus in inhibiting human immunodeficiency virus 1 (HIV-1), herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), hepatitis B virus (HBV) and hepatitis C virus (HCV) infections (Hoffmann et al., 2014).

The target sites of AVPs can be DNA and RNA and their action is to destroy the viral envelope and lead to its instability, as in Junin virus (JV), HIV-1 and HSV-2 (Matanic, V. C. A. and Castilla, V. 2004). Another way to inhibit viruses is to alter or interfere with cell signalling pathways (He, M., et al., 2018). Recently, an increasing number of studies have been conducted on the antiviral effect of AVPs on coronaviruses (such as SARS-CoV). In short, the existence of such research efforts is based on the inhibitory effect of AVP on the viral cell membrane and its inability to enter the host cell.

1.5.3. *Antifungal activity of AMPs*

Excessive and inappropriate use of immunosuppressive therapy, systemic antibiotics, chemotherapy and radiotherapy has increased the incidence of fungal infections in the general population. It is known that antifungal treatment options are limited. In addition, the increasing resistance of certain strains to systemic antifungal drug types commonly used in treatment, such as polyenes, azoles, echinocandins and flucytosines, has caused an increase in morbidity and mortality rates, creating an urgent need for alternative solutions in antifungal treatment.

Antifungal peptides (AFPs) may be a promising option for the treatment of fungal infections (Oshiro et al. 2019). AFPs can be classified according to a number of criteria such as structure, shape, activity and origin. Many AFPs exert their activity through membrane-associated mechanisms and specific targets. Specific targets such as glucosylceramides, mannosyldiinositol phosphorylceramide or fungal protein target provide high selectivity and prevent resistance to treatment (Rautenbach et al., 2016; Fernández et al., 2020)

Natural AFPs are classified as peptides rich in glycine, arginine, proline, histidine and tryptophan (Bondaryk et al., 2017). Semi-synthetic and synthetic peptides have been designed to improve pharmacological properties and reduce immunogenicity and side effects caused by natural peptides. Biophysical properties such as net charge, stereospecificity, hydrophobicity, secondary structure, peptide length and amphipathicity determine the antifungal activity of peptides (Akkam, Y. 2016). The most effective against fungal-mediated biofilms are mammalian peptides and are called defensins, cathelicidins and histatins (Oshiro, et al., 2019). Defensins are linked by disulfide bonds that ensure the stability of the structure

under all conditions and can be isolated not only from mammals but also from plants (Shafee, et al. 2016).

Defensins obtained from vertebrates are cationic and amphipathic peptides and can be divided into two subfamilies as α-defensins and β-defensins. They prevent Candida albicans from adhering to human intestinal epithelial cells and have the ability to prevent biofilm formation. β-defensin-1 shows inhibitory activity against germinating conidia of Aspergillus fumigatus. Synthetic defensin-like peptides such as α-defensin-3, β-defensin-1, β-defensin-3, and PG-1 exhibit antifungal activity against Cryptococcus neoformans biofilms, including both planktic cells and mature biofilm.

Cathelicidins are cationic peptides isolated from different mammalian species, consisting of 12–80 amino acids. They have been shown to inhibit Candida albicans cell adhesion. Furthermore, a bovine antimicrobial peptide, BMAP-28, was able to reduce the number of Candida albicans adherent cells on silicone surfaces and inhibit mature biofilm. Histatins are human salivary peptides, first isolated from the human parotid gland, and have polar and hydrophilic properties and an α-helical structural conformation in organic solutions (Oppenheim et al., 1988). Histatin-5 has been shown to inhibit biofilm formation by Candida albicans on acrylic dentures in vitro (Pusateri et al., 2009).

1.5.4. *Immunomodulatory activity of AMPs*

AMPs play an important role in immunomodulation and inflammation control. In humans, the three main families of AMPs are defensins, histatins, and cathelicidins. Based on the arrangement of disulfide bonds, defensins are divided into α-defensins and β-defensins and are produced by lymphocytes, neutrophils, and epithelial cells of mucosal membranes and skin

(13). The mechanisms of action of AMPs in immune modulation include various immune responses (Kumar, et.al. 2018).

1.5.5. *Anticancer peptides*

Antimicrobial peptides, also called anticancer peptides (ACPs), are α-helical or β-sheet peptides and can be divided into two groups. The first group includes peptides that are inactive against mammalian cells but active against cancer cells and bacterial cells, such as insect cecropins and frog skin magainins. The second group includes insect defensins that are toxic to normal cells, bacteria, and cancer cells, and the human LL-37 peptide bee venom mellitin. ACPs can cause cancer cell death by membranolytic or nonmembranolytic mechanisms, depending on their peptide properties and specific target membrane properties. Cancer cells are different from normal mammalian cells. In contrast, mammalian cell membranes have a zwitterionic character due to the molecules normally found in their membranes. In healthy cells, phosphatidylserine molecules are located in the inner leaflet of the plasma membrane, whereas in cancer cells, the asymmetry between the inner and outer membrane leaflets is lost, leading to the presence of PS in the outer leaflet. The negative net charge exposed in the cancer outer membrane resembles that of bacterial membranes, suggesting that AMPs and ACPs may share similar molecular principles for selectivity and activity. Dermaseptin B2 and B3 have been reported to be active against proliferation of human prostate, breast, and lymphoma cancer cells (Di Somma, et.al.2021).

1.6. Peptides Produced by *Galleria mellonella*

The simultaneous presence of a wide repertoire of antimicrobial peptides in the hemolymph of *G. mellonella* larvae has been demonstrated. These include linear α-helical peptides (cecropins and moricin-like peptides), cysteine-stabilized

peptides (defensins), proline-rich peptides, and glycine-rich peptides (gloverin) (Mak, et.al.2010).

1.6.1. *Gallerimycin*

Gm1 (Gallerimycin), a natural peptide with no net charge obtained from *G. mellonella,* was identified in 2003 as a defensin-like cysteine-rich peptide with the broadest spectrum of activity against different Gram-positive bacterial species and *Escherichia coli (E. coli)* D31 and filamentous fungi (Corre, et.al. 2014).

Drosomycin obtained from Drosophila melanogaster and heliomycin obtained from Heliothis virescens are antifungal peptides with amino acid sequences similar to gallerimycin. The recombinant protein has 76 amino acids and a molecular weight of 11.6 kDa when combined with the v-5 epitope and purified. This recombinant protein has activity against the entomopathogenic fungus *Metarhizium anisopliae*, but is inactive against bacteria such as *Micrococcus luteus*, Bacillus subtilis, and the yeast *Saccharomyces cerevisiae* (Küçük, M. E., & Kaplan, A. 2020).

1.6.2. *Galiomycin*

The cDNA consists of 622 nucleotides and contains an open reading frame of 216 nucleotides corresponding to a preprotein of 72 residues. A mature protein contains 43 residues and has a molecular weight of 4.7 kDa. Typically, insect defensins contain 6 cysteine residues forming 3 intramolecular disulfide bonds. It has 90.7% identity to heliomycin. This defense peptide is active against filamentous fungi and yeast, i.e. it has antifungal activity and prevents fungal infections, but it does not show antibacterial activity (Wojda, et.al. 2017).

1.6.3. *Moricins and gloverins*

There are 8 genes encoding 7 different moricin-like peptides in *G. mellonella.* Although they are effective to some

extent against yeast, Gram-positive and Gram-negative bacteria, they have been shown to be more effective against filamentous fungi. Moricins belong to the group of amphipathic α-helical antimicrobial peptides. They have broad antimicrobial effects. Gloverins are heat-stable antibacterial proteins enriched in glycine residues but devoid of cysteines, and have been identified in several lepidopteran species, including *Hyalophora gloveri, Helicoverpa armigera, Trichoplusia ni, Manduca sexta, Bombyx mori, Diatraea saccharalis, Plutella xylostella and Spodoptera exigua* (Cytryńska, et.al., 2007). They are effective against *E. coli,* Gram-positive bacteria, fungi and viruses, interact with LPS and prevent the formation of the bacterial outer membrane. Gloverins were first discovered in the silkworm *Hyalophora glover*i (Wojda, et.al., 2017). *G. mellonella* gloverin is a member of the Gloverin family. The general mechanism of their antimicrobial activity is not fully understood but is based on interactions with bacterial LPS, which either increase membrane permeability or inhibit the formation of the bacterial outer membrane. The activity of gloverins varies depending on the origin of each protein (Zitzmann, et.al. 2017). Little is known about the activity of GmGlv, but it has been demonstrated that the hemolymph of G. mellonella larvae exposed to LPS contains GmGlv and significantly inhibits the growth of *E. coli on* agar plates (Altincicek, et.al.,2007).

1.6.4. *Cecropinler(Cecropin A ve Cecropin D)*

Cecropins are linear, amphipathic peptides with an α-helical structure. A cecropin-like peptide was purified from the hemolymph of immune-stimulated Galleria mellonella larvae. The 37-residue peptide has a molecular weight of 4.16 kDa. This cationic peptide is active against Gram-positive and Gram-negative bacteria (Altincicek, et.al.,2007). This peptide is effective against filamentous fungi (*Aspergillus niger*) (Pereira, et.al., 2018).

1.6.5. *Cobatoxin*

Cobatoxin is strongly induced in *G. mellonella* after bacterial challenge but has not yet been characterized for antimicrobial activity. Furthermore, cobatoxin may have a synergistic effect when paired with AMPs other than Cecropin-A (Pereira, et.al., 2018).

1.6.6. *Defensins*

Defensin binds to the bacterial cell membrane, leading to leakage of intracellular contents and bacterial death. It is a small, cationic, cysteine-rich antimicrobial peptide that is particularly effective against Gram-positive bacteria. It binds to the bacterial cell membrane, forming pores, and Defensin production is increased in the event of infection with pathogens or when the immune system is stimulated. This allows the insect to respond rapidly to infections (Palusińska-Szysz, et.al., 2012).

A number of antimicrobial peptides have been isolated from the hemolymph of *G. mellonella* with activated immune systems. However, most of these peptides are known only at the peptide level and their structures have not yet been elucidated. These include apolipophoricin, anionic peptide-1, cecropin D-like peptide, and heliosin-like peptides (Kucuk, M. & Kaplan, A., 2020).

1.7. Properties of Peptides and Their Place in Treatment

Life-threatening infectious diseases have been an important health problem throughout human history. Today, these diseases are the second most common cause of death worldwide, while antibiotics are the third most sold drug. Resistant bacteria, for which antibiotics provide no benefit, continue to threaten health. This situation increases the search for antimicrobial treatments.

In recent years, the shortage of new antimicrobial drugs in the treatment of serious infections and the increasing prevalence of antibiotic resistance are considered serious threats to global health. Therefore, the urgent need for new antibiotics has accelerated the discovery of natural compounds with antimicrobial properties. In this context, genetically encoded antimicrobial peptides and proteins (AMPs) are of great interest because these compounds are considered evolutionarily conserved mechanisms used by the immune system of multicellular organisms to control or destroy microorganisms. The functions of AMPs go beyond directly killing microbes and include other biological processes such as defense, inflammation, and wound healing. They are considered evolutionarily conserved mechanisms used to control or destroy them. For example, some AMPs bind to and neutralize endotoxins, while others can regulate immune responses. In addition, some AMPs are considered to have potential in cancer treatment, as some of these compounds have cytotoxic effects against cancer cells. The therapeutic potential of these known AMPs has led to significant research for the discovery of new AMPs. As a result, almost all living species are included among the groups of organisms in which AMPs have been screened, with insects standing out as the most diverse.

The identification of effective immune-related molecules in *G. mellonella* and other insects has accelerated in recent years with the application of modern transcriptomic and proteomic approaches. These methods have significantly increased the efficiency of screening processes. The number of antimicrobial peptides and proteins reported from *G. mellonella* is quite high compared to other insects for which genetic sequences are available. Lepidoptera species such as *G. mellonella* possess glycine-rich AMPs such as attachins and gloverins, while proline-

rich AMPs such as lebocins are also present. Gloverins probably represent a group of antimicrobial peptides specific to Lepidoptera. In bacteria, these proteins have been shown to increase the formation and permeability of the outer membrane. IMPI, discovered in *G. mellonella,* stands out as a promising candidate for the development of its synthetic analogue, as it exhibits unique activity against microbial metalloproteinases that target important virulence and/or pathogenic factors of human pathogens (Vilcinskas, A., 2011).

Leishmaniasis is a very serious group of diseases caused by protozoan parasites belonging to more than 20 Leishmania genera. Epidemiological surveillance reveals that more than 350 million people in 98 countries are at risk of infection, making it one of the six endemic diseases considered to be of high priority worldwide. It has been declared a high priority disease by the World Health Organization (WHO). The main clinical manifestations of leishmaniasis in humans are cutaneous leishmaniasis (CL), which affects the skin; mucocutaneous leishmaniasis (MCL), which can have serious consequences on mucosal membranes; and visceral leishmaniasis (VL), which is considered life-threatening if untreated (Alemayehu, B., & Alemayehu, M. 2017). This disease is characterized by single or multiple ulcerative skin lesions resulting from infection of macrophages in the dermis. Ulcers can last for months, sometimes years, and eventually lead to disfiguring scars. While treatment options are limited, new treatment methods need to be discovered to reduce the side effects of current treatments (Torres-Guerrero, et.al.,2017). Therefore, there are studies aimed at evaluating *G. mellonella* hemolymph for potential peptides with anti-parasitic activity.

Antileishmanial activity of hemolymph fractions of G. mellonella larvae and the identification of four antimicrobial peptides in these antiparasitic fractions have been reported, two

of which showed antiparasitic activity and have potential as antileishmanial drugs. This suggests that peptides produced during the immune response of *G. mellonella*, especially those with low molecular weight, may have potential antiparasitic activities against *Leishmania promastigotes.* It is important to evaluate such peptides as new, effective and low-toxic alternatives for the treatment of diseases such as leishmaniasis (Patiño-Márquez,et.al., 2018).

Of particular interest is the insect metalloproteinase inhibitor (IMPI) discovered in *G. mellonella.* IMPI exhibits specific and potent activity against thermolysin-like microbial metalloproteinases, including a number of prominent virulence and/or pathogenic factors of human pathogens responsible for severe symptoms such as septicemia, hemorrhagic tissue hemorrhage, necrosis and increased vascular permeability. IMPI and antimicrobial peptides obtained from *G. mellonella* may provide promising templates for the rational design of new drugs, as there is evidence that the combination of antibiotics with pathogen-associated proteolytic enzyme inhibitors provides synergistic therapeutic effects. IMPI, discovered in G. mellonella and having unique activity against microbial metalloproteinases representing major virulence and/or pathogenic factors of human pathogens, is a promising candidate for the development of synthetic analogs. Although the parenteral use of AMPs requires more efforts to develop therapeutic strategies, topical application to infected and non-healing wounds, which is a major problem in patient care, seems to be a more feasible way to use insect-derived molecules in the treatment of infections (Ghazy,et.al., 2023; Hagemann, et.al, 2024).

Along with AMPs, several immune-related peptides have been identified that are involved in the immune response of G. mellonella: these molecules play an important role in the host defense system, are induced in response to an infection, and their

activity has been observed to be selective against different pathogenic species.

2. CONCLUSION

The place and use of peptides produced by *G. mellonella* in current scientific research maintains its importance and continues to grow as a subject of curiosity. There are several views on the number and diversity of peptides produced by *G. mellonella.*

Brown et al., (2009) showed in their studies that *G. mellonella* can produce at least 18 different antimicrobial peptides with a peptidomic approach, and this study revealed that the peptide diversity is wider than previously thought. Mak et al. (2010) examined the profile and induction kinetics of antimicrobial peptides produced in the hemolymph of *G. mellonella* when infected with different microorganisms, and in this study, they determined the diversity and amount of peptides produced as an immune response. Studies with different views on the microbial spectrum of *G. mellonella* antimicrobial peptides have also been conducted and different results have been obtained. Brown et al. (2009) stated that the peptides produced by *G. mellonella* are effective against a wide spectrum of microbial species, while Schöpf, et. al., (2025) examined the activity of three different antimicrobial peptides (cecropin A, gallerimycin and cobatoxin) obtained from *G. mellonella* and revealed that Cobatoxin was effective on *Micrococcus luteus* but not on *Escherichia coli.* In line with our research, it was observed that the antimicrobial, anticancer, antiviral and antifungal properties of *G. mellonella* peptides may be in broad and narrow spectrums. Similarly, different views have been presented on the synergistic effects of antimicrobial peptides. Some researchers, examined the individual activities of peptides isolated from *G. mellonella i*n

their studies and did not report any findings regarding synergistic effects. Schöpf, et. al., (2025) reported in their studies that cecropin A and cobatoxin peptides showed a synergistic effect when used together, meaning that they were more effective at lower concentrations when used together.

As a result of the research conducted on *G. mellonella* and the antimicrobial peptides it produces, it was seen that although there were studies that obtained different results, there were basically studies in the same direction. It can be said that *G. mellonella* contains a mystery that treats today's diseases, that studies and research are focused on this subject, that the antimicrobial, antifungal, antibacterial, anticancer etc. properties of *G. mellonella's* antimicrobial peptides are being investigated with importance and that this subject has the potential to produce a solution to the existing resistance problem.

G. mellonella attracts attention as an important model organism that protects itself against pathogens by producing various peptides as part of the innate immune system. As a result of the articles we reviewed, we saw that the data obtained revealed that these peptides are generally effective against bacteria, fungi and some viral agents. Among the antimicrobial peptides of *G. mellonella*, especially peptides such as gallerimycin, galiomycin, moricins, gloverins and defensins stand out for their antimicrobial and antifungal effects. These antimicrobial peptides have the ability to prevent infections by breaking down the cell membranes of pathogens or disrupting their cellular functions. In addition, it is thought that these compounds may offer new alternatives in the fight against microorganisms that have developed resistance to traditional antibiotics. As a result of studies conducted with the immune-related proteins of *G. mellonella,* it has been shown that these peptides are not only effective against infections but also have a potential role in cancer treatment. Some AMPs that show

anticancer activity can interact with cancer cell membranes and stimulate death mechanisms at the cellular level. Therefore, the potential use of these peptides in biomedical and pharmaceutical fields is a focus of great interest. Studies have shown that some *G. mellonella* peptides prevent the proliferation of cancer cells and even suppress tumor formation by causing programmed cell death. This is an important development for new biological agents to be developed for cancer treatment in the future. In addition, it has been determined that *G. mellonella* has advantages such as being easy to grow in temperate climates, having the potential to be genetically manipulated, and being a low-cost model organism that can be used in studies, especially when compared to species such as *Drosophila melanogaster* and *Bombyx mori*, as it can live in a wider temperature range and is suitable for modeling the interactions of the immune system with human pathogens. In conclusion, our research aimed to provide a holistic approach to the diversity of peptides synthesized by *G. mellonella* and their potential biotechnological applications. It is thought that the future research of these peptides in a broader scope in the fields of medicine, biotechnology and agriculture may provide significant contributions to the development of new treatment methods. Focusing more on the peptides produced by *G. mellonella*, especially on antimicrobial resistant microorganisms and cancer treatment, and adapting them to clinical applications will provide significant progress to the scientific world.

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