KARADENIZ TECHNICAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

BIOTECHNOLOGY DEPARTMENT

ISOLATION AND TAXONOMIC CHARACTERIZATION OF ACTINOMYCETES FROM *MELOLONTHA MELOLONTHA* AND PROFILING THEIR SECONDARY METABOLITES

MASTER THESIS

MUHAMMAD FATHALLAH ALBADRY ALI

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MUHAMMAD FATHALLAH ALBADRY ALI

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PREFACE

This study, entitled “Isolation and Identification of Actinomycetes from Melolontha melolontha and Detection and Characterization of Biotechnologically Significant Secondary Metabolite(s) was prepared as a “Master Thesis” at Karadeniz Technical University, Institute of Natural and Applied Sciences, Department of Biotechnology.

I would like to express my deep and sincere gratitude to my advisor, Prof. Dr. Zihni DEMIRBAG, for the invaluable guidance, unwavering support, and encouragement he has given me throughout this research. His cooperation and assistance have been invaluable in shaping my thesis.

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MUHAMMAD FATHALLAH ALBADRY ALI

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THESIS ETHICS DECLARATION

I declare that I have completed this study titled “Isolation and Identification of Actinomycetes from Melolontha melolontha and Detection and Characterization of Biotechnologically Significant Secondary Metabolites,” which is presented as a master's thesis and written under my advisor's supervision. I hereby declare that I have completed this study under the responsibility of Prof. Dr. Zihni DEMIRBAG and Ass. Prof. Mehtap USTA, that I have collected the data/samples myself, that I have performed the experiments/analyses in the relevant laboratories, that I have fully cited the information I have obtained from other sources in the text and bibliography, that I have acted in accordance with scientific research and ethical rules during the study process, and that I accept all legal consequences in case of any contrary results. 11/04/2025

MUHAMMAD FATHALLAH ALBADRY ALI

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Streptomyces, biyolojik olarak aktif bileşikler üretme yetenekleri nedeniyle büyük ilgi gören önemli bir mikroorganizma grubudur. Bu çalışmada, Doğu Karadeniz bölgesinde yer alan üç farklı bölgeden (Rize, Sinop ve Samsun) toplanan *M. melolontha* larvalarından Actinomycetes isolation agar besiyeri kullanılarak 8 aktinomiset izolatı seçilmiştir. Morfolojik, biyokimyasal ve genetik tanımlama testlerine dayanarak bu izolatlar *Streptomyces ambofaciens, Streptomyces mirabilis, Streptomyces pluricolorescens, Streptomyces araujoniae, Streptomyces durocortorensis, Streptomyces setonii, Streptomyces antibioticus* ve *Streptomyces caelestis* olarak tanımlanmıştır. Tüm izolatların sıvı (tam) ekstraktları antibakteriyel ve antifungal aktiviteler açısından test edilirken, *S. pluricolorescens* (M11) ve *S. antibioticus* (M15)'ın ham (kuru) ekstraktları antiviral ve antikanser özellikler bakımından taranmıştır. Antibakteriyel testler hem Gram-pozitif hem de Gram-negatif bakterilere karşı geniş bir aktivite spektrumu ortaya koymuştur. Ayrıca *C. albicans*'a karşı antifungal aktivite de gözlenmiştir. *S. pluricolorescens* ve *S. antibioticus*'in daha ileri HPLC analizi, antimikrobiyal bileşiklerin, antiviral bileşiklerin ve antitümör bileşiklerin yanı sıra başka bazı biyolojik olarak aktif metabolitlerin varlığını göstermiştir. HPLC analizi neticesinde, ham ekstraktlar kullanılarak yapılan testler HIV-1 RT'nin güçlü *in-vitro* inhibisyonunu ve antikanser MTT testlerinde kayda değer sitotoksisite ortaya koymuştur.

Anahtar Kelimeler: Streptomyces, *Melolontha melolontha*, böcek simbiyontları, antimikrobiyal bileşikler, antiviral ajanlar, antikanser maddeleri.

Master's Thesis

SUMMARY

ISOLATION AND IDENTIFICATION OF ACTINOMYCETES FROM MELOLONTHA MELOLONTHA AND DETECTION AND CHARACTERIZATION OF BIOTECHNOLOGICALLY SIGNIFICANT SECONDARY METABOLITES

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Streptomyces are an important group of microorganisms that have attracted great attention for their ability to produce bioactive compounds. In this study, eight actinomycete isolates were selected from *M. melolontha* larvae collected from three different locations (Rize, Sinop, and Samsun) in the Eastern Black Sea region, using Actinomycetes isolation agar medium. Based on morphological, biochemical, and genetic identification tests isolates were identified as *Streptomyces ambofaciens, Streptomyces mirabilis, Streptomyces pluricolorescens, Streptomyces araujoniae, Streptomyces durocortorensis, Streptomyces setonii, Streptomyces antibioticus,* and *Streptomyces caelestis*. Whole (liquid) extracts of all isolates were tested for antibacterial and antifungal activities, while crude (dry) extracts from *S. pluricolorescens* and *S. antibioticus* were screened for antiviral and anticancer properties. The antibacterial assays revealed a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. In addition, antifungal activity was observed against *C. albicans*. Further HPLC analysis of *S. pluricolorescens* and *S. antibioticus* showed the presence of antimicrobial compounds, antiviral compounds, and antitumor compounds, along with several other bioactive metabolites. Based on HPLC analysis, tests performed using the crude extracts demonstrated strong *in-vitro* inhibition of HIV-1 RT and showed notable cytotoxicity in anticancer MTT assays.

Key Words: Streptomyces, *Melolontha melolontha*, Insect symbionts, Antimicrobial compounds, Antiviral agents, Anticancer substances.

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ABBREVIATIONS

AIA:	Actinomycetes isolation agar media
ATCC:	The American Type Culture Collection
DMEM:	Dulbecco's Modified Eagle Medium
DNA:	Deoxyribonucleic acid
H_2O_2:	Hydrogen peroxide
H□S:	Hydrogen sulfide
HIV:	Human immunodeficiency virus
HPLC:	High-performance liquid chromatography
IC_{50}:	Half-maximal inhibitory concentration
MCF7:	Michigan Cancer Foundation-7
mL:	Milliliter
mm:	Millimetre
MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl
OD:	Optical density
PCR:	Polymerase chain reaction
RT:	Reverse transcriptase
rRNA:	Ribosomal ribonucleic acid
TSA:	Triple Sugar Agar

1. GENERAL INFORMATION

1.1. Introduction

Actinomycetes are prokaryotic organisms categorized as bacteria. They possess distinct characteristics that make them classified as a separate group (Gousia, 2023). They have aerobic and anaerobic organisms within the order *Actinomycetales* (Sullivan, 2010). It is a highly diverse group, with a t least of 350 genera identified currently described. They represent one of the largest bacterial phyla (Takahashi, 2018). They are a group of Gram-positive bacteria that possess a high content of guanine and cytosine (GC) in their genomes. They develop through the extension of the top and the branching of the hyphae (Barka, 2015).

Their given name indicates that in the Greek language, “atkis” is translated as “rays” and “mykes” as “fungus” because of their physical similarities with fungi; Also, they are the reason for the earth's smell (Saini, 2015). When they are cultivated on an agar medium, actinomycetes develop a branched structure of hyphae that multiply both on the surface and beneath it. The upper hyphae are termed aerial hyphae, whereas those beneath the upper layer are referred to as substrate hyphae. Septa typically partition the hyphae into elongated cells (20 micrometers or longer). Contains several bacterial chromosomes, which are nucleoids. These aerial hyphae extend above the substrate media and reproduce asexually. The majority of the Actinomycetales orders are not motile (Sharma, 2014).

They have a sophisticated radial mycelium that differentiates into the substrate and aerial mycelium. During their life cycle, substrate mycelium is cultivated in the media to absorb nutrients. Once Actinomycetes replicate in a nutrient-deficient environment, the hyphae coil and form a septum. Conidiospores are produced within the hyphae. Spores will be disseminated into the environment. Also they will germinate upon the achievement of appropriate conditions. During the germination phase, the spore will extend germ tubes, which subsequently progress to the vegetative development stage and repeat the life cycle (Ngamcharungchit, 2023).

Actinomycetes, for a very long time, have been taxonomically classified as fungi because of their morphological and filamentous shape (Madigan et al., 2009). Then later on, the classification and identification are according to other specific criteria, including

components of the cell, mycelia pigment color, use of carbon and nitrogen sources, spores that are produced, and molecular percentage of G + C of DNA, besides advanced molecular techniques such as DNA-DNA hybridization and 16S rRNA analysis (Adegboye, 2012), classified them as bacteria.

They are also found in the human body as normal flora in the oral cavity, the digestive system, and the urogenital tract. When tissue integrity is compromised by tissue damage, pathogens can infiltrate surrounding tissues and organs, causing internal infections called actinomycosis. It is an uncommon chronic condition that is a gradually progressing granulation condition that is induced by branched Gram-positive aerobic bacteria belonging to the genus Actinomyces; it's frequently incorrectly diagnosed. Due to its ability to resemble other illnesses, including malignancies and pneumonia (Wong, 2011).

1.2. Living Conditions of Actinomycetes

Actinomycetes can tolerate various environments and are abundantly widespread over ecosystems of nature (Selim, 2021). They adopt a vast range of habitats, and they frequently occur in both aquatic and terrestrial environments, particularly in soil. They are crucial for recycling refractory biomaterials through decomposing complicated polymeric material mixtures found in dying organisms such as animals and plants. They play a crucial role in soil biological degradation and soil fermentation by recycling nutrition-associated long-chain chemicals such as chitin and keratin (Sharma, 2014).

Since most Actinomycetes are mesophilic, they thrive best at temperatures between 25 and 30 degrees Celsius. Nonetheless, temperatures between 50 and 60°C are suitable for the growth of thermophilic Actinomycetes. Low humidity promotes the vegetative growth of actinomycetes in the soil, particularly when the spores are immersed in water. Growth is severely constrained and may even stop in arid environments with a high amount of moisture. The majority of actinomycetes thrive in pH-neutral environments, with their optimum growth at a pH value of 6 to 9; even so, some of their strains were found in acidic environments like pH 3.5 (Barka, 2016).

1.3. Secondary Metabolites

The definition of secondary metabolites refers to the products or intermediates that are produced by the organism, not needed for its growth, and the organism can survive without them. Those products are produced when the organism is exposed to attacks or changes in its growth elements and conditions, while the primary metabolites refer to the products that are produced by the organism and are essential for its growth process (Verpoorte, 2000). So after the inoculation of the microorganism into the media, it takes time to sense the media around and the new environment, so the organism shows no growth and no increase, which is called the lag phase. When the microorganism adapts to the media, it starts the log phase, which means a very significant increase in the number of cells and their size, which is essential for growth. When the organism senses a decrease in its elements, it starts to defend itself and produce secondary metabolites in the stationary phase. It's a phase that shows no increase in the number of cells, and then the microorganism starts to enter the death phase which is a phase that live cells start dying in (Fig. 1) (Mahmoudi, 2024). Secondary metabolites are naturally produced from diverse sources, including terrestrial plants, animals, marine organisms, terrestrial invertebrates, vertebrates, and microorganisms (Chin, 2006).

It has been proven that bacteria are a very rich reservoir of secondary metabolites like antibiotics, alkaloids, pigments, enzymes, antibacterials, anticancer agents, pesticides, microbial controls, cosmetics, etc. Those products have been used for the benefit of plants, humans, and animals (Abdelghani, 2021)

The first discovery of antibiotics was in 1928 by Alexander Fleming, the founder of the penicillin antibiotic from *Penicillium chrysogenum*, which was an event that put a big spotlight on secondary metabolite discoveries (Gaynes, 2017). Since that time, the application of chemicals generated from microorganisms has proliferated (Choi, 2015). Currently, thanks to advanced instruments, tools, and techniques, scientists can apply these products on the human body and show their effect on mankind and animals (Ji, 2009).

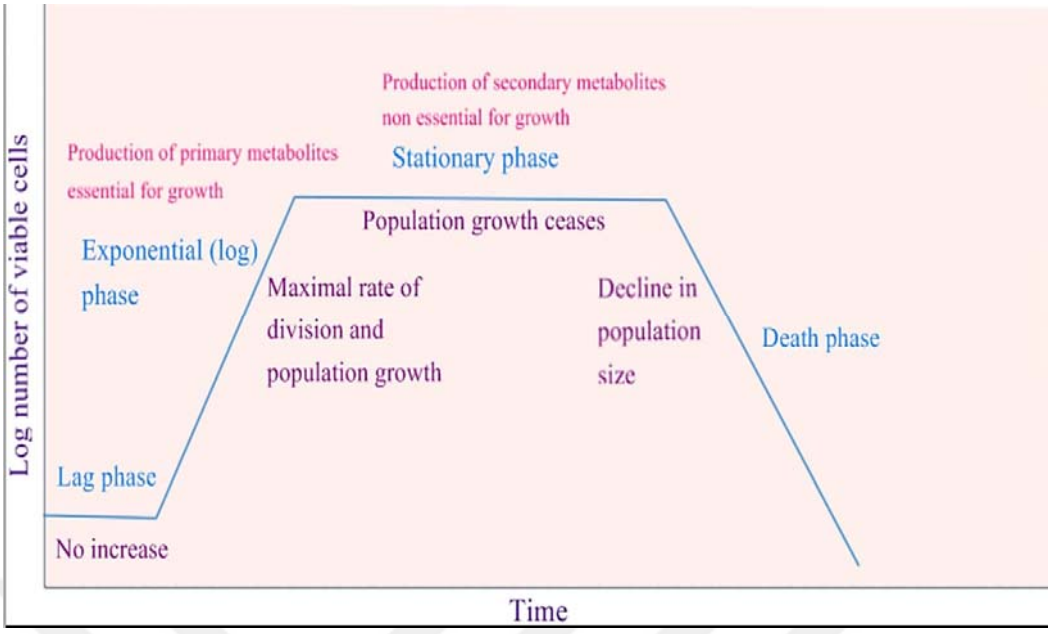


Figure 1. The growth curve of microorganisms. The production of primary and Secondary metabolites is shown (Mahmoudi, 2024)

1.4. Actinomycetes as The Main Source of Secondary Metabolites

Actinomycetes have been one of the most extensively utilized prokaryotes in biotechnology. Over the past 80 years, they have served as the origin of approximately two-thirds of currently used biologically active metabolites (Chinnathambi, 2023).

They serve as a reservoir of biologically active secondary metabolites, including antimicrobial agents, biopesticide agents, plant development regulators, antiviral compounds, anti-tumor agents, pharmaceutical substances, enzymes, inhibitors of enzymes, anti-inflammatory drugs, single-cell protein, and amphiphilic compounds (Selim, 2021).

These bacteria are very significant for medicine, biotechnology, and agriculture. Actinobacteria display numerous functions in their interactions with many higher living beings, as their individuals adapt to diverse lifestyles (Barka, 2016)

When an actinomycete's favorite environmental conditions are challenged by any type of stress, like temperature or pH, or even lack of nutrition, it starts to form spores to protect its generation and produce secondary metabolites to defend against other microorganisms (Van der Meij, 2017).

Numerous studies have examined the processes behind the activation and increase in the production of secondary metabolites via co-cultures between fungi and actinomycetes; for example, the study that included the culture of *Streptomyces rapamycinicus* with *Aspergillus nidulans* showed an increase in histone acetylation (Nützmann, 2011). Also, by co-cultivation between actinomycetes and bacteria or with another species of actinomycetes, the results show a very noticeable increment in secondary metabolites and antibiotics that was not even in the single cultures of actinomycetes by themselves (Bertrand, 2014).

1.4.1. Source of Antibiotics

Actinomycetes are responsible for the production of very large amounts of antibiotics. It is estimated that more than 70% of all known antibiotics come from this source, making it the most natural source of antibiotics (Behie, 2017). The production and synthesis of antibiotics by actinomycetes, especially, are related directly to the components of the media and their nutrients (Barka, 2016). It is well recognized that glucose and other beneficial carbon sources inhibit the morphological and chemical differentiation of antibiotics, as is the case with nitrogen and other elements (Reuther and Wohlleben, 2007).

In 1943, Albert Schatz, Elizabeth Bugie, and Selman Waksman discovered the first antibiotic produced by Actinomycetes, and it was used as a tuberculosis treatment (Behie, 2017). Since that date, thousands of antibiotics have been discovered from Actinomycetes.

Actinomycetes are regarded as being the predominant antibiotics among all microorganisms, generating approximately 55% of all known antimicrobial agents. *Streptomyces spp*. was responsible for the discovery of around 75% of these. While the other 25% have been identified as not *Streptomyces spp*., among the 22,000 identified bacterial secondary metabolic products, 70% are synthesized by actinomycetes, 20% by fungi. 7% by *Bacillus spp*. Also between 1 and 2% by other bacterial species (Bérdy, 2012).

1.4.2. Source of Antiviral Compounds

Viruses are the smallest form of life, and they have been the most threatening danger to the whole planet since they cause a wide range of diseases in all organisms, including AIDS, coronavirus, chickenpox, smallpox, herpes, etc. Moreover, some viruses are able to change their genome and require a new vaccine every year, as is common with influenza viruses. Occasionally, the mutations happen when a virus that causes infection in animals or birds then infects humans. The infections and diseases that are caused by viruses are countless, and the need for new treatments and vaccines becomes more pressing day after day. Fungal compounds have been known to be the most economical and effective secondary metabolites, including antiviral producers (Mohamed, 2023). Fattiviracins have been proven as new drugs that have activity against enveloped DNA viruses like the herpes family, HSV-1, and VZV, and enveloped RNA viruses like A and B viruses and the three strains of HIV-1. Fattiviracins are produced by one of the *Streptomyces* family, which is *Streptomyces microflavus. The* strain also produces at least 13 derivatives of fattiviracin, all with viral activities (Uyeda, 2004).

1.4.3. Source of Chemotherapeutic Agents

It's been recorded that cancer is the leading cause of death. In 1990, it was the third leading cause, and by 2013, it had become the second. Because the percentage of cancer is increasing rapidly every day, and its types have become more numerous. Some types of cancer are lethal, like brain and lung cancer; others might be cured by treatments, like prostate and breast cancer (Zaorsky, 2017).

Cancer is defined as a cell that divides randomly without control, transforming from one cell to another within the body (Brown, 2023). There are many ways to treat it, like surgery, radiation, chemotherapy, immunotherapy, and many other treatments. Besides, scientists are continually seeking new approaches. Most cancer treatments have side effects and symptoms (Lalitha, 2016). That's why they intend to extract cancer treatments from natural products and plants, besides microorganisms, which are a big source for cancer treatment (Tan, 2019)

Actinomycetes have been recorded as the most common phylum among all microorganisms that produce anti-cancer agents. Many useful compounds are currently

used to treat cancer, which are produced from actinomycetes, like bleomycin, which works on DNA damage and treats many types of cancer, like testicular cancer, Hodgkin's lymphoma, and ovarian cancer. This compound is extracted from *Streptomyces hindustanus* (Demain & Vaishnav, 2011). Besides many other compounds like peptides (bleomycin and actinomycin D), ureolic acids (mithramycin), and enediynes (neocarzinostatin), which are derived from actinomycetes and used particularly with other compounds as anti-cancer drugs (Krishnan & Jasmine, 2014).

1.5. Distribution of Actinomycetes

The actinomycetes ecosystem is so extensively vast and diverse (Fig. 2) that it's rich with many primary and secondary metabolites and able to survive in terrestrial, fresh, and marine water and deserts; it's even found in environments with severe conditions (Kurtböke, 2017).

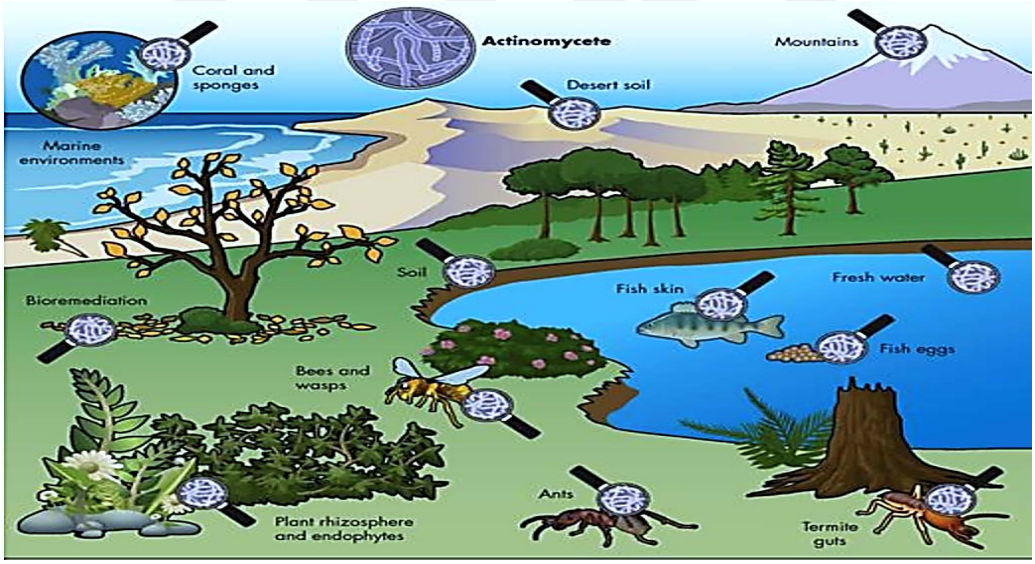


Figure 2. Possible ecosystems of *Actinomycetes* (Van der Meij, 2017).

1.5.1. Insects as a Reservoir of Actinomycetes

Several insects have a high potential to be hosts of actinomycetes. Actinomycetes microbiomes have lately become a study for many scientists. Bacteria isolated from such environments are rich in novel compounds. Metagenomic analysis (DNA sequenced in the

whole sample) showed that the most common organisms inside insects are bacteria (Douglas, 2022). The skeleton of all insects is mostly full of microorganisms. Some insects even produce toxins to protect their microorganisms from other organisms. Their bacteria mostly provide them with essential compounds (amino acids) and vitamins (B vitamins) that help the insects maintain their lifestyle and are present in many variant environments. Some insects are designed to carry bacteria; for example, many attine ants bear Actinobacteria: (*Pseudonocardiaceae)*.

Insects have a lack of nitrogen, and since microorganisms have a higher nitrogen and protein content, insects need to keep them in their diet not only as a nitrogen source but also for the nitrogen fixation process.

It's also common in some insects, such as beetles, to have a specialized structure for hosting bacteria and fungi, known as a mycangium. This structure differs from insect to insect; in some, it's a simple surface structure, while in others, it has a more complicated anatomical structure (Douglas, 2022).

Ants are the most popular insects in our sight; they are eusocial insects with more than 22,000 species and are also recorded to be a source of Actinomycetes. Even with significant active products, a study in Thailand of about 18 ants collected from different places, after applying 16S rRNA tests, showed that about 72 Actinomycetes have been isolated. By applying antimicrobial tests on those 72 Actinomycetes, it showed inhibiting effects (Tunvongvinis, 2024).

A new actinomycete strain has been discovered in ants after identification tests. It was shown that the ODS25^{T} strain that was not found before, also by applying its extracts against different strains of gram-negative and gram-positive bacteria and yeast, showed inhibiting activities against them (Tunvongvinis, 2024).

- The Symbiosis of Actinomycetes and Insects

Symbiosis is an interaction that occurs between two different organisms in which one or both of the symbionts depend on the other, partially or completely, to survive. We can notice that reactions between insects and actinomycetes are so frequent, with different shapes and forms, for example.

- Bacterial Ectosymbioses

This type of symbiosis occurs between some insects and bacteria in which the insects carry the bacteria upon their outer surface. For example, the relationship between *Streptomyces sp*. and bees is so important that the bacteria have specific channels on the

insect antennae. The female puts the larvae in a nest that contains many chambers underground. Also, it lays down the *Streptomycetes sp.* When each larva grows, it takes the bacteria with it, which produces antibiotics that make a defense for the new larva (Kaltenpoth, 2014).

Another example of ectosymbiosis is *Pseudonocardia sp.* bacteria and different types of attine ants. The ants are born with the bacteria, which are located in different places of the ant's body, like the forelegs or on the surface of the prothorax. According to the ant type, the bacteria produce antibiotics that help the ants defend against different types of fungi (Mattoso, 2012).

- Gut Symbiosis

The gut of most insects contains many different types of microorganisms that are gained through insect nutrition. The insect microbiota for each insect is different from another insect, even between the same type of insects that are kept inside the same field or inside the same lab conditions. Also, it is different between different stages (egg, larva, pupa, adult) for the same insect. Perhaps some variety comes from the different food sources for each insect and the different environments for each insect (Douglas, 2022).

- Endosymbiosis

Refers to endosymbiotic microorganisms, which are related to the bacteria that are localized and live inside the insect within the internal organs of the insect. The bacteria that are localized inside insects are found in cells that are known as bacteriocytes. It's reported that those cells are passed from one generation to another. It's also reported that they're found in more than one insect order. Bacteriocytes are related more to the insects that don't have a fixed diet system, which means the endosymbiotic relationship that's found between the bacteria and insects is a nutritional relationship. It's also reported that some of these relationships are endosymbiotic defense relationships (Douglas, 2022).

1.6. Introduction for *Melolontha melolontha*

Melolontha melolontha is one member of the biggest subfamily, *Scarabaeidae,* the subfamily that has around 11 thousand species in 750 genera of 28 tribes. The adult stage has many different common names: June beetles, May beetles, or cockchafers (Jia, 2021).

In the nineteenth century, it's reported that the first attempt to decrease the population of *Melolontha melolontha was made;* by then, it was well known that the insect

was a severe forest pest. Its life cycle lies down on two different stages during its life period: larvae, which live for about 4 years and feed on roots, and adults, which feed on leaves of the plants (Niemczyk, 2017). *Melolontha melolontha* is an insect that causes severe damage to plants. Additionally, it's reported that this insect is one of the most threatening in European countries, as each larva feeds on the roots of many plants for a period of between 3 and 4 years, slowing down the plant growth process and, in some cases, causing the death of the plants. (Woreta, 2015).

Lifecycle of *Melolontha melolontha* like most insects, its lifecycle consists of 4 different stages. The first stage is the adult stage, which is characterized by flying in the dark and is most common in the fourth and fifth months. They feed on the leaves of some plants for about two weeks. The female completes her sexual cycle and starts to lay her eggs, about 24 eggs deep, like 20 cm in the soil. Some of the eggs die, and some hatch out and become the second stage.

The second stage, egg: cockchafer females prefer the moist soils, which makes it easier for them to dig through hard soils to lay their eggs. Females go deep into the soil and lay their eggs. It keeps increasing its size by taking water up from the soil, and after about one month, larvae come out.

The third stage is larvae, which is the most dangerous stage in the insect life cycle since it lives a long time, between 3 and 5 years, and keeps feeding on the plant roots. It looks like a big, fluffy, white worm. Temperature and moisture are very important elements during the larval life period. In the hot weather during summer, its size increases, and in cold weather, it hibernates until next summer. Each season, its size and weight increase; it might reach 70 mm with a weight of 4 g. After 3 years or 4 years, the larvae became mature and moved to the fourth stage, the pupa.

The fourth stage is pupa. During the summer, after the larvae become fully mature, at a depth of 15 cm up to one meter, they go into pupation. According to the temperature, they change into the pupa. For example, at 25°C, it takes only 25 days to become a pupa, and at low temperatures, it takes more days, up to 100 days.

The pupa becomes inactive until the next summer, then the adult comes out of the pupa, flies, and forms a new cycle (Huiting, 2006; Pedrazzini, 2023; Szmidla, 2019).

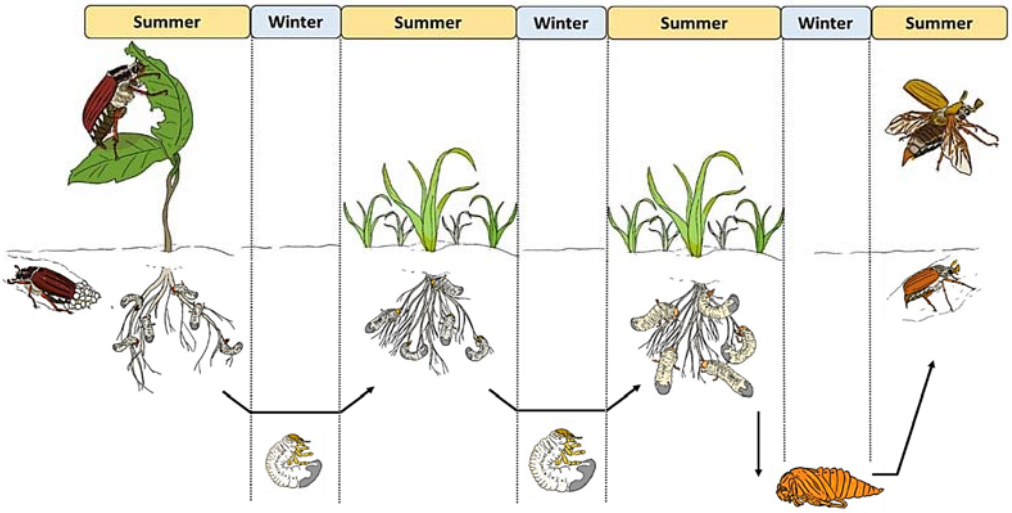


Figure 3. Life cycle of *Melonentha melonentha* (Pedrazzini, 2023).

1.7. Aim of the Study

In this study, which was carried out to identify secondary metabolites of biotechnological importance, our objectives were to collect Melelontha melenontha larvae from different parts of the Eastern Black Sea region of Türkiye, to isolate Actinomycetes from the collected larvae, to identify the Actinomycetes to be isolated morphologically, biochemically and molecularly, and to test whether the extractions specific to each isolate have antimicrobial activity, antiviral activity and anticancer activity.

2. MATERIALS AND METHODS

2.1. Collection of *Melolontha melolontha* Larvae

The samples were collected during the summer of 2023 by digging 50 cm to 100 cm deep in the soil from the Rize, Sinop, and Samsun vicinities of the Black Sea region of Türkiye. The larvae were collected in clean 50 ml Falcon tubes and then carried to the lab for work. Larvae have been examined and observed physically for their morphological features according to the *Melolontha melolontha* larvae features (Huiting, 2006).

For surface sterilization, firstly, larvae were washed with 70% alcohol, followed by washing with distilled water. The larvae were smashed and homogenized in 10 ml of sterilized phosphate buffer solution. The homogenized suspension was filtered (Sezen, 2007), and the filtrate was diluted to three different dilutions: 90%, 60%, and 30% dilutions. From each dilution, 100 microliters were taken to starch glucose agar media (give the recipe of the media or reference for it) plates and incubated at 28°C for 7 days.

2.2. Bacterial Isolation from *Melolontha melolontha*

After the incubation period, actinomycetes have been chosen according to their morphological shape; the color, size, and reverse of the colony; see the details at 2.4.1. The single colonies have been transferred and streaked to actinomycetes isolation agar media (ingredients g/L: sodium caseinate 2 g, L-asparagine 0.1 g, sodium propionate 4 g, dipotassium phosphate 0.5 g, magnesium sulfate 0.1 g, ferrous sulfate 0.001 g, agar 15 g, final pH (at 25°C) 8.1±0.2), and incubated at 30°C for 3 days. To follow the identification steps, 11 actinomycete isolates were isolated. The first isolation was done according to morphological characters of actinomycetes, followed by other types of identification.

2.3. Identification of Bacterial Isolates

Identification of actinomycetes has been done in three different types of identification: morphological identification, biochemical identification, and genetic identification (Pérez-Corral, 2022).

2.3.1. Morphological Identification

The morphological identification has been done in many ways. The entire plate is observed with the naked eye and then examined under the microscope to determine the colony color, reverse colony color, colony size, convexity, shape, margin, and elevation (Li, 2016).

After brief isolation and identification, Gram staining was performed and observed under a light microscope, first using a 40x objective, followed by a 100x immersion oil objective. The shape of the bacteria on the agar plate was also examined, as were the shapes of the aerial mycelia, substrate mycelia, and sporangia (Tripathi, 2020).

2.3.2. Biochemical Identification

A couple of biochemical tests have been done to identify the isolates to be able to differentiate actinomycete bacteria from other types, such as the following tests.

- Catalase Enzyme Production Test

It is a standard biochemical assay used to determine whether an organism, such as actinomycetes, produces the catalase enzyme. Catalase plays a key role in breaking down hydrogen peroxide into water and oxygen, protecting cells from oxidative damage. Catalase-positive organisms can decompose hydrogen peroxide (H_2O_2) into water and oxygen, producing bubbles when exposed to H_2O_2. Studies have consistently found that many actinomycetes, particularly Streptomyces species, produce catalase enzyme (Adeyemo, 2021).

In order to perform the catalase test, a small amount of the actinomycete culture was transferred to a clean slide. Then, a drop of 3% H_2O_2 solution was added to that slide. The formation of bubbles indicates a positive catalase reaction, indicating the production of the enzyme.

- The Amylase Enzyme Production Test

The amylase test is one of the tests that are used to identify actinomycetes biochemically. The media used was Mueller-Hinton agar medum (Naligama, 2022), a famous medium that is used to detect antibiotic activity since it has a large amount of starch, 0.15%, which can be used for the amylase test too.

A heavy inoculum of the actinomycetes strain was streaked onto a Mueller-Hinton agar plate. After incubation overnight at 30°C, the plates were flooded with iodine solution. After a few minutes, a clear zone was formed under the bacterial growth, which means the bacteria consumed the starch; other areas were blue (Lee, 1976).

- Triple Sugar Agar Tests

The triple sugar agar (TSA) test is used to test H_2S production, gas production (CO_2 and H_2), and sugar fermentation. The media contains ferric ammonium citrate, which indicates the production of H□S if the color turns black. The media also contains three sugar sources, the amount of which indicates the fermentation process (Jadon, 2014).

A fresh single colony was picked up with a needle and streaked onto the surface of the TSA tube and stabbed to the bottom of the agar butt (Åhman, 2020). After incubation, the tube with the loosened cap was maintained at 37°C for 24 hours, allowing the results of the fermentation reaction, gas production, and H□S production to be observed (Lehman, 2005).

- The Casein Hydrolysis Test

Casein is one of the main milk proteins that is water-soluble, which is hydrolyzed to smaller amino acids by the caseinase enzyme. Some microorganisms produce their enzyme, and according to them, some organisms are identified, like actinomycetes (Attimarad, 2012).

Skim milk agar media (Gebreyohannes, 2013) has been prepared. The freshly prepared samples, a loopful, have been streaked to SMA plates. The plates have been incubated at 28°C for 5–7 days. (Duddu, 2015). After the incubation period, a clear zone was observed for positive casein hydrolysis by actinomycetes.

2.3.3. Molecular Identification

For molecular identification, 16S rRNA gene sequencing was done. By comparing it to the microbial gene library, we were able to complete the identification without needing to pass through the DNA isolation step. The method has proven its effectiveness since it saves time and effort. A loopful of fresh, cultured actinomycetes has been transferred to an Eppendorf tube containing 50 µL of distilled water. After mixing well, we transferred it to a 95°C heat for ten minutes, then put it directly in the ice for 2 minutes. The tubes were centrifuged for 5 mins at 4200 x g, then put in the ice for 2 mins and proceeded for the

PCR cycle. A PCR reaction has been done using the kit FIREPol® DNA Polymerase fromSolis BioDyne. Universal primers UNI16SRNA-L (5′-ATTCTTAGAGTTTGA TCATGGCTCA-3′) and UNI16SRNA-R (5′-ATGGTACCGTGTGACGGGCGGT GTGTA-3′) (Kai, 2019) were used for amplification. The 16S rRNA genes of bacterial isolates were amplified in polymerase chain reaction (PCR), and then the amplified DNA samples were sent to the Macrogen company (https://www.macrogen-europe.com) for sequencing.

2.4. Primary Screening of Active Compounds by Culture Methods

The total number of isolates that have proven they belong to actinomycetes was 18, then by the end of the identification steps, it came to 8 isolates. A stock from each isolate has been kept at -80°C in 15% glycerol to be used for further analysis (Chand, 1994). The following tests have been applied to detect antimicrobial, antiviral, and anticancer compounds. The detection has started with general screening for active compounds for the actinomycetes that have been identified. The general primary screening has been done on all the isolates to test the antimicrobial activities by two techniques.

2.4.1. Antimicrobial Activity of Bacreial Isolates by Plate Streak Method

The streak method was done by streaking one line of each actinomycete isolate on Muller-Hinton agar media plates, and then the plates were incubated at 28°C for 4 days. Then, on both sides of each isolate, predetermined specific serial dilution concentrations for those organisms—three Gram-negative bacteria (*E. coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, and *Pseudomonas aeruginosa* ATCC 27853), three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Entoerococcus faecalis* ATCC 29212, and *Bacillus subtilis* ATCC 6633), and one yeast (*Candida albicans* ATCC 10231) were streaked (Kumar, 2010).

Serial dilution has been prepared for each tested microorganism to get to the most appropriate concentration for each one by using a spectrophotometer when it reached an OD of 200 nm to give a starting inoculum of 1×10^8 bacteria/ml. Plates had been incubated at 37°C for 24 hours, and then the results were observed (Othman, 2011).

2.4.2. Antimicrobial Activity of Bacreial Isolates by Agar Diffusion Method

To apply the test, it has started with the preparation of crude extract for each actinomycete isolate. The steps were as follows: each isolate was inoculated into 10 ml of AF media (components for one litre: glucose, H_2O 20 g, soybean meal 8 g, yeast extract 2 g, $CaCO_2$ 4 g, and NaCl 1 g), and the pH was adjusted to 7.3 in a 15 ml tube as a refreshment step for isolates., The tubes have been incubated at 30 °C in a shaking incubator (200 rpm) for 72 hours. After that a 900 µL of AF media for each isolate has been inoculated into two 15 ml tubes, each containing different types of media, media one, which is M8 media (components for one litre: soluble starch 20 g, glucose H_2O 10 g, yeast extract 2 g, meat extract 2 g, casein hydrolysate 4 g, and $CaCO_3$ 3 g, pH was adjusted to be 7.2), media two, which is SV2 media (components for one litre: glucose, H_2O 15 ml, glycerol 12 ml, soya peptone 15 g, NaCl 5 g, and $CaCO_3$ 2 g, pH was adjusted to be 7), both media have been incubated in a 30 °C shaking incubator (200 rpm) for 72 hours for *Streptomyces sp* and one week for non-Streptomyces strains, 500 µL of both harvest media was added to 1ml of 100% ethanol in 2ml tubes, the tube was shaken horizontally at room temperature for 1 hour, then centrifuged for 5 minutes at maximum speed. The supernatant was applied as a crude extract (Hili, 1997). The extracts of both media were dropped 10 µL on the plates that were previously inoculated and spread with the seven microorganisms individually that were used during the primary screening step. The media used for the bacterial strains was Muller-Hinton agar media, while for the yeast strain it was Sabouraud Dextrose Agar media. The method used was the diffusion method. For each plate, 5 µL of 50% ethanol was used as a negative control; the positive control was kanamycin (5 mg/mL) for bacterial samples, and for yeast, it was 5 µL of amphotericin (1 mg/mL). The plates that were inoculated with bacterial strains were incubated at 37°C for 24 hours, while the Candida sample was incubated at 28°C for 72 hours. Then the inhibition diameter (ID) was measured and recorded with a caliper; each test was done and repeated 3 or 4 times.

According to the two primary screening test results, the two most active samples were selected for chromatography analysis, followed by anticancer and antiviral tests.

2.5. Detailed screening of Active Compounds by Chromatographic Methods

The crude extract for M11 and M15 samples was prepared as mentioned in 2.5.2.1 steps and separated using HPLC. The separated substances were read, and their retention time were recorded (Intra, 2011). The analysis was done at Naicons labs in Italy, and the whole extracts of isolates M11 and M15 were used. Results were provided by Dr. Mariana LORIO.

2.6. Antiviral Tests

According to the results of chromatography, two isolates (M-11 and M-15) have been tested. The same steps have been done to prepare the powder extract, except that the media used for extraction was M-8.

The extracts were suspended in 30% DMSO. Potential reverse transcriptase (RT) inhibitors were assessed for their in vitro inhibitory activity using a non-radioactive HIV-1 RT colorimetric ELISA kit from Roche Diagnostics (Germany).

The RT inhibition assay was performed as described in the kit protocol. Briefly, the inhibitor diluted with lysis buffer was added to the reaction mixture containing the primer complex, dNTPs, and reverse transcriptase. The final concentrations of the extracts after this process were 50 µg for M-11 and M-15. A reaction mixture containing lysis buffer without HIV-1 RT was used as a negative control. After a 1-hour incubation at 37°C, the reaction mixture was transferred to a streptavidin-coated microplate (MP) and incubated at 37°C for 1 hour. After incubation, the solution was completely removed and washed. After washing, anti-DIG-POD (200 mU/mL) was added. Incubation was continued for 1 hour at 37°C. After incubation, peroxidase substrate (ABST) was added to the MP and left at room temperature for approximately 10 to 30 minutes for photometric determination. The absorbance of the samples was measured at a wavelength of 405 nanometers using a microplate ELISA reader. Inhibitory activity was calculated as percent inhibition compared to a sample containing no inhibitor (Dube, 2015).

2.7. Cytotoxicity Tests

According to the results of chromatography, two isolates (M-11 and M-15) have been selected to be tested against breast cancer cells (MCF7, ATCC: HTB-22).

The steps to prepare the extract to be tested against MCF7 cells M-11 and M-15 samples have been inoculated into a 2-liter flask that contains 400 ml of starch casein broth media (casein powder 1.0, starch 10.0, seawater 37.0, agar 15.0, final pH 7). The flasks have been incubated at 30°C for 7 days at 200 rpm. Each flask has been divided into 50 ml Falcon tubes to be centrifuged at 13500 rpm for 5 minutes. The supernatant has been measured and transferred to 1-liter flasks, and the same volume of pure ethyl acetate has been added. The flasks have been shaken at 200 rpm for 24 hrs at room temperature. After leaving the samples to settle, the upper layer has been taken to be evaporated using, A powder extract has been formed and is ready to be dissolved with ethyl acetate to be tested against MCF7 cells (Balakrishnan, 2017; Muthusamy, 2019). After the powdered extract has been prepared, it has been tested against breast cancer cells as follows: Dulbecco's Modified Eagle's medium was used to culture human mammary gland cancer cell line MCF7 cells (ATCC: HTB-22) at 37°C and 5% CO_2 atmosphere, including 10% FBS and 100 units/mL penicillin and 100 µg/mL streptomycin.

The safety of the extract was evaluated using the MTT assay. The MTT assay is widely applied to measure cytotoxicity under in vitro conditions; it is a fast, easy, and highly accurate quantitative colorimetric method (Mosmann, 1983). The MTT assay is based on viable cells reducing yellow, water-soluble tetrazolium salt to insoluble purple-colored formazan precipitate by NADH-dependent succinate dehydrogenase (Riss & Moravec, 1992).

The stock solutions of M-11 and M-15 were prepared by dissolving the extracts in ethyl acetate to 1000 mg/mL. Dilution series of 100, 10, and 1 mg/mL were prepared from these stock solutions (logarithmic doses were chosen). Three concentrations, including the stock solutions, were added to the prepared medium at a final concentration of 0.4% (v/v). The cells were sown in a 96-well microplate, and after 24 h, the cells were incubated with ethyl acetate extracts with a concentration range (1–1000 mg/mL). Cells incubated with 0.4% ethyl acetate were used as a solvent control. Cells incubated with 1 µM doxorubicin were used as a positive control. After 24 h, MTT solution was added to the 96-well plate in DMEM with a final concentration of 0.5 mg/mL. After 3 h of incubation, the plate was

shaken on an orbital shaker for 10 min. The absorbance values of the formed formazan crystals were measured at 570 and 690 nm wavelengths (Cloos et al., 2011; Mosmann, 1983). The absorbance values measured for each concentration of the extracts were proportioned as a percentage of the absorbance value of the solvent control group. Thus, the percentage of cell viability was calculated for the studied concentrations of each extract. The studies were carried out in duplicate. The results were given as the mean % viability value ± standard deviation for each extract.

3. RESULTS

3.1. Identifying the Bacterial Isolates

Eight Streptomycetes were isolated from *Melolontha melolonta*, and the following identification steps were done.

3.1.1. Morphological Identification

The shapes of plates, their reverse, and their shapes under a 40x light microscope have been photographed and recorded in Figure 4.

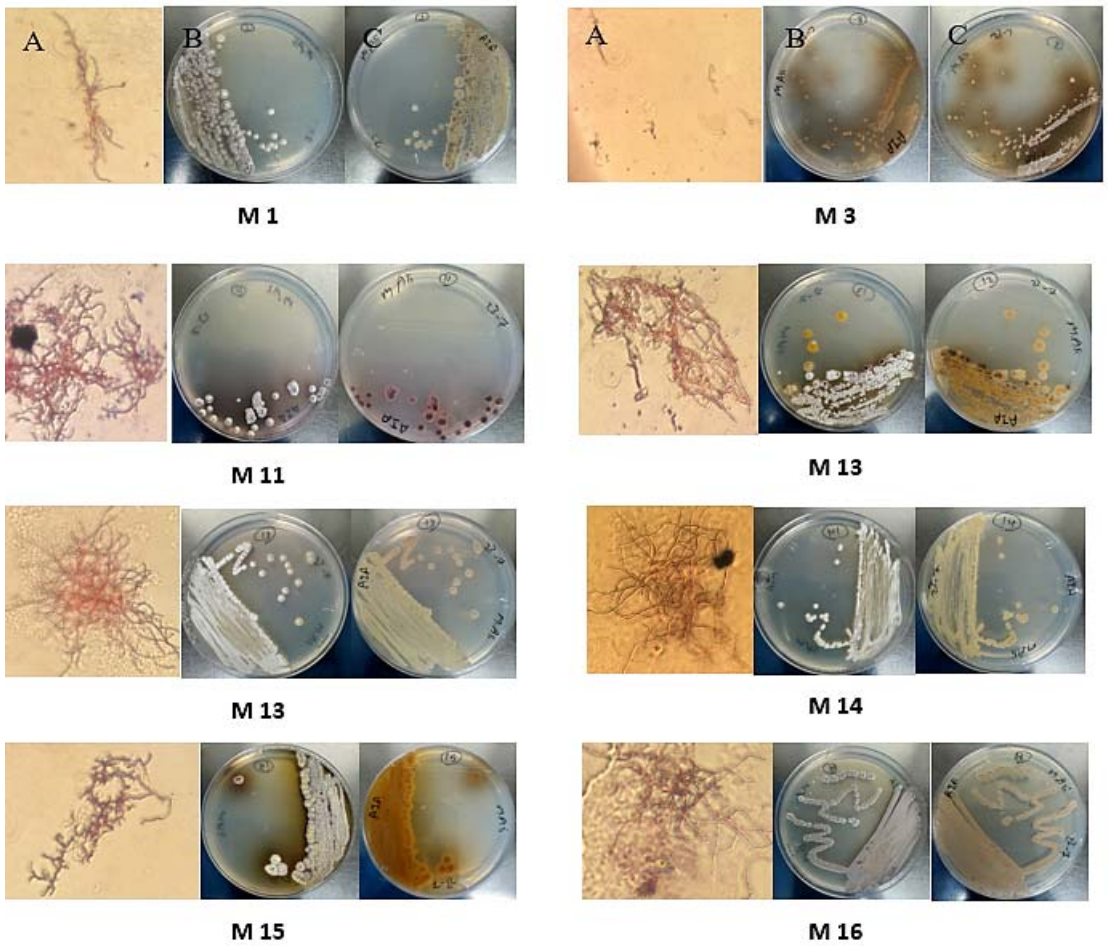


Figure 4. Morphological features of bacterial isolates. Column A) results of Gram staining 40x, Column B) face of the plates. Column C) back of the plates of each isolate.

The figures show the ideal shapes of Streptomyces shalky with different colors, and the reverse for each plate was colored, which is common on them. During observation under a light microscope, actinomycete mycelia and branches were so clear, as shown in Figure 5. The macroscopic description of each colony (form, elevation, margin, color, reverse color, and size) has been recorded in Table 1.

Table 1. Colony morphology of bacterial isolates

Isolate	**Form**	**Elevation**	**Margin**	**Pigment color**		**Size**
				Front	**Reverse**	
M 1	Round	Crateriform	Entire	Chalk gray	Gray	Medium
M 3	Round	Crateriform	Entire	Chalk gray	Light brown	Small
M 11	Round	Convex	Undulate	Chalk	Deep red	Medium
M 12	Round	Unboned	Entire	Chalk	yellow	Medium
M 13	Round	Unboned	Entire	Chalk	Pale brown	Medium
M 14	Round	Convex	Scalloped	Chalk	Pale brown	Medium
M 15	Irregular	Unboned	Serrate	Chalk Yellow	Orange-brownish	Large
M16	Round	Crateriform	Entire	Chalk gray	Chalk bag	Medium

Small < 2 mm (diameter), medium between 2 mm & 5 mm (diameter), and large > 5 mm (diameter).

3.1.2. Spore Chain Morphology

All the samples have shown a round shape for colonies, except M15, which was irregular in shape. For the elevation of the samples, it was crateriform for M1, M3, and M16; for M11, convex; and for M12, M13, and M15, umbonate. The margin was entire for all the samples except M14, which was scalloped, and for M15, which was serrate. The sizes were all medium except large for M15 and small for M3.

3.1.3. Biochemical Identification of Bacterial Isolates

The following tests have been repeated 2-3 times: the results are mentioned in Table 2. The results of the Gram stain showed a violet color under the microscope at 40x and catalase enzyme production. After being done on a slide, all the samples showed a purple color, which means that the catalase enzyme was produced, and split H_2O_2 into H_2O and O_2, and amylase enzyme production has been observed in all the colonies.

Table 2. Biochemical tests of bacterial isolates.

Isolate	Biochemical Test Results				
	Gram Stain	**Catalase Production**	**Starch hydrolysis**	**H_2S production**	**Casein hydrolysis**
M 1	+	+	+	+	+
M 3	+	+	+	-	+
M 11	+	+	++	-	+++
M 12	+	+	++	-	+++
M 13	+	+	++	-	++
M 14	+	+	++	-	++
M 15	+	+	+	-	+
M16	+	+	++	-	++

+ = positive (the number of pluses according to the severity of the test). - = Negative results.

Colonies have shown a clear zone after adding the iodine to the plates. Samples M11, M12, M13, M14, and M16 showed bigger clear zones, but the rest showed smaller zones. Production of caseinase enzyme samples M1, M3, and M15 showed a small colorless zone for the test (3 mm); samples M13, M14, and M16 showed an average zone (5 mm); but samples M11 and M12 showed very big clear zones (8 mm). Production of H_2S gas was carried out for all samples; only for sample M1 was the gas produced. The results for all the tests together are represented in Table 2.

3.1.4. Molecular Identification Results

Sequencing results have been read and analyzed by ezbiocloud.net, which researches against quality-controlled databases of 16S rRNA sequences. The top-hit information for each identification is the top hit against all prokaryotic names, as shown in Table 3.

Table 3. Identification of bacterial isolates based on 16S rRNA gene sequence.

Name	Top-hit taxon	Percentage (%)	The name of The located city
M 1	*Streptomyces ambofaciens*	99.38	Samsun
M 3	*Streptomyces mirabilis*	99.92	Sinop
M 11	*Streptomyces pluricolorescens*	99.76	Rize
M 12	*Streptomyces araujoniae*	99.61	Rize
M 13	*Streptomyces durocortorensis*	99.92	Rize
M 14	*Streptomyces setonii*	100	Rize
M 15	*Streptomyces antibioticus*	99.92	Rize
M16	*Streptomyces caelestis*	99.22	Rize

The results showed that all eight isolates were Streptomyces, also showing that all of them had above 99% similarity.

Based on the 16S rRNA gene sequence, the M1 sample is *Streptomyces ambofaciens*, which was isolated from Samsun city, and the M3 sample is *Streptomyces mirabilis, which* was isolated from Sinope city. The M11 sample is *Streptomyces pluricolorescens*, the M12 sample is *Streptomyces araujoniae*, the M13 sample is *Streptomyces durocortorensis*, the M14 sample is *Streptomyces setonii*, the M15 sample is *Streptomyces antibioticus*, and the M16 sample is *Streptomyces caelestis*. All samples from M11 to M16 were isolated from Rize city.

3.2. Primary Screening of Active Compounds by Cultural Methods

Primarily, screening has been done with two different techniques: the streak method, in which after each isolate was streaked, eight different strains of bacteria and yeast from ATCC were incubated for different times, and the results are shown in Table 4.

Table 4. Primary screening for antimicrobials by streaking technique

Isolate	**The tested organisms with their inhibition zone (in mm)**							
	Yersinia pseudotuberculosis	***Enterococcus faecalis***	***Bacillus subtilis***	***Klebsiella pneumoniae***	***Staphylococcus aureus***	***Escherichia coli***	***Salmonella Typhi***	***Candida albicans***
M 1	-	-	5	-	-	-	-	2
M 3	3	3	3	-	-	-	-	-
M 11	3	-	4	-	4	-	4	-
M 12	-2	-	-	2	-	3	-	-
M 13	3	-	-	-	3	-	-	-
M 14	-	3	-	-	-	-	3	-
M 15	5	3	4	-	4	-	3	3
M16	-	-	3	-	-	-	-	-

The observed and recorded results showed that all the Streptomyces isolates showed activity at a minimum, with two or more organisms. Some isolates showed activity against the most significant isolate, which was the M11 sample, which showed activity against four organisms. Also, sample M15 showed activity against 6 tested organisms.

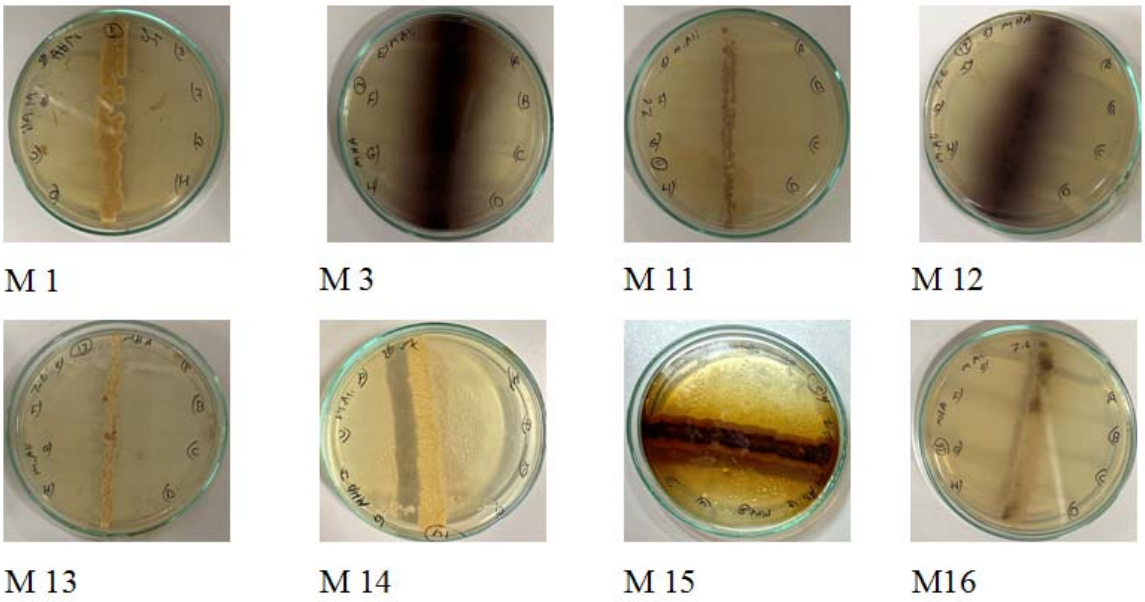


Figure 5. The streaking method results are followed by the tested organisms. A) *Yersinia pseudotuberculosis*, B) *Enterococcus faecalis*. C) *Bacillus subtilis.* D) *Klebsiella pneumoniae*. E) *Staphylococcus aureus*. F) *Escherichia coli.* G) *Salmonella Typhi.* H) *Candida albicans*.

The observed and recorded results showed that all the Streptomyces isolates showed activity at a minimum, with two or more organisms. Some isolates showed activity against the most significant isolate, which was the M11 sample, which showed activity against four organisms. Also, sample M15 showed activity against 6 tested organisms.

The plates were photographed and recorded in Figure 5, which shows that beside each Streptomyces strain causing an effect against bacteria or yeast, the inhibition zones for samples M11 and M15 were clearer, and a noticeable streaking test was a startup step that led to a second type of primary screening by cultures.

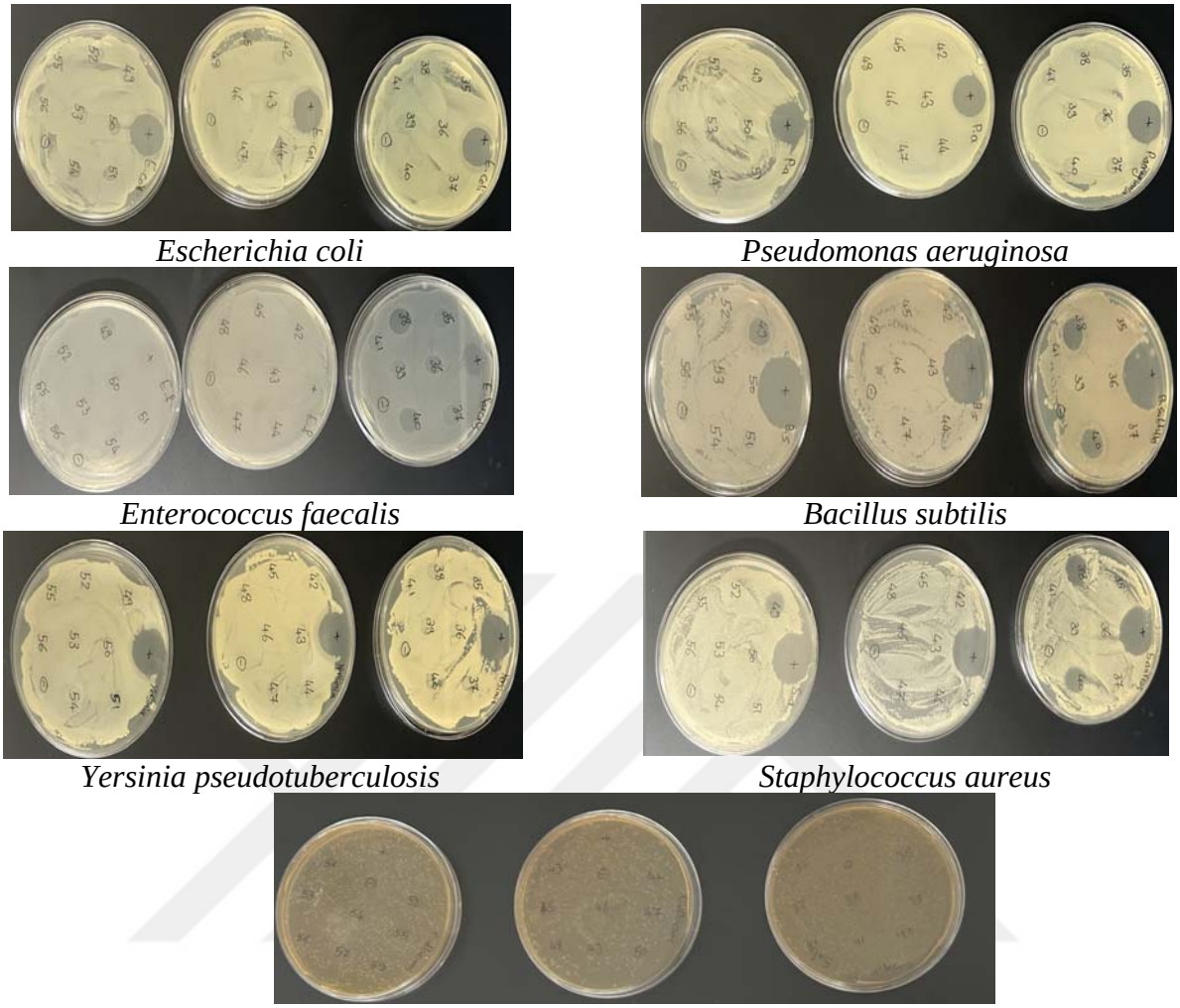
Diffusion technique results were recorded in Table 5, which showed activity against three Gram-negative and three Gram-positive bacteria and one yeast. The inhibition zones' diameters for each sample were recorded. The noticeable thing was the extra activity for the M11 and M15.

Table 5. Primary screening with the diffusion technique.

ISOLATE	The Tested Organisms Inhibition zone (in mm)													
	Yersinia pseudotuberculosis		*Bacillus subtilis*		*Klebsiella pneumoniae*		*Staphylococcus aureus*		*Escherichia coli*		*Salmonella Typhi*		*Candida albicans*	
Media Type	M8 media	S V 2 media	M8 media	S V 2 media	M8 media	S V 2 media	M8 media	S V 2 media	M8 media	S V 2 media	M8 media	S V 2 media	M8 media	S V 2 media
M1	-	-	8	4	-	-	-	-	-	-	-	-	-	-
M3	5	-	10	6	7	-	-	-	4	-	-	-	6	-
M11	-	5	10	4	-	4	10	-	5	5	11	6	-	4
M12	-	5	5	5	-	-	-	-	5	5	-	-	-	-
M13	4	-	-	5	4	4	-	-	5	5	-	4	-	-
M14	-	5	6	7	-	5	-	-	5	5	-	-	-	-
M15	3	-	7	3	3	-	5	-	5	5	-	-	4	-
M16	-	5	5	-	-	4	-	-	-	5	-	-	-	-

The recorded inhibition zones for M11 were many against many organisms in their two media. It was against *Yersinia pseudotuberculosis* in SV2 media, against *Bacillus subtilis* in both media, against *Klebsiella pneumoniae* in SV2 media, against *Staphylococcus aureus* in M8 media, and against *Escherichia coli* in both media. *Salmonella typhi* was tested in both media, and *Candida albicans* was tested in SV2 media only.

The recorded inhibition zones for M15 were many against many organisms in their two media. It was against *Yersinia pseudotuberculosis* in M8 media.



Escherichia coli *Pseudomonas aeruginosa*

Enterococcus faecalis *Bacillus subtilis*

Yersinia pseudotuberculosis *Staphylococcus aureus*

Candida albicans

Figure 6. Antimicrobial tests of bacterial isolates by diffusion method.

Against *Bacillus subtilis* in both media, against *Klebsiella pneumoniae* in M8 media, against *Staphylococcus aureus* in M8 media, and against *Escherichia coli* in both media. *Salmonella Typhi* was tested in none of the media, and *Candida albicans* was tested in M8 media only, as shown in Figure 6.

The results of primary screening of active compounds by streaking and diffusion methods were so successful and showed activity, especially for samples M11 and M15, which is why only those two samples were carried out for further detailed analysis using HPLC, which was performed in Italy, Naicons labs.

3.3. Results of Detailed Screening of Active Compounds by Chromatographic Methods (Performed in Italy)

The HPLC results for M11 and M15 were recorded in Table 6. It was a very convincing result for the shown antimicrobial effect against bacteria and yeast, which was done in 3.3 screening, since the results contain actinomycin D, antimycin A3, antimycin A5, antimycin A11, nikkomycin D, platensimycin, geldanamycin, blasticidin H, phenazine-1-carboxylic acid, and enterobactin, all of which are used as antibiotics against gram-positive and gram-negative bacteria, antifungal activity, and siderophore-mediated iron chelation.

Table 6. Shows the HPLC results for samples M11 and M15 with their usage.

Category	Representative Compounds	Most Common Use/Activity	References
Antimicrobial	Actinomycin D, Antimycin A3, Antimycin A5, Antimycin A11, Nikkomycin D, Platensimycin, Geldanamycin, Blasticidin H, Phenazine-1-carboxylic acid, Enterobactin	Antibiotics against Gram-positive and Gram-negative bacteria, antifungal activity, siderophore-mediated iron chelation	(Demain, 2014; Shen, 2019)
Anticancer	Actinomycin D, Fredericamycin A, Fredericamycin B, Fredericamycin C, Geldanamycin, Migrastatin, Epicarine	Cytotoxic effects, DNA intercalation, HSP90 inhibition, tumor suppression, anti-metastatic activity	(Pommier, 2010; Whitesell & Lindquist, 2005)
Antiviral	Actinomycin D, Physostigmine, Indole-3-acetaldehyde	Inhibition of viral replication, modulation of host cell metabolism	(De Clercq, 2004; Shen, 2019)
Other / Bioactive Metabolites	**Ornithine-containing lipid, Biotin, Cyclo(L-Leu-Gly), Bestatin, Nicotianamine, Myxochelin, Daidzein**	**Cofactor activity, metal chelation, immunomodulation, enzyme inhibition**	**(Blunt et al., 2018; Newman & Cragg, 2020)**

The results also showed some other activities for anticancer compounds (Actinomycin D, Fredericamycin A, Fredericamycin B, Fredericamycin C, Geldanamycin, Migrastatin, and Epicarine), which are already used to treat cancer cells, such as cytotoxic

effects, DNA intercalation, HSP90 inhibition, tumor suppression, and anti-metastatic activity, besides the presence of antiviral agents (Actinomycin D, Physostigmine, and Indole-3-acetaldehyde). used for the inhibition of viral replication and modulation of host cell metabolism.

The HPLC results also have shown the results of bioactive compounds (ornithine-containing lipid, biotin, cyclo(L-Leu-Gly), bestatin, nicotianamine, myxochelin, and daidzein), which are used for cofactor activity, metal chelation, immunomodulation, and enzyme inhibition.

The results of HPLC led the researchers to go further, to detect and apply some further tests, such as cytotoxicity tests and antiviral tests.

3.4. Results of Antiviral Tests

In vitro RT inhibitory activities of crude extracts obtained from bacteria were repeated 3 times each, and then the average was calculated. Inhibition percentages were determined to be 97.3% and 96% for M-11 and M-15 samples, respectively. The results have been represented in Figure 7.

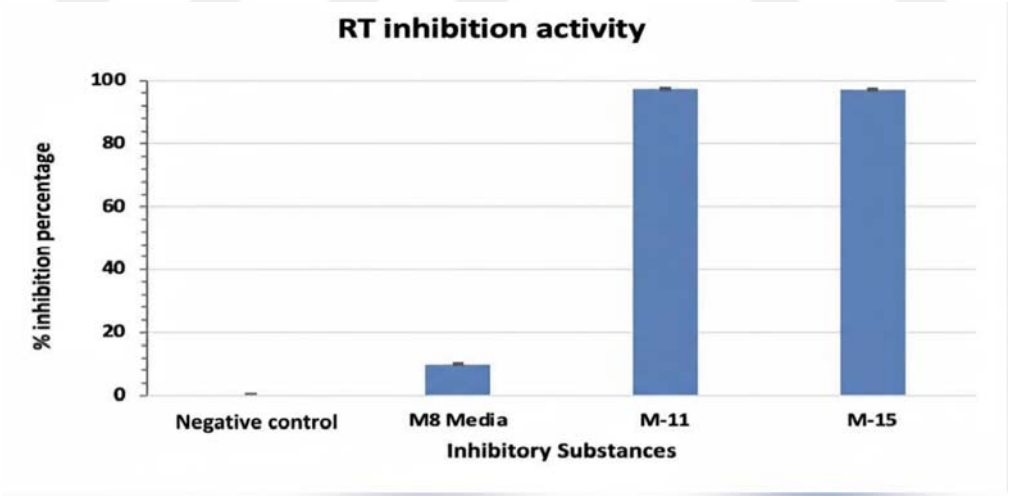


Figure 7. Inhibition rates of HIV-1 RT for M11 and M15 samples.

The results of the antiviral have proved the results of HPLC, which are actinomycin D, physostigmine, and indole-3-acetaldehyde substances.

3.5. Results of Cell Culture Tests

According to the results of the duplicate MTT assay, the M-11 extract concentration of 1000 mg/mL significantly reduced MCF-7 cell viability compared to the other test groups, as shown in Figure 8. The M-11 extract concentrations of 100, 10, and 1 mg/mL significantly reduced MCF7 cell viability compared to the negative control group (post-test Tuckey: $p<0.01$; ***: $p<0.001$, $R^2=0.9925$).

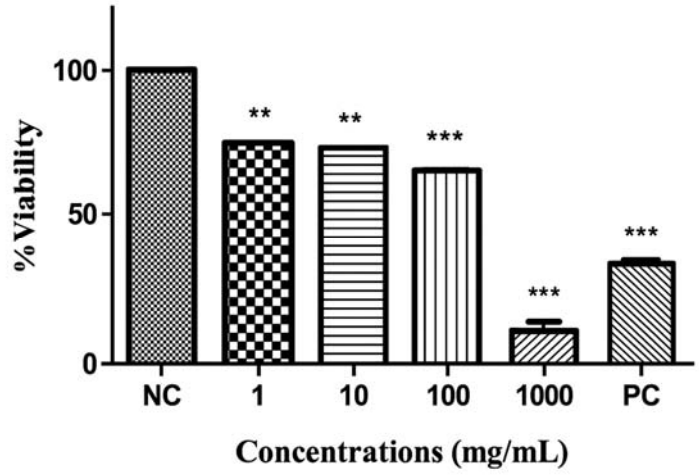


Figure 8. shows the effect of M-11 extract with different concentrations against breast cancer cells. NC: Negative control group. PC: Positive control group.

According to duplicate MTT results, the IC_{50} value of the M-11 extract was calculated as 395.1 mg/mL, as shown in Figure 9.

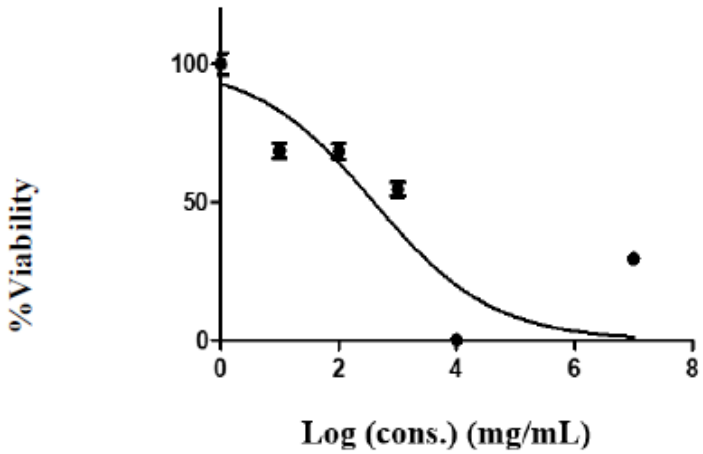


Figure 9. The IC_{50} value diagram for M-11 extract.

According to the results of the duplicate MTT assay, the M-15 extract concentration of 1000 mg/mL significantly reduced MCF7 cell viability compared to the other test groups. The M-15 extract concentrations of 100, 10, and 1 mg/mL significantly reduced MCF7 cell viability compared to the negative control group (post-test Tukey, *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, $R^2=0.9862$) as shown in figure 10.

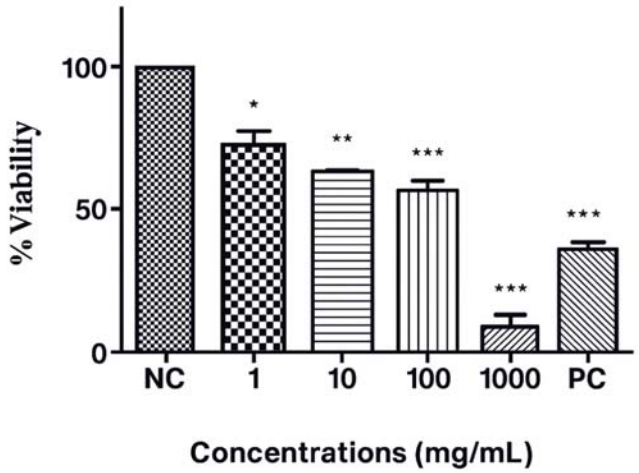


Figure 10. The effect of M-15 extract with different concentrations against breast cancer cells. NC: Negative control group. PC: Positive control group.

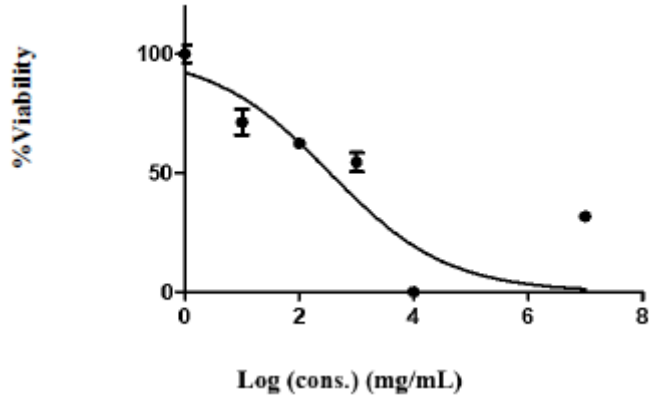


Figure 11. The IC50 value diagram for M-15 extract

According to duplicate MTT results, the IC_{50} value of the M15 extract was calculated as 345.2 mg/mL, as shown in Figure 11.

3.6. Statistical Analysis

The log(inhibitor) vs. normalized response-variable slope test was used to calculate the IC_{50} value of M-11 and M-15 extracts. A one-way ANOVA test was used to assess whether there was a statistically significant difference between the control group and the M-11 and M-15 extracts. The Tukey test was used as a posttest. All tests were performed using the Graphpad Prism 5.1 program. $p<0.05$ was considered statistically significant.

4. DISCUSSION

Actinomycetes have always been a source of active, important compounds (De Simeis, 2021), especially those that are extracted from severe environmental conditions (Tiwari, 2013). Since the source of the isolates that have been worked on was *Melolontha melolontha* larvae, which live deep down in soils with this type of temperature, moisture, and pH, it was planned to be the source of isolates. The isolation of actinomycetes from this has not been studied frequently recently. The current study has been based on 16 actinomycete isolates from some of the Black Sea region cities (Rize, Sinope, and Samsun). The area is famous for the production of hazelnuts; it contains plenty of its trees and *Melolontha melolontha*, one of the most common pests that causes a very big danger to the production of hazelnuts in this area (Sezen & Demirbag, 2006), which underwent many identification processes. Each step has reduced the total number of isolates. The morphological tests gave the tests a quick discovery survey for the plates, and under the microscope, many other contaminations were discarded. Actinomycetes are so famous for their different shapes, it's easy to recognize them among other bacteria. Only shaky samples have been isolated for biochemical tests; all of the samples were actinomycetes. Testing their ability to produce amylase enzyme, catalase enzyme, hydrogen sulfide, or even β-galactosidase enzyme was very remarkable to confirm the actinomycetes samples (Lwin, 2020)

By reaching the last identification (16S rRNA sequences) and comparing the results with the Genebank database, the number has been reduced to 8 isolates only. All samples have proved that they come under Streptomyces *(Streptomyces ambofaciens, Streptomyces mirabilis, Streptomyces pluricolorescens, Streptomyces araujoniae, Streptomyces durocortorensis, Streptomyces setonii, Streptomyces antibioticus, and Streptomyces caelestis*). The samples have been coded as M1, M2, M11, M12, M13, M14, M15, and M16, respectively.

Followed by the detection of essential active compounds, which has been started with the general primary screening, which detects the priority or comparison between the isolates. The results of the primary screening were obtained using two types of tests. The streaking method and disc diffusion method are applied and effective methods according to (Skowronek 2020), which isolated 900 bacteria from the midgut of Mentha longifolia;

among those numbers, two samples showed antibacterial activity. For the current study, the streaking method has been used to test all 8 Streptomyces samples against one yeast (*Candida albicans* ATCC 10231), three gram-positive bacteria *(Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 25923), and four gram-negative bacteria (*Yersinia pseudotuberculosis* ATCC 29833, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, and *Salmonella typhi* ATCC 6539). The test has been repeated twice, and the results for that test showed activity with all samples, especially samples M11 and M15. Those two samples showed activity against all the strains mostly. Also, the antimicrobial test has been done with the disk diffusion technique (Ertürk, 2016). The eight Streptomyces isolates were incubated and extracted using two different media M8 media and SV2, and the extracts have been tested against three Gram-negative bacteria (*E. coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 27853), three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633), and one yeast (*Candida albicans* ATCC 10231). This test was more confirmation for the previous one; it also approved the antibacterial activity for all eight streptomyces isolates. The results for M11 and M15 have been showing big activity with big inhibition zones, which led the article to take further steps for deeper detection, chromatography tests, which made the research go further for more specific detection chromatography

Because samples M11 and M15 have been showing massive activity, the chromatography test has been done for both samples, and results have been done for those two samples. HPLC results have shown the presence of plenty of active compounds, a group of them related to antimicrobial activity (Actinomycin D, Antimycin A3, Antimycin A5, Antimycin A11, Nikkomycin D, Platensimycin, Geldanamycin, Blasticidin H, Phenazine-1-carboxylic acid, and Enterobactin) (Demain, 2014; Shen, 2019), which explains the reason for the presence of big inhibition zones during antimicrobial tests.

Besides the presence of antiviral compounds (actinomycin D, physostigmine, and indole-3-acetaldehyde) (De Clercq, 2004; Shen, 2019), which explains the activity that has been proved within antiviral tests

The anticancer compounds that have been detected by HPLC are actinomycin D, fredericamycin A, fredericamycin B, fredericamycin C, geldanamycin, migrastatin, and epicarine (Pommier, 2010; Whitesell & Lindquist, 2005). Besides some other active

compounds (Ornithine-containing lipid, Biotin, cyclo(L-Leu-Gly), Bestatin, Nicotianamine, myxochelin, and Daidzein), those compounds have further usage as cofactor activity, metal chelation, immunomodulation, and enzyme inhibition.

The MTT assay is widely applied to measure cytotoxicity under in vitro conditions; it is a fast, easy, and highly accurate quantitative colorimetric method (Mosmann, 1983). MCF7 cells are widely used to test the activity of actinomycete isolates (Silva et al., 2020; Fahmy et al., 2021; Barreca et al., 2023; Lee et al., 2023). In the MTT assay, the 1000 mg/mL concentration of M-11 isolate significantly reduced the viability of MCF7 cells compared to the other test groups ($p<0.001$, $R^2=0.992$). M-11 isolate concentrations of 100, 10, and 1 mg/mL significantly reduced the viability of MCF7 cells compared to the negative control group ($p<0.01$; $R^2=0.992$). The IC50 value is calculated as 395.1 mg/mL. In the MTT assay, the 1000 mg/mL concentration of M-15 isolate significantly reduced the viability of MCF7 cells compared to the other test groups ($p<0.001$, $R^2=0.9862$). The M-15 isolate concentrations of 100 and 10 mg/mL significantly reduced the viability of MCF7 cells compared to the negative control group ($p<0.01$; $R^2=0.9862$). 1 mg/mL of M-15 isolate significantly reduced MCF7 cell viability compared to the negative control group ($p<0.05$; $R^2=0.9862$). The IC_{50} value is calculated as 345.2 mg/mL. Both isolates have strong inhibitory effects on MCF7 cells. According to IC_{50} values, the inhibitory effect of the M-15 isolate on breast cancer cells is slightly higher than that of the M-11 isolate.

5. CONCLUSION

Insects, especially those living in severe conditions, are so rich with magnificent organisms and compounds. 8 Streptomyces samples have been isolated from the Melonetha melonetha larvae in the Black Sea region, and HPLC results showed the presence of anticancer, antibacterial, antiviral, and other bioactive compounds. Finding all those compounds just from a single organism is quite important. This indicates the importance of insect microbiomes as a reservoir for medical and biotechnological compounds. There are more and more types that need to be researched and discovered.

In this study, the activity of the whole extracts was tested against gram-positive bacteria, yeast, gram-negative bacteria, yeast viruses, and cancer cells. However, the activity of the fractions from each extraction was not examined, which would require more detailed HPLC work.

The world is now in need of new antibiotics, as it is full of diseases. The main reason for this is viruses, bacteria, yeast, and different cancers, so it's in need of antibiotics, anticancer drugs, and antiviral compounds. Microorganisms have always been an economic source for that, especially isolates extracted from insect sources

There are many, many insects waiting to be researched and examined, and Streptomyces needs to be on the study spots.

Further research could be done to detect and test the activity of each component separately, test its activity functioning to separate substances by substance, and test each compound individually to be sure which compound the activity came from exactly or if the inhibition was because of the presence of more than one compound together. The reason not to fractionate for each substance is that it costs a lot of time and money, but it would be great for other researchers to continue from this point and follow up after.

This article is quite important from many sides. First, it makes the spots isolated from insects, especially those found in different and severe conditions of temperature, pressure, and humidity, since they are not studied much so far, and they are such rich organisms with vital active compounds. One more thing: microorganisms are the cheapest source that can be used.

6. RECOMMENDATION

1. We recommend applying forward fractionations for HPLC results since it's rich with many active compounds that need to be discovered.
2. We suggest that researchers conduct more studies on other bacteria that we have found and tested.
3. Putting into consideration that Streptomyces is a source of active biotechnological compounds.
4. It is recommended that a study be conducted and applied to each field or section of our isolates in all different fields, such as pharmaceutical, biochemical, and biotechnological
5. Search and dig for more research for the area of the black sea region to be considered for study, since it is full of different biological organisms.
6. It's good for new researchers to find and isolate organisms from different severe environments.
7. It's so important to consider and focus on the insects since they're a very rich source of organic compounds, which are full of bioactive compounds.

7. REFERENCES

Adegboye, M. F., & Babalola, O. O. (2012). Taxonomy and ecology of antibiotic-producing actinomycetes. African Journal of Agricultural Research, 7(15), 2255–2261.

Abdelghani, Z., Hourani, N., Zaidan, Z., Dbaibo, G., Mrad, M., & Hage-Sleiman, R. (2021). Therapeutic applications and biological activities of bacterial bioactive extracts. Archives of Microbiology, 203(8), 4755–4776.

Attimarad, S. L., Ediga, G. N., Karigar, A. A., Karadi, R., Chandrashekhar, N., & Shivanna, C. (2012). Screening, isolation, and purification of antibacterial agents from marine actinomycetes. International Current Pharmaceutical Journal, 1(12), 394–402.

Balakrishnan, S., Santhanam, P., & Srinivasan, M. (2017). Larvicidal potency of marine actinobacteria isolated from mangrove environments against Aedes aegypti and Anopheles stephensi. Journal of Parasitic Diseases, 41(2), 387–394.

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J. P., Klenk, H. P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2015). Taxonomy, physiology, and natural products of Actinobacteria. Microbiology and Molecular Biology Reviews, 80(1), 1–43. https://doi.org/10.1128/MMBR.00019-15

Barreca, M., Buttacavoli, M., Di Cara, G., D'Amico, C., Peri, E., Spanò, V., Li Petri, G., Barraja, P., Raimondi, M. V., Cancemi, P., & Montalbano, A. (2023). Exploring the anticancer activity and the mechanism of action of pyrrolomycins F obtained by microwave-assisted total synthesis. European Journal of Medicinal Chemistry, 253, 115339. https://doi.org/10.1016/j.ejmech.2023.115339

Behie, S. W., Bonet, B., Zacharia, V. M., McClung, D. J., & Traxler, M. F. (2017). Molecules to ecosystems: Actinomycete natural products in situ. Frontiers in Microbiology, 7, 2149.

Bérdy, J. (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. The Journal of Antibiotics, 65(8), 385–395.

Bertrand, S., Bohni, N., Schnee, S., Schumpp, O., Gindro, K., & Wolfender, J. L. (2014). Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. Biotechnology Advances, 32(6), 1180–1204.

Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H. G., & Prinsep, M. R. (2018). Marine natural products. Natural Product Reports, 35(1), 8–53. https://doi.org/10.1039/C7NP00052A

Brown, J. S., Amend, S. R., Austin, R. H., Gatenby, R. A., Hammarlund, E. U., & Pienta, K. J. (2023). Updating the definition of cancer. Molecular Cancer Research, 21(11), 1142–1147.

Chand, S., Lusunzi, I., Veal, D. A., Williams, L. R., & Karuso, P. (1994). Rapid screening of the antimicrobial activity of extracts and natural products. The Journal of Antibiotics, 47(11), 1295–1304.

Chin, Y. W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. The AAPS Journal, 8, E239–E253.

Chinnathambi, A., Salmen, S. H., Al-Garadi, M. A., Wainwright, M., & Alharbi, S. A. (2023). Marine actinomycetes: An endless source of potentially therapeutic novel secondary metabolites and other bioactive compounds. Journal of King Saud University–Science, 35(9), 102931.

Choi, H., & Oh, D. C. (2015). Considerations of the chemical biology of microbial natural products provide an effective drug discovery strategy. Archives of Pharmacal Research, 38, 1591–1605.

De Clercq, E. (2004). Antiviral drugs in current clinical use. Nature Reviews Drug Discovery, 3(9), 781–796. https://doi.org/10.1038/nrd1472

De Simeis, D., & Serra, S. (2021). Actinomycetes: A never-ending source of bioactive compounds—An overview on antibiotic production. Antibiotics, 10(5), 483.

Demain, A. L. (2014). Importance of microbial natural products and the need to revitalize their discovery. Journal of Antibiotics, 67(9), 661–663. https://doi.org/10.1038/ja.2014.109

Demain, A. L., & Vaishnav, P. (2011). Natural products for cancer chemotherapy. Microbial Biotechnology, 4(6), 687–699.

Douglas, A. E. (2022). Insects and their beneficial microbes. Princeton University Press.

Dube, P. N., Mokale, S. N., Shaikh, S. I., Patil, Y., Yadav, B., Deshmukh, P., & Sabde, S. (2015). Synthesis and molecular docking analysis of imidazol-5-one derivatives as anti-HIV NNRTIs. Pharmaceutical Chemistry Journal, 49(2), 125–131.

Duddu, M. K., & Guntuku, G. (2015). Isolation and partial characterization of Actinomycetes from a mangrove sediment sample. Journal of Global Biosciences, 4, 2921–2929.

Dogancı, M. A., Ay Sal, F., Guler, H. I., Katı, H., Ceylan, E., Belduz, A. O., ... & Canakcı, S. (2022). Investigation of potential inhibitor properties of violacein against HIV-1 RT and CoV-2 Spike RBD: ACE-2. World Journal of Microbiology and Biotechnology, 38(9), 161.

Elshafie, H. S., & Camele, I. (2022). Rhizospheric actinomycetes revealed antifungal and plant-growth-promoting activities under a controlled environment. Plants, 11(14), 1872.

Ertürk, Ö., Kati, H., Yayli, N., & Demirbağ, Z. (2006). Antimicrobial properties of Silene multifida (Adams) Rohrb. plant extracts. Turkish Journal of Biology, 30(1), 17–21.

Fahmy, N. M., & Abdel-Tawab, A. M. (2021). Isolation and characterization of marine sponge-associated Streptomyces sp. NMF6 strain producing secondary metabolite(s) possessing antimicrobial, antioxidant, anticancer, and antiviral activities. Journal of Genetic Engineering & Biotechnology, 19(1), 102. https://doi.org/10.1186/s43141-021-00203-5

Gaynes, R. (2017). The discovery of penicillin—new insights after more than 75 years of clinical use. Emerging Infectious Diseases, 23(5), 849.

Gousia, J., Ishfaq, S., Uqab, B., & Mudasir, S. (2023). Actinomycetes as biofertilizers for sustainable agriculture. In Microbiomes for the Management of Agricultural Sustainability (pp. 183–192). Cham: Springer Nature Switzerland.

Hili, P., Evans, C. S., & Veness, R. G. (1997). Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. Letters in Applied Microbiology, 24(4), 269–275.

Ibnouf, E. O., Aldawsari, M. F., & Waggiallah, H. A. (2022). Isolation and extraction of some compounds that act as antimicrobials from actinomycetes. Saudi Journal of Biological Sciences, 29(8), 103352.

Jadon, R., Pandey, S., & Singh, V. (2014). Screening of potential actinomycete isolates against selective pathogenic microorganisms. International Journal of Advanced Technology in Engineering and Sciences, 2, 631–642.

Jia, Z. C., Fang, H., & Jiang, L. (2021). Morphological description of the white grub Melolontha incana (Coleoptera: Scarabaeidae: Melolonthinae: Melolonthini). Microscopy Research and Technique, 84(5), 921–928.

Ji, H. F., Li, X. J., & Zhang, H. Y. (2009). Natural products and drug discovery: Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO Reports, 10(3), 194–200.

Kai, S., Matsuo, Y., Nakagawa, S., Kryukov, K., Matsukawa, S., Tanaka, H., ... & Hirota, K. (2019). Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinION™ nanopore sequencer. FEBS Open Bio, 9(3), 548–557.

Kaltenpoth, M., Roeser-Mueller, K., Koehler, S., Peterson, A., Nechitaylo, T. Y., Stubblefield, J. W., ... & Strohm, E. (2014). Partner choice and fidelity stabilize coevolution in a Cretaceous-age defensive symbiosis. Proceedings of the National Academy of Sciences, 111(17), 6359–6364.

Krishnan, K., Mani, A., & Jasmine, S. (2014). Cytotoxic activity of bioactive compound 1,2-benzene dicarboxylic acid, mono 2-ethylhexyl ester extracted from a marine-derived Streptomyces sp. VITSJK8. International Journal of Molecular and Cellular Medicine, 3(4), 246.

Kumar, N., Singh, R. K., Mishra, S. K., Singh, A. K., & Pachouri, U. C. (2010). Isolation and screening of soil Actinomycetes as a source of antibiotics active against bacteria. International Journal of Microbiology Research, 2(2), 12.

Kurtböke, D. İ. (2017). Ecology and habitat distribution of actinobacteria. In Biology and Biotechnology of Actinobacteria (pp. 123–149).

Lal, A., & Cheeptham, N. (2012). Starch agar protocol. American Society for Microbiology, 1, 1–9.

Lalitha, P., Veena, V., Vidhyapriya, P., Lakshmi, P., Krishna, R., & Sakthivel, N. (2016). Anticancer potential of pyrrole (1,2, a), pyrazine 1,4, dione, and hexahydro 3-(2-methyl propyl) (PPDHMP) extracted from a new marine bacterium, Staphylococcus sp. strain MB30. Apoptosis, 21, 566–577.

Lee, B., Lee, G. E., Hwang, G. J., Heo, K. T., Lee, J. K., Jang, J. P., Hwang, B. Y., Jang, J. H., Cho, Y. Y., & Hong, Y. S. (2023). Rubiflavin G, photorubiflavin G, and photorubiflavin E: Novel pluramycin derivatives from Streptomyces sp. W2061 and its anticancer activity against breast cancer cells. The Journal of Antibiotics, 76(10), 585–591. https://doi.org/10.1038/s41429-023-00643-w

Lee, W. S. (1976). Use of Mueller-Hinton agar as an amylase testing medium. Journal of Clinical Microbiology, 4(3), 312–312.

Lehman, D. (2005). Triple sugar iron agar protocols. American Society for Microbiology, 1–7.

Li, Q., Chen, X., Jiang, Y., & Jiang, C. (2016). Morphological identification of actinobacteria. In Actinobacteria – Basics and Biotechnological Applications (pp. 59–86).

Lynch, J. M., Brimecombe, M. J., & De Leij, F. A. (2001). Rhizosphere. eLS.

Madigan, M. T., & Jung, D. O. (2009). An overview of purple bacteria: Systematics, physiology, and habitats. In The Purple Phototrophic Bacteria (pp. 1–15).

Mahmoudi, F., Jalayeri, M. H. T., Montaseri, A., MohamedKhosroshahi, L., & Baradaran, B. (2024). Microbial natural compounds and secondary metabolites as immunomodulators: A review. International Journal of Biological Macromolecules, 134778.

Mattoso, T. C., Moreira, D. D., & Samuels, R. I. (2012). Symbiotic bacteria on the cuticle of the leaf-cutting ant Acromyrmex subterraneus subterraneus protect workers from attack by entomopathogenic fungi. Biology Letters, 8(3), 461–464.

Meenakshi, S., Hiremath, J., Meenakshi, M. H., & Shivaveerakumar, S. (2024). Actinomycetes: Isolation, cultivation, and its active biomolecules. Journal of Pure & Applied Microbiology, 18(1).

Mohamed, H. F., & Sun, X. (2023). Enhanced antiviral activity of isolated Streptomyces avermitilis extract in conjugation with gold nanoparticles. Egyptian Journal of Chemistry, 66(6), 1–11.

Muthusamy, S., Selvan, S. T., Arunachalam, P., & Grasian, I. (2019). Bioconversion and bioethanol production from agro-residues through the fermentation process using mangrove-associated actinobacterium Streptomyces olivaceus (MSU3). Biofuels, 10(2), 167–179.

Ngamcharungchit, C., Chaimusik, N., Panbangred, W., Euanorasetr, J., & Intra, B. (2023). Bioactive metabolites from terrestrial and marine actinomycetes. Molecules, 28(15), 5915. https://doi.org/10.3390/molecules28155915

Niemczyk, M., Karwański, M., & Grzybowska, U. (2017). Effect of environmental factors on occurrence of cockchafers (Melolontha spp.) in forest stands. Baltic Forestry, 23(2), 334–341.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Nützmann, H. W., Reyes-Dominguez, Y., Scherlach, K., Schroeckh, V., Horn, F., Gacek, A., ... & Brakhage, A. A. (2011). Bacteria-induced natural product formation in the fungus Aspergillus nidulans requires Saga/Ada-mediated histone acetylation. Proceedings of the National Academy of Sciences, 108(34), 14282–14287.

Othman, M., San Loh, H., Wiart, C., Khoo, T. J., Lim, K. H., & Ting, K. N. (2011). Optimal methods for evaluating antimicrobial activities from plant extracts. Journal of Microbiological Methods, 84(2), 161–166.

Pedrazzini, C. (2023). The population genetic structure of the European cockchafer Melolontha melolontha and its fungal pathogens Beauveria brongniartii and Beauveria pseudobassiana in an Alpine region (Doctoral dissertation, ETH Zurich).

Pérez-Corral, D. A., Ornelas-Paz, J. D. J., Olivas-Orozco, G. I., Acosta-Muñiz, C. H., Salas-Marina, M. Á., Berlanga-Reyes, D. I., ... & Rios-Velasco, C. (2022). Molecular, morphological, and biochemical characterization of actinomycetes and their antagonistic activity against phytopathogenic fungi. Revista Fitotecnia Mexicana, 45(1), 103–115.

Pommier, Y. (2010). DNA topoisomerases and cancer. Nature Reviews Cancer, 10(10), 654–670. https://doi.org/10.1038/nrc2910

Reuther, J., & Wohlleben, W. (2007). Nitrogen metabolism in Streptomyces coelicolor: Transcriptional and post-translational regulation. Journal of Molecular Microbiology and Biotechnology, 12(1–2), 139–146.

Saini, A., Aggarwal, N. K., Sharma, A., & Yadav, A. (2015). Actinomycetes: A source of lignocellulolytic enzymes. Enzyme Research, 2015(1), 279381.

Sezen, K. A. Z. I. M., Demir, Ý., & Demirbağ, Z. (2007). Identification and pathogenicity of entomopathogenic bacteria from the common cockchafer, Melolontha melolontha (Coleoptera: Scarabaeidae). New Zealand Journal of Crop and Horticultural Science, 35(1), 79–85.

Sezen, K., & Demirbag, Z. (2006). A new isolate of Melolontha melolontha entomopoxvirus in Turkey: Morphology, infectivity, and prevalence in the field. Applied Entomology and Zoology, 41(3), 471–477.

Selim, M. S. M., Abdelhamid, S. A., & Mohamed, S. S. (2021). Secondary metabolites and biodiversity of actinomycetes. Journal of Genetic Engineering & Biotechnology, 19(1), 72. https://doi.org/10.1186/s43141-021-00156-9

Sharma, M., Dangi, P., & Choudhary, M. (2014). Actinomycetes: Source, identification, and their applications. International Journal of Current Microbiology and Applied Sciences, 3(2), 801–832.

Shen, B. (2019). A new golden age of natural product drug discovery. Frontiers in Microbiology, 10, 1404. https://doi.org/10.3389/fmicb.2019.01404

Silva, L. J., Crevelin, E. J., Souza, D. T., Lacerda-Júnior, G. V., de Oliveira, V. M., Ruiz, A. L. T. G., Rosa, L. H., Moraes, L. A. B., & Melo, I. S. (2020). Actinobacteria from Antarctica as a source for anticancer discovery. Scientific Reports, 10(1), 13870. https://doi.org/10.1038/s41598-020-69786-2

Skowronek, M., Sajnaga, E., Pleszczyńska, M., Kazimierczak, W., Lis, M., & Wiater, A. (2020). Bacteria from the midgut of common cockchafer (Melolontha melolontha L.) larvae exhibiting antagonistic activity against bacterial symbionts of entomopathogenic nematodes: Isolation and molecular identification. International Journal of Molecular Sciences, 21(2), 580.

Smati, M., Bramki, A., Makhlouf, F. Z., Djebaili, R., Farda, B., Abdelhadi, F. Z., ... & Pellegrini, M. (2025). Isolation of Actinobacteria from date palm rhizosphere with enzymatic, antimicrobial, antioxidant, and protein denaturation inhibitory activities. Biomolecules, 15(1), 65.

Sullivan, D. C., & Chapman, S. W. (2010). Bacteria that masquerade as fungi: Actinomycosis/Nocardia. Proceedings of the American Thoracic Society, 7(3), 216–221.

Takahashi, Y., & Nakashima, T. (2018). Actinomycetes are an inexhaustible source of naturally occurring antibiotics. Antibiotics, 7(2), 45.

Tan, L. T., Chan, C., Chan, K., Pusparajah, P., Khan, T. M., Ser, H., ... & Goh, B. (2019). Streptomyces sp. MUM256: A source for apoptosis-inducing and cell cycle-arresting bioactive compounds against colon cancer cells. Cancers, 11(11), 1742.

Tiwari, K., & Gupta, R. K. (2013). Diversity and isolation of rare actinomycetes: An overview. Critical Reviews in Microbiology, 39(3), 256–294.

Tripathi, N., & Sapra, A. (2020). Gram staining.

Tunvongvinis, T., Jaitrong, W., Samung, Y., Tanasupawat, S., & Phongsopitanun, W. (2024). Diversity and antimicrobial activity of the tropical ant-derived actinomycetes isolated from Thailand. AIMS Microbiology, 10(1), 68.

Tunvongvinis, T., Jaitrong, W., Suriyachadkun, C., Sripreechasak, P., Tanasupawat, S., & Phongsopitanun, W. (2024). Streptomyces odontomachi sp. nov., a novel actinobacterium with antimicrobial potential isolated from ants (Odontomachus simillimus Smith, 1858). The Journal of Antibiotics, 77(11), 727–736.

Uyeda, M. (2004). Metabolites produced by actinomycetes are antiviral antibiotics and enzyme inhibitors. Yakugaku Zasshi, 128, 469–479.

Van der Meij, A., Worsley, S. F., Hutchings, M. I., & van Wezel, G. P. (2017). Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiology Reviews, 41(3), 392–416.

Verpoorte, R. (2000). Secondary metabolism. In Metabolic Engineering of Plant Secondary Metabolism (pp. 1–29). Dordrecht: Springer Netherlands.

Whitesell, L., & Lindquist, S. L. (2005). HSP90 and the chaperoning of cancer. Nature Reviews Cancer, 5(10), 761–772.

Wong, V. K., Turmezei, T. D., & Weston, V. C. (2011). Actinomycosis. BMJ, 343.

Woreta, D. (2015). Control of cockchafer Melolontha spp. grubs—a review of methods. Folia Forestalia Polonica, Series A (Forestry), 57(1).

Xie, F., & Pathom-Aree, W. (2021). Actinobacteria from the desert: Diversity and biotechnological applications. Frontiers in Microbiology, 12, 765531.

Yaman, M., Algı, G., Güner, B. G., Ertürk, Ö., Ünal, S., & Radek, R. (2016). First record, occurrence, and distribution of entomopathogens in populations of the European cockchafer, Melolontha melolontha (Coleoptera: Scarabaeidae), in Turkey. North-Western Journal of Zoology, 12(1), 192–195.

Zaorsky, N. G., Churilla, T. M., Egleston, B. L., Fisher, S. G., Ridge, J. A., Horwitz, E. M., & Meyer, J. E. (2017). Causes of death among cancer patients. Annals of Oncology, 28(2), 400–407.

CURRICULUM VITAE

In 2017, I graduated with a Bachelor of Science degree in Microbiology-Chemistry from Al-Azhar University, Egypt, where I earned a "Very Good with Honors." After completing my undergraduate studies, I pursued a Diploma in Microbiology at Suez Canal University, Egypt, starting in September 2018. I have worked at Mamiba Cosmetics for two years as a microbiologist in the quality control section. Then, after I worked for the Arma company for one and a half years as a quality control microbiologist, I later moved to Turkey three years ago to continue my studies in biotechnology. I am currently enrolled in the master's program in biotechnology at Karadeniz Technical University (KTU) in Trabzon, Turkey. This opportunity has allowed me to immerse myself in cutting-edge research and biotechnology applications.