République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université A. MIRA - Béjaia

Faculté des Sciences de la Nature et de la Vie
Département de Microbiologie
Spécialité Biotechnologie Microbienne

Réf :.....

Mémoire de Fin de Cycle
En vue de l'obtention du diplôme

MASTER

Thème

Evaluation du potentiel pharmacologique des actinobactéries isolées à partir d'un sol de décharge

Présenté par :
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Soutenu le : **13 Septembre 2022**

Devant le jury composé de :

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Année Universitaire: 2021 / 2022

Democratic and Popular Republic of Algeria
Ministry of Higher Education and Scientific Research
University A.MIRA-BEJAIA

Faculty of Nature Science and Life
Department of Microbiology
Microbial Biotechnology

Reference:.....

End of Cycle Dissertation

In view of obtaining the Master's degree

Theme

Evaluation of the pharmacological potential of actinobacteria strains isolated from discharged soil

Presented by:
Yahiaoui Sarah
Supported on: **13 Septembre 2022**

Ahead the jury composed of:

Mme. Laincer Firdousse	MCB	President
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Academic Year: 2021 / 2022

Dedication

To my mother who believed in me more than anyone ever did.

Acknowledgments

I would like to express my feelings of gratitude to all the people who participated in this work, who taught me an infinite number of things and who helped, advised and supported me at all times in order to carry out this work in the best conditions.

I would first like to thank Mrs. DJINNI Ibtissem, who has agreed to be my promoter, for her patience, support and most importantly her help and guidance. My Thanks go to Mrs. LAINCER for agreeing to chair the jury and Mrs. KERAMAN for agreeing to examine my work.

I would like to thank Mrs. YANAT and Mrs. SOUFI for their help during my internship.

I thank all the staff of the Mycology laboratory for allowing me to carry out the various tests and analyzes and for having made available to me the equipment and means necessary to carry out my work. Special thanks go to Mrs. KERRACHE Lydia, for her kindness and advices, I wish there was more people like her in this world.

I would like to express my feelings of gratitude to my mother who taught me perseverance, to my grandmother for her infinite love and support, thanks to my brothers, to my best friend Kenza whom I wish lots of success, thanks to my aunts Faroudja and Zoubida, my uncles Karim, Larbi, and Brahim, to my cousins Sabrina and Souad for their support, I'm extremely grateful to have you all in my life.

LIST OF ABBREVIATIONS

CFU : Colony-forming unit

DPPH : 2,2-diphenyl-I-picrylhydrazyl

FRAP : Ferric Reducing Antioxidant Power

GC : Guanine and Cytosine content

LPSN : The List of Prokaryotic names with Standing in Nomenclature

MH : Mueller-Hinton medium

MHB : Mueller Hinton Broth

MHBG : Mueller Hinton Broth Glucose

NIH : National Institute of Health

NP : Natural products

OD : Optic density

PCD : Programmed cell death

WHO : World Health Organization

Acknowledgments

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Introduction

In recent decades, we are confronted with the emergence of bacteria that are multidrug-resistant to commonly used antibiotics, and the toxicity of synthetic antioxidants (Hengzhuang et al., 2011; Penesyan et al., 2015). Free radicals induce oxidative damages to biomolecules such as lipids, proteins, and DNA. This damage is involved in the development of many diseases, such as cardiovascular diseases, atherosclerosis... etc (Valko et al., 2007; Ferreira et al., 2009; Cavar et al., 2012).

Biofilm-associated infections are usually difficult to treat as the body's immune system is incapable of penetrating the biofilms to eliminate the bacteria. Furthermore, the current available antibiotics are only effective against planktonic bacteria (Azman et al., 2019). According to a public announcement by the US National Institutes of Health (NIH), "Biofilms are medically important, accounting for over 80% of microbial infections in the body". Yet bacterial biofilms remain poorly understood and strategies for their control remain underdeveloped. Standard antimicrobial treatments typically fail to eradicate biofilms, which can result in chronic infections and the need for surgical removal of afflicted areas. The need to create effective therapies to counter biofilm infections presents one of the most pressing challenges in anti-bacterial drug development (Davies et al., 2003).

A recent publication in *The Lancet* shows that, in 2019, the global burden associated with drug-resistant infections was estimated at approximately 5 million deaths, of which antimicrobial resistance was the direct cause of around 1.3 million deaths (The lancet, 2022).

Antimicrobial resistance caused by microbes which are silently gaining power by getting resistant to most drugs, was already a global concern even before the Covid pandemic had arrived. That was evident by the fact that the World Health Organization (WHO) had named it a global priority for public health for the year 2020. Antimicrobial resistance is caused naturally but is compounded due to overuse of antibiotics, non-compliance with antibiotic doses, improper dumping of untreated chemicals from factories and hospitals in the environment, random dosing of antibiotics to livestock, and excessive spraying of pesticides on crops (Dailypioneer, 2022).

After the revolution in the "golden era", when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides, and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in

microbial resistance (Mayers et al., 2009). Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health (Guschin et al., 2015; Martin et al., 2015).

This has prompted scientists to look for other sources in search for effective natural molecules without any side effects. Among the sources studied of natural origin, the microbial world is one of the promising alternatives in this field.

Actinobacteria encompass a well-defined clade of high GC bacteria, the members of which have a wide range of niches. Representatives of the group can be found in various habitats, including soil, rhizosphere, marine, and freshwater ecosystems. (Ul-Hassan et al., 2009). Various ecological habitats have been reported as a potential source of highly useful natural products such as antibacterial, and antifungals (Rakotoniriana, 2006; Djinni et al., 2019).

Of all the *Actinobacteria* genera, the genus *Streptomyces* is represented in nature by the largest number of species. These species can produce a large number of antibiotics and active secondary metabolites (Thomas et al., 2010). Indeed, the activities described and associated with this genus are very diverse: antimicrobial, anti-inflammatory, cytotoxic, antiviral, antioxidant as well as many other activities.

Actinobacteria are potentially interesting for the discovery of new bioactive secondary metabolites (Hamed et al., 2013; Djinni et al., 2019). It is in this approach that this study is inscribed, which provides antagonist tests, in order to look for a pharmacological potential in *Actinobacteria* strains.

This study is organized around three parts: the first, presents the description of bibliographic data concerning *Actinobacteria*, their ecology and distribution in nature, their importance in different fields, and their useful bioactive molecules. The second chapter concerns the experimental part which is dedicated to highlighting the antibacterial and antifungal activities as well as antioxidant and antibiofilm activities of 4 strains of *Actinobacteria* isolated from discharged soils. The third and final part discusses the results obtained during this study followed by a general conclusion.

Chapter I
Bibliographic review

I. Actinobacteria

Actinobacteria from a cosmopolitan phylum that includes both rod-shaped and filamentous bacteria are one of the largest taxonomic units among the major lineages currently recognized within the bacteria domain (Van der Meij et al., 2017). They thrive in soil environments as well as in marine and freshwater ecosystems. Besides their success as free-living microbes, they are increasingly recognized as important interaction partners of higher eukaryotes (Barka et al., 2016).

Actinobacteria are highly versatile natural product (NP) producers. As producers of a wealth of secondary metabolites, they have the potential to produce chemically diverse and relevant metabolites, including two-thirds of all known antibiotics as well as many anticancer, antifungal, antitumor, and anti-inflammatory agents, along with plant-growth-promoting substances and regulators, and immunosuppressive agents, these bacteria are of utmost importance for human health, agriculture, and biotechnology. In addition to the industrially relevant enzymes (e.g., cellulases, chitinases, and xylanases) responsible for the production of biofuels and biochemicals (Barka et al., 2016, Djinni et al., 2019).

For almost a century, *Actinobacteria* have contributed significantly to the development of the antibiotic arsenal required for human health, they are responsible for the production of more than 70% of relevant anti-infective natural products. Antimicrobial agents have been the first isolated natural compounds, starting with actinomycin from *Streptomyces antibioticus* in 1940, followed by a significant number of antibiotics discovered in the so-called “golden age” corresponding to the period 1940s–1960s, when the production of about half of all known antibiotics is due to *Streptomyces* (Genilloud, 2017).

The genus *Streptomyces* with 1125 species (The List of Prokaryotic names with Standing in Nomenclature (LPSN), 2022) is the largest genus of *Actinobacteria* and is a natural inhabitant of soils and decaying vegetation. *Streptomyces* are characterized by its complex morphological differentiation and their ability to produce a variety of secondary metabolites (Polkade et al., 2016). Other relevant secondary metabolites produced by *Streptomyces* also exhibit many kinds of biological activities and are used as pharmacological agents and agrobiologicals (Chemoh et al., 2021).

Actinobacteria may be inhabitants of soil or aquatic environments (e.g., *Streptomyces*, *Micromonospora*, *Rhodococcus*, and *Salinispora* species), plant symbionts (e.g., *Frankia* spp.),

plant or animal pathogens (e.g., *Corynebacterium*, *Mycobacterium*, or *Nocardia* species), or gastrointestinal commensals (e.g., *Bifidobacterium* spp.) (Barka et al., 2016).

1. General properties of actinobacteria

Actinobacteria are Gram-positive filamentous bacteria with a high Guanine+Cytosine (GC) content in their genomes. They grow by a combination of tip extension and branching of filaments. They form a mycelium consisting of narrow hyphae is what gave them their name, which derives from the Greek words for ray (aktis or aktin) and fungi (mũkes) for "Ray Fungi". Traditionally, actinomycetes were considered transitional forms between fungi and bacteria. Indeed, like filamentous fungi, many *Actinobacteria* produce a mycelium, and many of these mycelial actinomycetes reproduce by sporulation. However, the comparison to fungi is only superficial: like all bacteria, actinomycetes cells are thin with a chromosome that is organized in a prokaryotic nucleoid and a peptidoglycan cell wall; furthermore, the cells are susceptible to antibacterial agents (Barka et al., 2016).

The class *Actinobacteria* comprises a heterologous group of procaryotes, branching filaments of less than 1 μm in diameter. However, fungi are eucaryotes and their filaments (hyphae) are always greater than 1 μm in width (Veteriankey. 2016).

The less evolved *Actinobacteria* have an incomplete mycelial development, which occurs only during active growth. However, most developed ones have two types of mycelium, in the substrate, the rhizoids; and outside substrate, the aerial mycelium. The *Actinobacteria*, which produce mycelium using this structure for attachment and penetration, can release enzymes that degrade essential compounds in order to obtain nutritional supplements (Araujo-Melo et al., 2019). Spores may be formed on the substrate and/or the aerial mycelium as single cells or in chains of different lengths. In other cases, spores may be harbored in special vesicles (sporangia) and endowed with flagella. Thus, in the genera *Micromonospora, Micropolyspora,* and Thermoactinomycetes, spore formation occurs directly on the substrate mycelium, whereas in *Streptomyces* the spores grow out from the aerial mycelium. The *Actinoplanes* and *Actinosynnema* groups are characterized by motile spores, while *Thermoactinomyces* has unique heat-resistant endospores (Barka et al., 2016).

This group of bacteria are aerobic microorganisms but some forms are optional aerobic or even anaerobic (Lechevalier, 1988), mesophilic. However, there are thermophilic species such as the genus *Thermoactinomyces*, whose optimal temperature is between 50 and 60 °C, and grow optimally in the pH range 5.0 to 9.0 with optimal proximity to neutrality (Williams

and Wellington, 1982; Goodfellow and Williams, 1983). They have a slow growth compared to other bacteria, the average generation time is about 2 to 3 hours (Beckers et al., 1982).

The *Actinobacteria* have a characteristic odor of "wet earth", which is related to volatile compounds produced by its secondary metabolism, such as geosmin. Besides that, feature intense metabolic activity, producing terpenoids, pigments and extracellular enzymes with which degrade organic matter of plant and animal origin producing secondary metabolites of economic importance (Araujo-Melo et al., 2019).

2. *Streptomyces* genus life cycle

The *Streptomyces* lifecycle starts with germination of a spore by growing one or two germ tubes which further develop into hyphae. The hyphae grow by branching and tip extension, thereby establishing a network of hyphae that jointly form the vegetative mycelium. In response to stresses such as nutrient depletion, a proportion of the mycelium is sacrificed, following autolytic degradation via programmed cell death (PCD); this leads to the release of nutrients in the environment which will be used for the formation of aerial hyphae and spores. The onset of cell differentiation coincides with antibiotic production, which provides protection against competing microorganisms attracted by the nutrients released during PCD (Van der Meij et al., 2017).

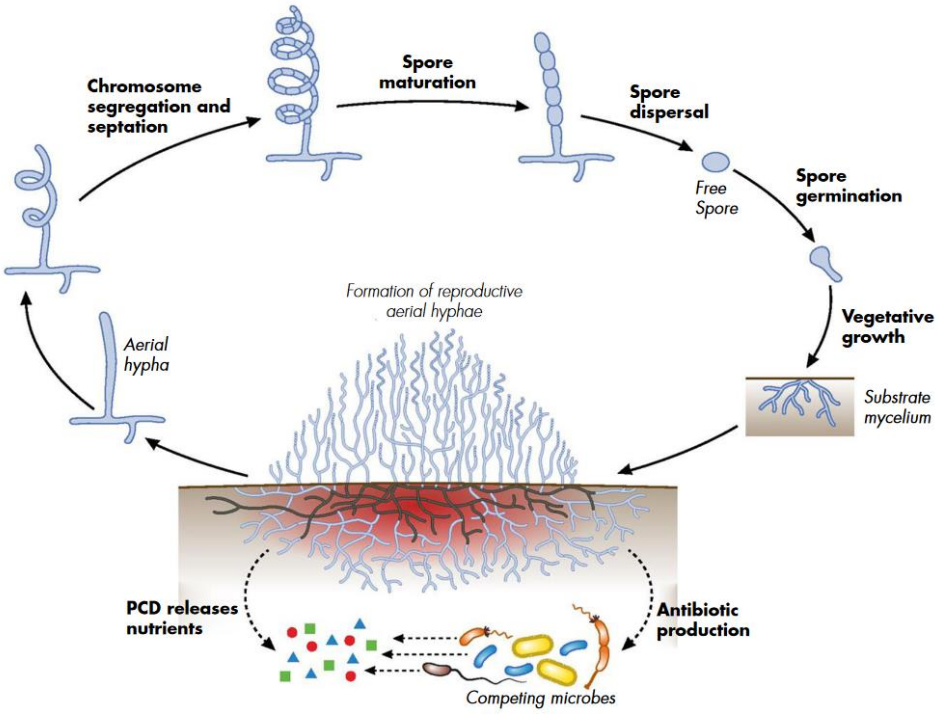


Figure 1: Streptomyces genus life cycle (Barka et al., 2016).

3. Ecology and distribution of actinobacteria in nature

Actinobacteria are a group of omnipresent bacteria that occur in the multiplicity of natural and synthetic environment. They are found in different niches such as soil, air, freshwater, oceans and on a variety of materials such as fertilizer, compost plant residues and food products (Table 1) (Kumar et al., 2003). They're almost everywhere in nature. They constitute an important part of the telluric microflora: 10% to 20% or sometimes more (Dommergues and Mangenot, 1970; Ishizawa and Araragi, 1976). Usually the genus *Streptomyces* is the one that predominate in the grounds and diverse other substrates. It represent 80% to 90% of total *Actinobacteria* (Lacey, 1973; Elwan et al., 1985). After *Streptomyces*, the most common genera are *Nocardia* and *Micromonospora* (Dommergues and Mangenot, 1970).
Other genera are only a small fraction and are sometimes infrequent or even quite rare.

Most *Actinobacteria* are saprophytic but a few can be pathogenic or symbionts of plants and animals (Suzuki et al., 1994). In general, *Actinobacteria* are heterotrophs, but several species are also capable of chemoautotrophic growth (Ensign et al., 1993).

Physiologically, it is possible to distinguish between aerobic forms, which are by far the most numerous, and anaerobic types found in animals and humans. *Actinobacteria* prefer a neutral or low alkaline pH, they are generally mesophilic, others are thermophilic tolerant of temperatures around 50 °C and can go up to 60 °C (Omura, 1992). *Actinobacteria* are widely distributed throughout the world. Their number depends on many factors, including the nature and abundance of organic matter, depth, pH, aeration and humidity (Theilleux, 1993).

Table 1: Habitats of selected *Actinobacteria* (Grigorova and Norris, 1990).

Actinobacteria	Habitats
Actinoplanes	Fresh water, plant litter, soil.
Frankia	Root nodules of non-legumes.
Micromonospora	Fresh water, sediments, wet soils.
Nocardia amarae	Activated sludge.
Rhodococcus coprophilus	Animal droppings, water, soil.
Saccharopolyspora rectivirgula	Hay mold.
Streptomyces	Soil, plant litter, water.
Thermoactinomyces	Compost.

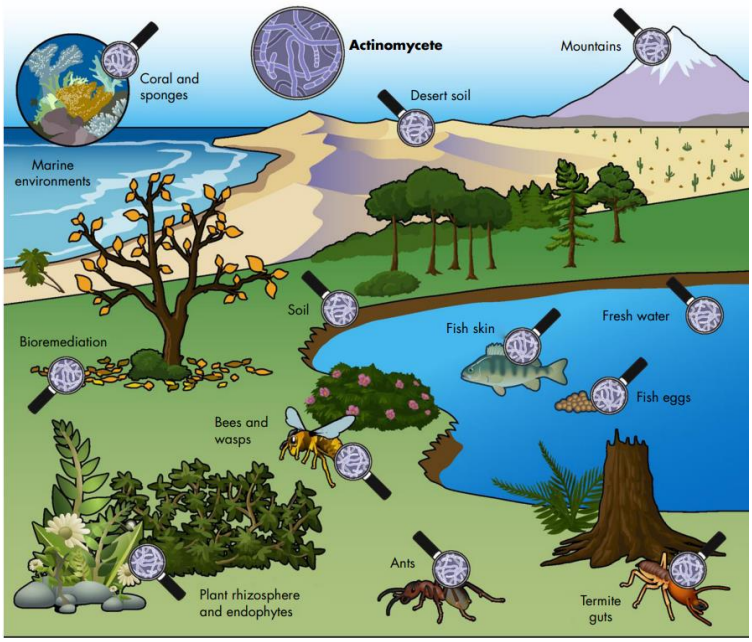


***Figure 2**: Ecology and distribution of Actinobacteria (Van der Meij et al. 2017).*

3.1. Actinobacteria in soils

Although the first strains of Actinobacteria were isolated from human and animal sources respectively by Cohn in 1875 and Nocard in 1888, the soil is certainly the richest reservoir, from which these microorganisms can invade many biotopes. They can be found as 10^6–10^9 cells per gram of soil (Goodfellow and Williams, 1983). They produce specific substances such as geosmin and 2-methyl isoborneol which is responsible for the humus odor characteristic of soils (Zaitlin et al., 2003). They are found in permanently frozen polar soils as well as in hot and dry desert soils, in soils highly contaminated with heavy metals, polluted by hydrocarbons and natural caves (Moncheva et al., 2002). In the Saharan soils of Algeria, *Streptomyces* constitute between 15 and 60% of all microorganisms and can even exceed 85% in the deep horizons of palm grove soils (Sabaou et al., 1992).

The most abundant actinomycetes in soil are *Streptomyces spp* (Lechevalier, 1964). Other genera have been isolated but at low and variable percentages (Table 2). Low numbers of actinobacteria have been recorded in Antarctica, acid peat, and saturated soils. In alkaline dry soils, their abundance is relatively high. Their presence is greatest in the first centimeters of the soil and reduced with depth (Arvind et al., 2003).

Table 2: Frequency of various genera of Actinobacteria in soil (Lechevalier et Lechevalier, 1967).

Genera	**Percentage of isolates**
Streptomyces	95,34
Nocardia	1,98
Micromonospora	1,4
Thermomonospora	0,22
Actinoplanes	0,20
Microbispora	0,18
Mycobacterium	0,14
Streptosporangium	0,10
Actinomadura	0,10
Micropolyspora	0,10
Pseudonocardia	0,06
Microellobosporia	0,04

3.2. Actinobacteria in aquatic ecosystems

Actinobacteria are present on the surface water, and in the deep sea, and also in sediments located more than 4000 m deep, with varying pH, temperature, salinity, and other extreme physical conditions. Marine *Actinobacteria* hold an important position due to their adaptation capability to various extreme conditions and resultantly the production of different compounds used for various applications (Khattabi et al., 2002 ; Djinni et al., 2013; Manivasagan et al., 2014 ; Sarkar et al., 2022).

Compared to terrestrial and freshwater habitats, *Actinobacteria* are less numerous in marine waters. Among several genera that have been isolated from the seas, we can mention *Streptomyces, Microbispora, Micropolyspora, Nocardia, Streptoverticillium, Thermoactinomyces*... (Silini, 2012).

Actinobacteria are also present in extremely alkaline lakes, salt lakes, however it seems that they are absent in very acidic mining waters (pH <1) and very hot thermal springs of volcanic origin (Lechevalier, 1981).

4. Importance of Actinobacteria

These microorganisms hold an extremely rich and diverse metabolism, producing secondary metabolites of extraordinary chemical variety, which attracted the attention of the biotechnology branch (Vicente et al., 2013) with applications in human medicine, animals, and agriculture (Tian et al., 2004; Ballav et al., 2012; Soares et al., 2012; Rao et al., 2015). The most studied and representative genera with this potential are *Microbispora*, *Micromonospora*, *Nocardia*, and *Streptomyces* (Oliveira et al., 2010).

Products from *Actinobacteria* include antibiotics, antifungals, extracellular enzymes, enzyme inhibitors, neurotransmitters, terpenoids, pigments, anti-tumor, plant growth promoters, pesticides, antioxidant molecules, among others (Franco-Correa et al, 2010; Tan et al., 2019). From 45% of composites with biological activities derived from filamentous Actinobacteria, approximately 80% of 7600 compounds are produced by *Streptomyces* sp. characterizing this genus as one of the most important in producing bioactive compounds (Berdy, 2005). Even with this metabolic diversity, only about 10% of the total number of natural products synthesized by these organisms were discovered (Watve et al., 2010).

4.1. In the veterinary and industrial medical fields

Actinobacteria have provided a considerable number of bioactive compounds of high commercial value, and are usually sought for the discovery of new bioactive substances (Vijayakumaret al., 2007). Antibiotics have also found applications in industrial animal farms. They are used not only to combat animal and plant diseases, but also in feeding to increase zootechnical yields (Khachaturians, 1998).

Secondary metabolites produced by species of *Streptomyces* genus presenting a biological activity of interest to human and animal health: antibacterial (streptomycin, tetracycline, chloramphenicol), antifungal (nystatin), antiviral (tunicamycin), antiparasitic (avermectin), immunosuppressive (rapamycin), antitumor (actinomycin, mitomycin C, anthracyclines), enzyme inhibitor (clavulanic acid) (Demain, 2000).

4.2. In ecology

The ecological roles of *Actinobacteria* in plant biomass degradation, in soil, in compost, and in association with animals have selected for the evolution of high cellulolytic abilities. Free-living *Actinobacteria* contribute to carbon cycling in other environments enriched in decaying plant material, including leaf litter, compost, and manure (Lewin et al., 2016).

Actinobacteria are also implicated in the cycling of a variety of other carbon sources. This includes chitin, the second most abundant organic carbon source in nature. As with cellulose, few organisms can degrade chitin, but some *Actinobacteria*, including most *Streptomyces* and *Arthrobacter* species, have this ability (Lewin et al., 2016).

Bioremediation, can be defined as "the use of living organisms to clean up pollutants from soil, water, or wastewater" (EPA, 2016). Currently, members of the phylum *Actinobacteria* are being studied for their efficacy in the bioremediation of pesticides and heavy metals (Polti et al., 2014; Aparicio et al., 2015).

4.3. In biotechnology

Actinobacteria are excellent producers of enzymes with multiple biotechnological applications (Table 3) (Tanaka and Omura, 1990; Vonothini et al., 2008). They are able to produce various types of biosurfactants that have antibacterial activity and play an important role in bioremediation (Voytsekhovskaya et al., 2018).

Enzymes carry extremely interesting thermostability properties and good activity in a wide range of pH, and their solvent tolerance potentials made it a clear choice for industrial processes. These special properties of the enzymes have turned beneficial in terms of medical and biotechnological perceptions. The enzymes such as kinases, nucleases, and polymerases produced by *Streptomyces thermoviolaceus* can retain their properties in adverse conditions like the presence of high detergent concentrations. Cellulase recovered using *Thermomonospora fusca* was used for degradation of cotton and Avicel. Their applications are not only limited for biotechnological perspective but also inevitable for economical production due to the use of cheap substrates like fruit peels, wheat straw, etc. (Kontro et al., 2021). **(Table 1, Annex I).**

4.4. In the pharmaceutical fields

Actinobacteria are a rich and tremendous source for screening of novel metabolites with potential pharmaceutical applications (Voytsekhovskaya et al., 2018). Many bioactive compounds with various biological activities have been isolated from *Actinobacteria* as molecules with various activities such as antibiotics, antifungals, anticancer, antitumors, anti-inflammatories, antioxidants... etc (Lam, 2006). Twenty-nine new species and one novel genus have been isolated, mainly from the Algerian Saharan soil and palm groves, where 37.93% of the most abundant genera belong to *Saccharothrix* and *Actinopolyspora*. Several of these strains were found to produce antibiotics and antifungal metabolites, including 17 new

molecules among the 50 structures reported, and some of these antibacterial metabolites have shown interesting antitumor activities (Djinni et al, 2019).

4.5. In agronomic fields

In addition to the production of a large number of commercially important metabolites, *Actinbacteria* make up a high percentage of soil microbial biomass. They have the ability to produce a wide variety of extracellular hydrolases, which confer a role in the decomposition of organic matter in the soil. In addition to their highly active decomposition function, *Actinobacteria* appear to have importance among the microflora of the rhizosphere (Valois, 1996). The genus *Frankia* is well known in forestry for its role in the fixation of symbiotic atmospheric nitrogen in the root nodules of certain dicotyledonous trees (other than legumes) such as casuarina, elm, alder, etc. (Becking, 1974).

Their pronounced antagonistic power gives them a role in the ecological distribution of microorganisms and in the biological control of certain soil plant pathogens (Goodfellow and Williams, 1983). *Actinobacteria* play an important role in the recycling and biodegradation processes of organic matter and mineral elements and thus contribute to soil fertilization (Goodfellow et al., 1984). They have a great power to transform complex organic substances that are difficult or not degradable by other microorganisms, such as complex polymers, polysaccharides, lignocelluloses, chitin, etc. (Lechevalier, 1981; Goodfellow and Williams, 1983).

5. Secondary metabolites

Secondary metabolites are synthesized during the end or near the stationary phase of growth and are not involved in cell growth, development, or reproduction (Abdel-Aziz et al., 2017). Secondary metabolites are produced by microorganisms when one or more of the nutrients in the culture media is depleted. Many of the identified secondary metabolites have a role in ecological function, including acting as a defense mechanism(s), by serving as antibiotics and by producing pigments. The composition of culture media also can affect the type of secondary metabolites produced by microorganisms (Djinni et al., 2018; Djinni et al., 2022). **(Table 2, Annex I).**

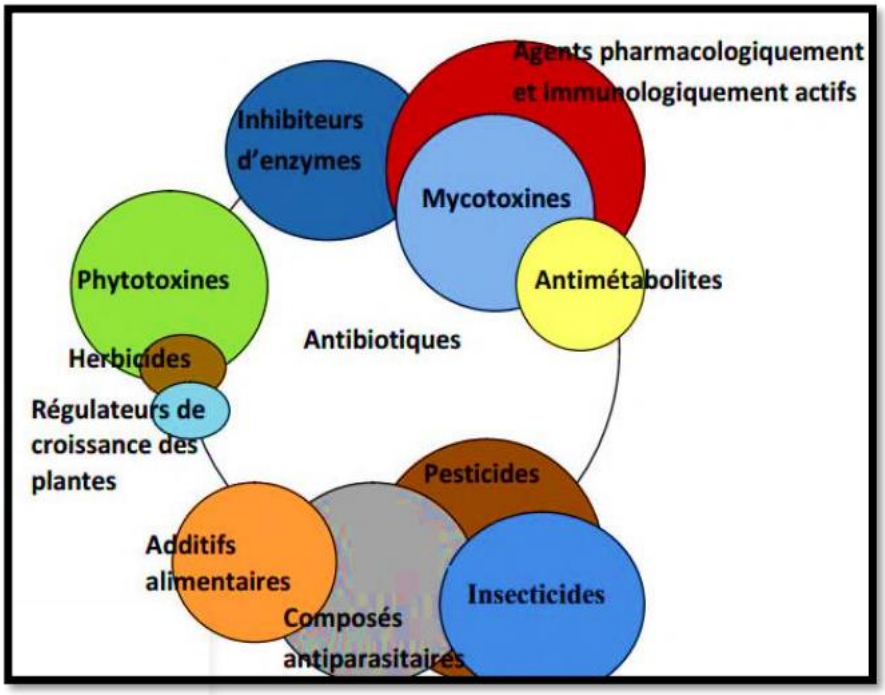


***Figure 3**: Different secondary metabolites of Actinobacteria (Conn, 2005)*

6. Antimicrobial compounds

Over 1 million natural compounds are available in the world, among which 5% are of microbial origin (Das et al., 2018). Microbial antagonists are widely used in the biological control of plant pathogenic fungi. The antagonistic activity of *Streptomyces* against fungal pathogens is generally related to the production of extracellular antifungal compounds and hydrolytic enzymes (Prapagdee et al., 2008). In addition to *Streptomyces*, other genera belonging to *Actinobacteria* are also producers of molecules with antifungal activities (Boar and Trujillo, 1997). For example, ramicidins are antifungals produced by a strain of *Actinomadura bibisca* (Tomita et al., 1990).

Secondary metabolites produced by the phylum *Actinobacteria* can be used not only as antibiotics, but also as herbicides, antifungal, antititumor or immunosuppressant and anthelmintic agents (Gohain et al., 2020).

Table 3: Some examples of antibiotics produced by *Actinobacteria* (Loucif, 2011).

Actinobacteria producers	**Antibiotics**
1. Antibacterial agents	
Micromonospora sp.	Clostomycin.

Streptomyces griseus.	Candicidin.
Streptomyces lydicus.	Streptolydigin.
Streptomyce lindensis.	Retamycin.
Marinispora sp.	Marinomycin.
Verrucosispora sp.	Abyssomycin.
2. Antifungal agents	
Streptomyces griseochromogenes.	Blasticidin.
Streptomyces humidus.	Phenylacetate.
Nocardia transvalensis.	Transvalenecin.
Streptomyces nodosus.	Amphotericin B.
3. Bioherbicides and bioinsecticides produced by actinomycetes	
Saccharopolyspora spinosa.	Spinosad, Insecticide, Neurotoxic.
Actinomadura sp.	Herbicides.
Streptomyces hygroscopicus.	Herbimycin.

7. Antioxidant compounds

Actinobacteria produce a large number of unique bioactive compounds such as natural antioxidant molecules (**Table 4**) (Hassan et al., 2017).

Table 4: Examples of antioxidant molecules produced by some species of *Actinobacteria*.

Molecules	**Species**	**References**
Ageloline A	*Streptomyces* sp.	Cheng et al. (2016).
5-(2,4-dimethylbenzyl) pyrrolidin-2-one	*Streptomyces sp.*	Sauvar et Kannabiran, (2011).
2-allyoxyphenol	*Streptomyces sp.*	Arumugam et al. (2010).
Protocatechualdehyde	*Streeptomyces lincolnensis.*	Jakim et al. (2008) .
Streptopyyrolidin	*Streptomyces sp.*	Shin et al. (2008).
JBIR-94 et JBIR-125	*Streptomyces sp.*	Kawahara et al. (2012).

5-(2,4-dimethylbenzyl)-2-one (DMBPO)	*Streptomyces sp.*	Saurvar et Kannabiran et al. (2012).

8. Biofilm and antibiofilm compounds

Biofilms can be found widely in nature (glaciers, hot vents, rocks), industrial process (cosmetic, food), and aquaculture. It can also be found in the human body, either in human tissues or on body surfaces as well as on medical devices and implants (catheters, mechanical cardiac valves, and prostheses which may lead to life-threatening biofilm-associated infections. Biofilms have been found to be involved in at least 80% of the microbial infections in the body including Gram-positive pathogens (*Staphylococcus aureus, Enterococcus faecalis),* and Gram-negative pathogens (*Pseudomonas aeruginosa, Escherichia coli*), and yeast such as *Candida albicans* (Azman et al., 2019).

A biofilm is a community of microorganisms attached to a surface and embedded in a matrix of extracellular polymeric substances. Biofilms confer resistance towards conventional antibiotic treatments; thus, there is an urgent need for newer and more effective antimicrobial agents that can act against these biofilms. Due to this situation, various studies have been done to investigate the antibiofilm effects of natural products including bioactive compounds extracted from microorganisms such as *Actinobacteria*. Most bacteria can detect changes in the conditions of the surrounding environment and adapt to survive in the new environment. The formation of biofilm is an evolutionary adaptation of bacteria to enable survival in unfavorable environmental conditions (Azman et al., 2019).

Studies have shown that most of *Streptomyces* species exhibits anti-biofilm activities (Azman et al., 2019).

Chapter II
Materials & Methods

II. Materials and Methods

This work was carried out in the Mycology Laboratory of the A. Mira of Bejaia University during the period from 11/04/2022 to 17/06/2022.

In the following, the materials and methods of the main parts of this work relating to the study of the morphological characteristics of actinobacteria strains and the extraction of active molecules will be discussed, as well as the demonstration of their respective antimicrobial and antioxidant and anti-biofilm activities.

1. Analytical material

The material used in this study is reported in the annex II.

2. Biological material

2.1. Actinobacteria strains

Four actinobacteria strains were kindly provided by BELABBAS Hanane, a PhD student at Laboratory of Applied Microbiology (LMA). These actinobacteria were isolated from soil samples collected in the public landfill on Boulimate and PET bottle surface from the shore of Sidi Ali Lebhar, purified, and then preserved by freezing and underwent revivification by several successive subculturing on the Gauss medium followed by incubation at 28 °C for 10 days.

Table 5 gives the studied strains and their origin.

Table 5: Origin of the studied actinobacteria strains.

Actinobacteria strains	Isolated ecosystems
YS1	Sidi Ali Lebhar
YS15	Boulimate
YS56	Boulimate
YS155	Boulimate

2.2. Target germs

The antagonistic activity of the four actinobacteria strains was tested against a pathogenic germs listed on the following Table 6, provided from the collection of the Laboratory of Applied Microbiology.

Table 6: Target germs used in the antagonistic activity evaluation

Bacteria	**Fungi**
Bacillus cereus	*Aspergillus flavus*
Bacillus subtilis	*Aspergillus niger*
Staphylococcus aureus	*Penicillium sp*
MRSA	
Escherichia coli	
Salmonella Typhi	
Pseudomonas aeruginosa	
Klebsiella pneumoniae	
Acinetobacter baumanii	
Enterobacter sp.	
Vibrio cholerae	

3. Morphological study of actinobacteria strains

3.1. Macromorphology

The 4 strains of actinobacteria were seeded in tight streaks on the surface of Gauss medium for YS1, YS15, and YS56, and of ISP4 medium for YS155. This study consists in determining the color of the aerial and substrate mycelia as well as the soluble pigments production.

3.2. Micromorphology

The micromorphological study is essential for the recognition of the genera. The strains were observed on the culture mediums mentioned above, using a Zeiss optical microscope, with magnifications (10x10) and (10x40). It consists in seeing the structures in place and the characteristic sporulation of the strains, as well as the fragmentation or not of the substrate mycelium.

4. Antimicrobial activity evaluation of actinobacteria strains isolated from a discharge ground

4.1. Standardization of the inoculum of target germs

Bacterial and fungal cultures aged 24h and 72h, respectively, seeded on a nutrient agar were used. 3 to 4 colonies are taken with a sterile Pasteur pipette and introduced into tubes containing 5mL of physiological sterile water, the cell load was subsequently adjusted by dilution so as to obtain a concentration of 10^7 CFU / ml. After rigorous agitation at the vortex, the suspension is seeded by swabbing on the Muller-Hinton medium (MH) **(Annex III)**.

4.2. Agar cylinders method

Isolated and cultivated actinobacteria strains obtained from the two different ecosystems were initially screened for bioactivity using a cylinder agar assay **(Figure 4)** (Bastide et al. 1986) against a standard Gram-positive and Gram-negative test bacteria and fungal strains: listed in table 2 above. The purified actinobacteria strains were grown for 7-10 days in gauss and ISP4 culture medium at 28° C. After the strains were grown properly with well-established mycelia, agar cylinders (6 mm diameter) were formed and shifted to the surface of Mueller-Hinton plates uniformly inoculated with a lawn of the test bacterial strains on the agar surface (10^7 CFU/mL). The cylinders were then pressed lightly on the agar surface in equidistant positions and kept at 4°C for 30 minutes then incubated for 24h at 37°C. Zones of inhibition were noted by measuring the diameter (in mm) of the clearing zones around the cylinders.

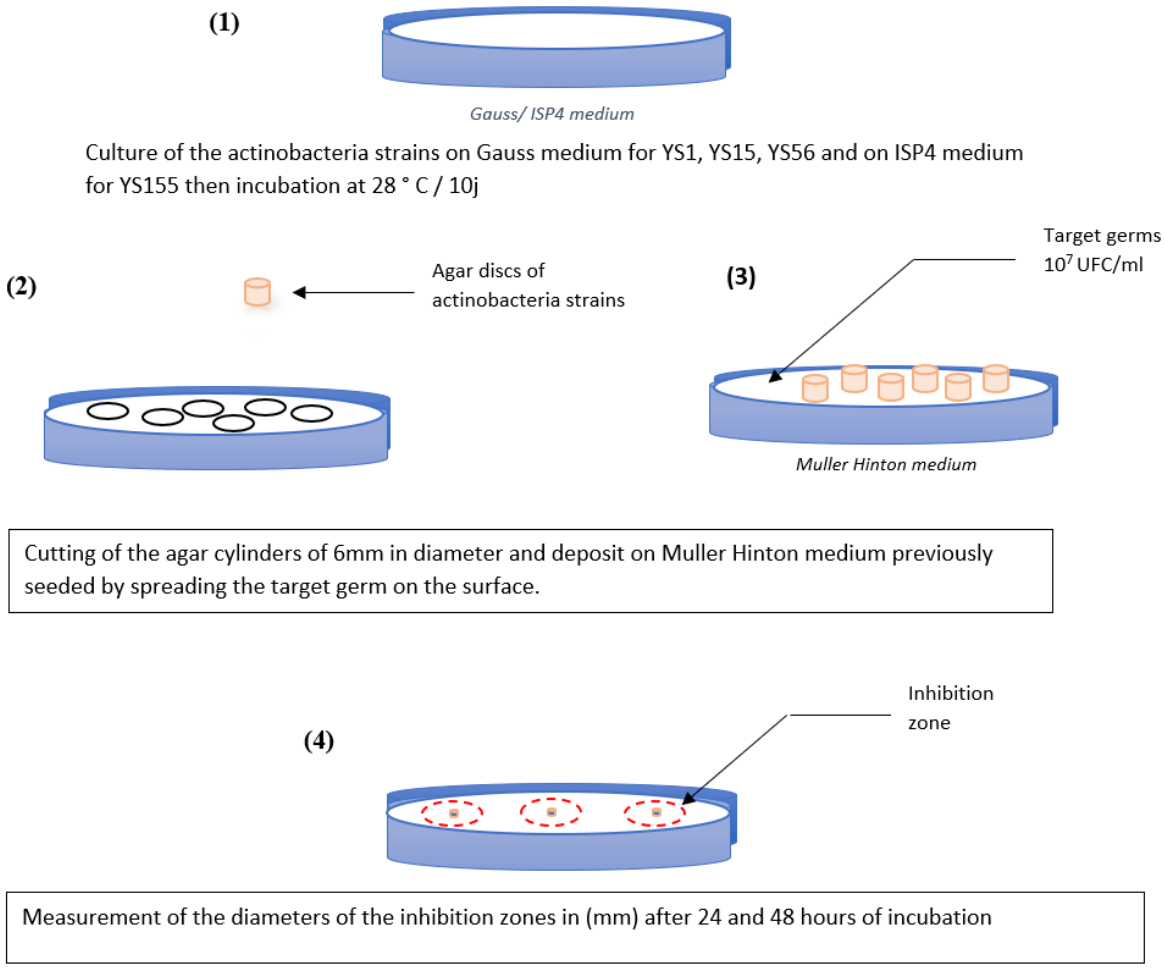


Figure 4 : Demonstration of the antibiotic activity of actinobacteria isolates on Mueller-Hinton medium by the agar cylinder method (Bastide et al., 1986).

4.3. Agar well diffusion method

4.3.1. Extraction of active molecules and culture of the strains

In order to obtain the crude extracts of the four strains necessary for our study, a solid medium culture was undertaken. Indeed, the strains are seeded in tight streaks on the surface of their corresponding mediums, YS1, YS15, and YS56 on gauss medium, YS55 on ISP4 medium, with 35 Petri dishes for each strain. The plates are incubated at 28 °C for 7 days.

After culture, the extraction of the active molecules is carried out by maceration. For this, the mycelia and agars are cut into small pieces of 1 cm^2 and then introduced into an Erlenmeyer's

with the addition of 500 ml of ethyl acetate **(Figure 5).** The mixture is macerated overnight, away from light at room temperature. The crude extract is obtained after filtration through Whatman Paper N°= 1 to separate the solvent from the agar blocks and mycelium, then evaporated dry using a Rotavapor. The dry extract obtained is then recovered in 7ml of methanol and the mass concentration is determined. The extract is stored at 4°C for later use.

Standardization of the inoculum of target germs (review page 17). The target germs were spread on plates, then holes were bored (6mm diameter). Each hole was filled with 50µl of each solvent extracts of the strains.

Inhibition zones were measured after 24 h incubation at 37 °C.

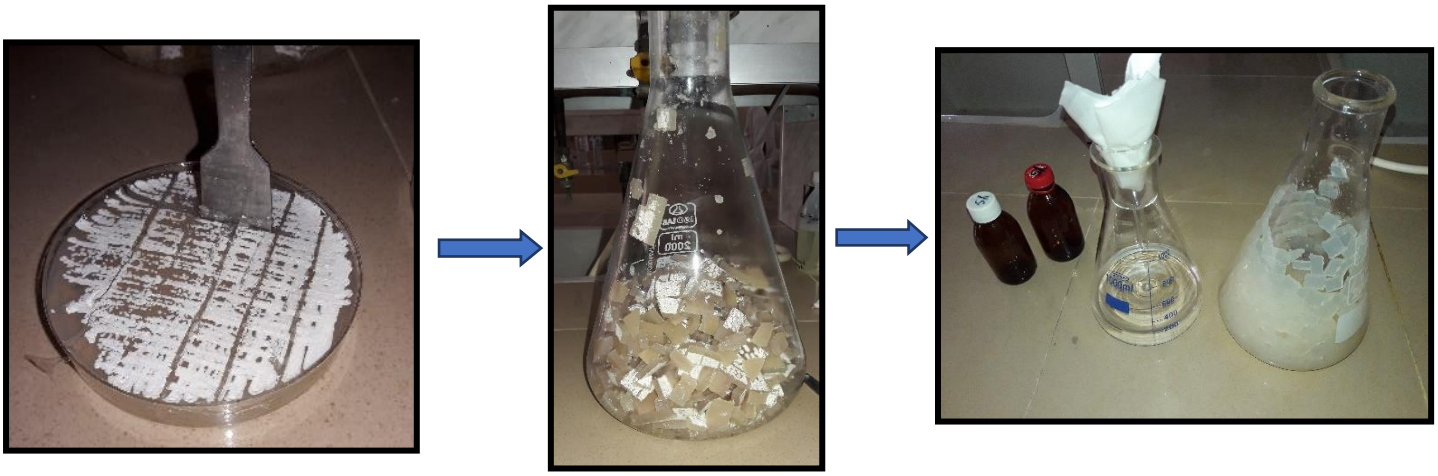


Figure 5 : The method used for extraction with maceration

5. Determination of total polyphenols

5.1. The principle of the method

The Folin-Ciocalteu reagent consists of a mixture of phosphotungstic acid ($H_3PW12O40$) and phosphomolybdic acid ($H_3PMO12O40$). It is reduced by phenols to a mixture of blue oxides of tungsten and molybdenum (Junaid et al., 2013). The staining produced is proportional to the number of polyphenols present in the extracts (Boizot and Charpentier, 2006). The procedure the determination of the total polyphenols present in the extract is carried out by the Folin-ciocalteu method described by (Kahkönen et al., 1999).

5.2. The operating mode

200µl of extract are mixed with 1000µl of the Folin-Ciocalteu reagent. The mixture is stirred for 3 minutes and then added 800µl of sodium carbonate (7.5%). After 30 minutes of incubation, absorbance is measured at 725nm. The phenolic compounds content is expressed in mg gallic acid equivalent per 100g dry matter (mg EAG/100 g DM), by reference to a calibration curve **(Figure 1, Annex IV).**

6. Study of antioxidant activity

6.1. Determination of DPPH free radical (2,2-diphenyl-I-picrylhydrazyl) scavenging

6.1.1. The principle of the method

DPPH radical (2,2-diphenyl-I-picrylhydrazyl) is one of the most stable organic nitrogen radicals, which bears a purple color. It is a colorimetric method based on color loss at 515 nm due to DDPH reduction. The concentration of antioxidants is proportional to the decrease in absorbance due to the decrease in the intensity of the staining of the DPPH solution. DPPH, a purple-colored free radical, is reduced to a yellow compound in the presence of anti-radical compounds (Molyneux, 2004). The DPPH method (1,1-di-phenyl-2-picrylhydrazyl radical) is used to determine the ability of extracts to yield protons and/or electrons in order to neutralize radicals. The anti-radical activity of crude extracts is evaluated according to the Lesage-Meessen et al. (2001) method.

6.1.2. The operating mode

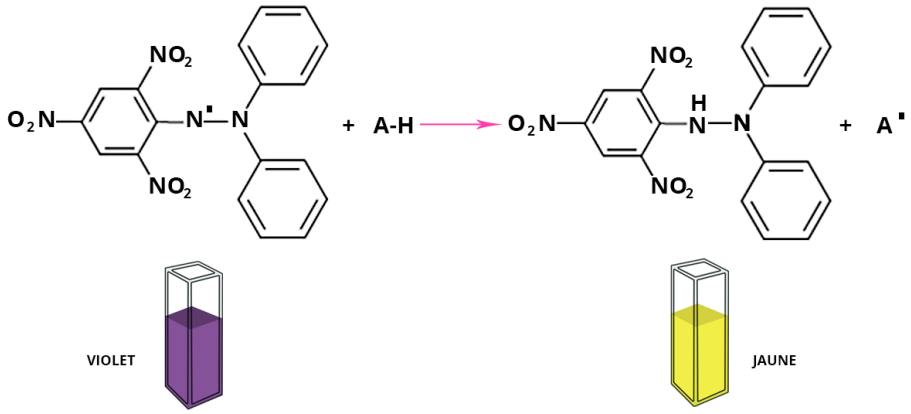
A volume of 100 µl of extract is added 900 µl of the DPPH solution. The stirred mixture is incubated in the dark for 30min and the absorbance is measured at 517 nm. The reduction of DPPH radicals implies a decrease in absorbance. The anti-radical activity expressed in mg ascorbic acid equivalent/100g dry matter (mg EAA/100g MS) is calculated by reference to a calibration curve **(Figure 2, Annex IV).**

$$\mathbf{PI\%} = [(\frac{\text{Abs T} - \text{Abs Ech}}{\text{Abs T}}) \mathbf{X\ 100}$$

Where:

Abs T: Control absorbance after 30 min. Abs

Sample: Extract absorbance after 30 min.



6 Figure: A reaction of an antioxidant and a DPPH radical (ChimActiv).

6.2. Ferrozine assay

6.2.1. The principle of the method

Ferrozine can form a complex with a red color by forming chelates with Fe^{2+}. This reaction is restricted in the presence of other chelating agents and results in a decrease of the red color of the ferrozine-Fe^{2+} complexes. Measurement of the color reduction determines the chelating activity to compete with ferrozine for the ferrous ions (Soler-Rivas et al., 2000).

The chelating capacity of extracts is measured by following inhibition of fe^{2+}-ferrozine complex formation after incubation of samples with divalent iron according to the method of Wang et al. (2008).

6.2.2. The operating mode

A volume of 250 µl of extract is supplemented with 25 µl of ferrous chloride (2 mM) and 0,8 ml of methanol. The mixture is staged and then incubated in the dark at room temperature for 5 min; 50 µl ferrozine (5 mM) are added to the reaction mixture; after 5 min of incubation, the absorbance of the Fe^{2+}–ferrozine complex is measured at 562 nm. The sequestering effect of crude extracts against iron is expressed as a percentage according to the following equation:

$$\text{Chelating capacity (\%)} = [1-(A_1 - A_2)/A_0] \times 100$$

A0: Absorbance in the absence of extract;

A1: Absorbance in the presence of the extract;

A2: Absorbance without ferrozine.

6.3. Ferric reducing antioxidant power (FRAP)

6.3.1. The principle of the method

At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593nm. The reaction is non specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half-reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is, therefore, directly related to the combined or "total" reducing the power of the electron-donating antioxidants present in the reaction mixture (Mayank et al., 2009). The reducing power is estimated by the method of Gülçin et al. (2002).

6.3.2. The operating mode

A volume of 350 µl of extract are mixed with 350 µl of phosphate buffer (0.2M; pH6.6) and 350 µl ferricyanide potassium (1%). After incubation, at 50 ° C / 20min, 350 µl of trichloroacetic acid (10%) are added to the mixture; after 5min, a volume of 300 µl of ferric chloride (0.1%) were

added and the absorbance is measured at 700nm. The reducing power of the extracts is expressed in mg ascorbic acid equivalent per 100g of dry matter (mg EAA/100g MS) **(Figure 3, Annex IV).**

7. Antibiofilm activity evaluation

The detection of antibiofilm activity is done in several steps according to a well-established protocol using flat-bottomed microplates containing 96 wells. The test used is based on the formation of biofilm at the solid-liquid interface (Driche et al., 2017). It should be noted that the choice of target germs used have been selected for their good results in antibacterial activity in previous tests.

Day1:

- The germs have been inoculated in 10ml of Muller Hinton Broth (MHB) supplemented with glucose to 2,5% Muller Hinton Broth Glucose (MHBG) then les suspensions has been incubated at 37°C for 24H with agitation.

Day2:

- The inoculum has been standardized at 10^8 UFC/ml in the medium MHBG.
- A volume of 200µl of the bacterium suspension has been distributed in each well of the microplate.
- Four crude extracts were prepared with their respective concentrations YS1 (9,42mg/ml); YS15 (17,85mg/ml); YS56 (14,28mg/ml); YS155 (3,71mg/ml), distributed in a row of wells containing the bacterium suspension.
- A row containing only MHBG is used like a negative control and an other one containing MHBG + the inoculum without crude extract is used like a positive control.
- The microplate has been incubated at a 37°C for 24H.

Day3:

- The content in the microplate has been removed then washed with 350µl of sterile water 3 time to eliminate planktonic bacteria.
- The microplate has been incubated in the incubator at 60°C for 45min to fix the biofilm.

- A volume of 200µl of Crystal violet at 0,2% has been added to the microplate and left at room temperature for 15min.
- Washing with sterile water has been done 3 times in order to eliminate every trace of the not fixed color.
- A volume of 150µl of ethanol solution at 95% has been added in order to free the color fixed within the cells imprisoned thus formed.
- Optic density (DO) of all wells is determined by a microplate reader BIOTEK at a wavelength of 630 nm **(Figure 7).**

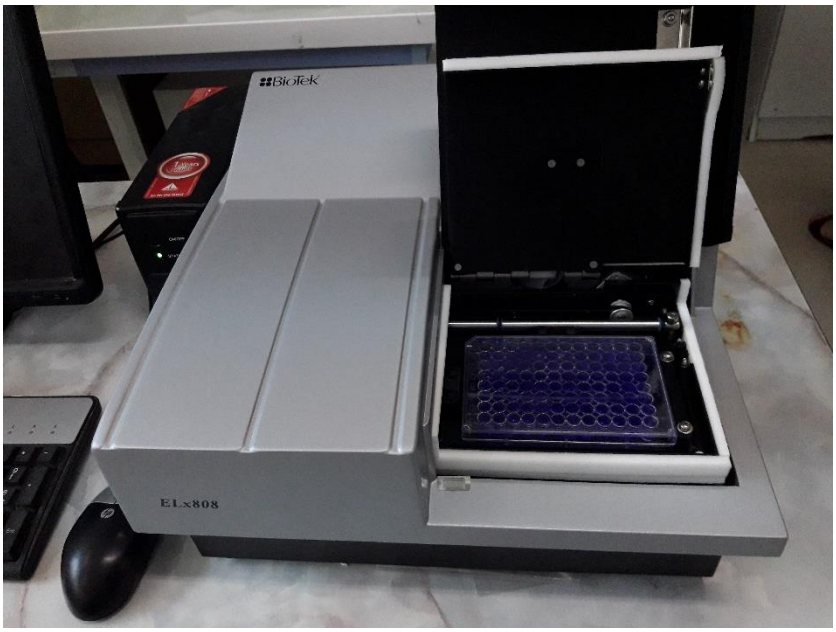


Figure 7: Microplate reader BIOTEK used for the lecture of the microplate.

Chapter III
Results & Discussion

III. Results and discussion

The results of this study will be presented and discussed in this chapter which includes, in the first step, the demonstration of the antagonistic potential of the four studied actinobacteria strains, and the evaluation of their biological activities through the antibacterial, antifungal, antibiofilm and antioxidant potential will be addressed.

1. Study of morphological characteristics of actinobacteria strains

1.1. Macromorphological features

The study of the morphological characteristics of the four-strains have been conducted on their respective culture media:, YS1, YS15, YS56 on Gausse medium, and YS155 on ISP4 for 7days at 28°C. The obtained results are presented in the following **Table 7.** Figure 8 illustrates the macromorphological aspects of the strains on Gausse and ISP4 culture media.

Table 7 : Macromorphological characteristics of strains YS1 YS15 YS56 YS155 after 7 days of incubation at 28 ° C.

Character / **Strains**	**Growth**	**Color of aerial mycelium (MA)**	**Production of diffusible Pigments**	**Appearance of colonies**
YS1(Gausse)	++	White	-	Smooth colonies with chalk-like aspect
YS15 (Gausse)	++	Cream white	-	Smooth colonies with chalk-like aspect
YS56 (Gausse)	++	Cream white	-	Smooth colonies with chalk-like aspect
YS155 (ISP4)	++	Green cream	-	Rough colonies

+ Very good growth; + average growth - No pigment production

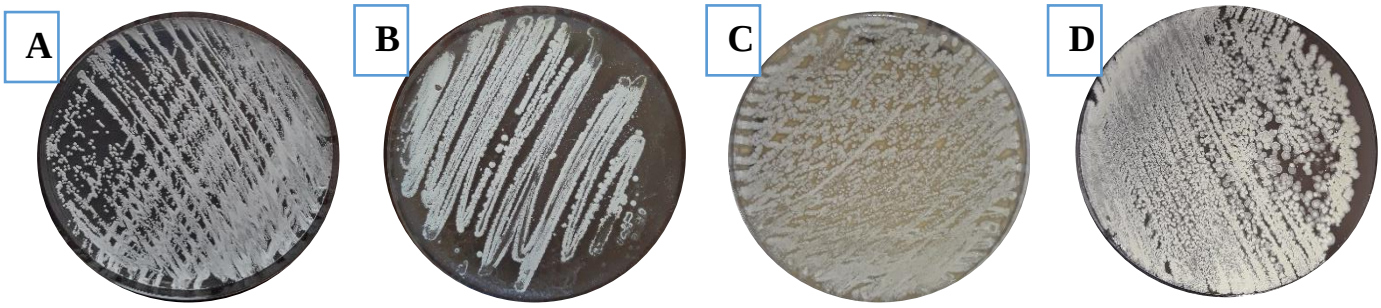


Figure 8: Macromorphology characteristics of actinobacteria strains from left to the right respectively; YS1 strain on Gausse medium. YS15 strain on Gausse medium. YS56 strain on Gausse medium. YS155 strain on ISP4 medium, after 7 days of incubation at 28°C.

1.2. Micromorphological characters

The petri plates containing the Gausse and ISP4 culture media used for the macromorphological study of the four isolates were also the subject of a micromorphological study. The colonies are directly observed on petri plates under optical microscope with x40 lens after 7 days of incubation at 28 ° C. Figures 9, 10 and 11 below illustrate the micromorphologies of the four strains observed under an optical microscope.

The filaments of the aerial mycelium's of the strains are branched, straight having immobile spore chains. All other sporophores, sporangia, mobile spores, synnemata and sclerotia are absent in all isolates.

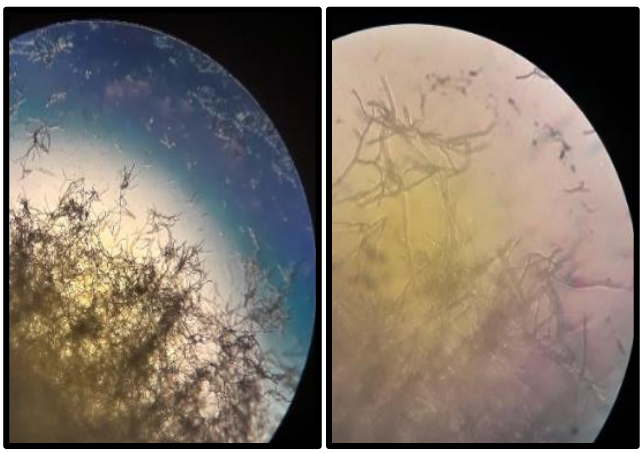


Figure 9: Microbiological observation of the strain YS15 under optical microscope with x40.

Figure 10: Microbiological observation of the strain YS155 under optical microscope with x40.

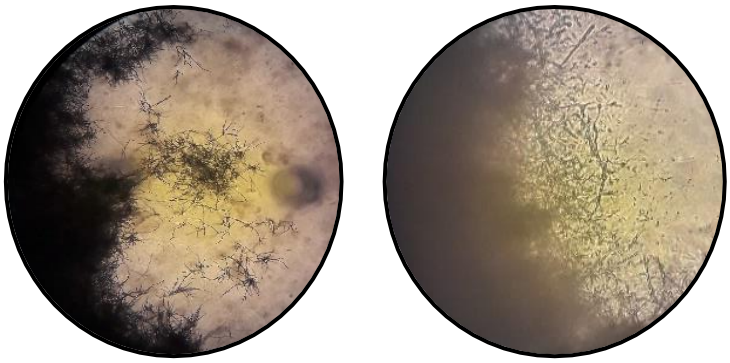


Figure 11: Microbiological observation of the strain YS1 under optical microscope with x40.

2. Production and extraction of active molecules

The extraction of the active molecules produced by the 4 isolates allowed the obtention of 4 crude extracts, as specified in table 8, reconstituted in 7ml of methanol for each isolate. The obtained crude extracts are reported in figure 12.

Table 8: Concentration of the crude extracts of the 4 isolates studied obtained after maceration.

Crude extract	Color	Concentration (mg/ml)
YS1	Transparent light yellow	9.42
YS15	Transparent light pink	17.85
YS56	Orange	14.28
YS155	Brown	3.71

Figure 12: Crude extracts of the four strains.

3. Antimicrobial activity evaluation of the strains

Antimicrobial activity of the actinobacteria strains (YS1, YS15, YS56, YS155) were evaluated against pathogenic germs. The results were obtained after incubation at 37 ° C for 24 hours for bacteria and at 28 °C for 48 hours to 5 days for fungi.

3.1. Evaluation of antibacterial activity

The antibacterial effects of the tested strains are reported in table 13, figures 14, 15 and 16 below.

Table 9 : Antibacterial evaluation of the strains against target germs.

	YS1	**YS15**	**YS56**	**YS155**
E.coli	-(6)	-(6)	+(7)	-(6)
S. typhi	+(10)	+(7)	-(6)	+(8)
B. cereus	-(6)	-(6)	-(6)	-(6)
B. subtilis	-(6)	+(6)	-(6)	-(6)
V. cholera	-(6)	+(8)	-(6)	-(6)
Acinetobacter	-(6)	+(7)	-(6)	-(6)
Enterobacter	-(6)	-(6)	-(6)	-(6)
S. aureus	-(6)	-(6)	-(6)	-(6)
Klebsiella	-(6)	-(6)	-(6)	-(6)
Pseudomonas	-(6)	+(10)	-(6)	-(6)
S.A.R.M	-(6)	-(6)	-(6)	-(6)

= 6mm: negative activity, > 6mm: positive activity

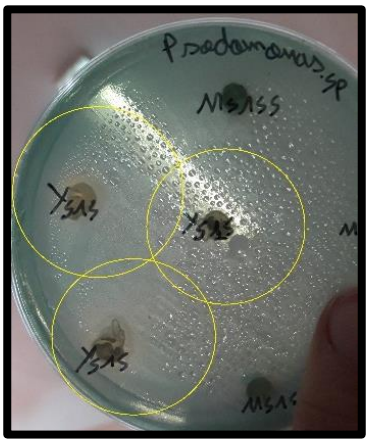


Figure 13: Antibacterial activity of YS15 strain against *Pseudomonas aeruginosa*.

Figure 14: Antibacterial activity of YS1 strain against *Staphylococcus aureus*

Figure 15: Antibacterial activity of YS1 and YS155 against *Salmonella Typhi.*

The tested strains showed variable antibacterial activity against the used target germs.

According to the obtained result we noticed that this activity varies according to the tested strains and the target germs. Some had a significant antagonistic effect, others none.

YS15 strain presented a moderate antagonistic activity against *Pseudomonas aeruginosa* and a weak activity against Vibrio cholerae with inhibition zones of 10 mm and 8 mm respectively and a particularly weak activity against *E. coli, S. Typhi, B. subtilis, B. cereus,* and *Acinetobacter.*

YS1 strain showed a moderate antibacterial effect against *S. Typhi* with inhibition zone of 10 mm. YS 155 strain exhibited a weak antibacterial effect against *S. Typhi* with inhibition zone of 8 mm. According to the data **(table 9)**, YS15 showed the best antagonistic activity against most target germs, except for, *MRSA, Klebsiella pneumoniae, Staphylococcus aureus, Enterobacter* sp., *Bacillus cereus,* whereas, the strongest activity was presented against *Pseudomonas aeruginosa* and *V. cholerae.* YS56 presented no antibacterial activity.

3.2. Evaluation of antifungal activity

The antifungal effects of the tested strains are reported in table 10, fugures 16, 17, 18, 19 below.

Table 10: Evaluation of antifungal activity of the strains with agar cylinders method.

	YS1	**YS15**	**YS56**	**YS155**
Penicillium aeruginosa	-	-	+	+
Aspergillus niger	-	+	+	+
Aspergillus flavus	+	-	+	+

(-) : no inhibition , (+) : presence of inhibition

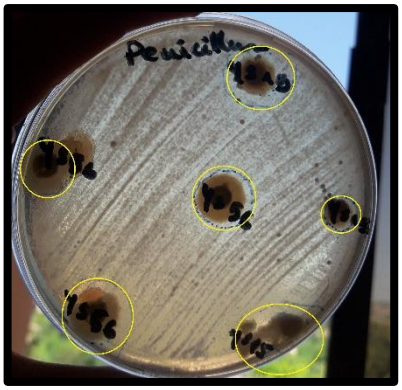


Figure 16: Antifungal activity of YS56 strain against *Penicillium aeruginosa.*

Figure 17: Antifungal activity of YS15 and YS56 strains against *Aspergillus niger.*

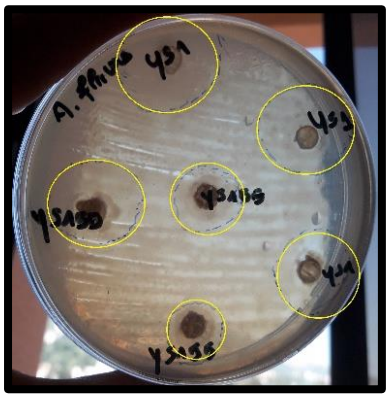


Figure 18: Antifungal activity of YS1 and YS155 against *Aspergillus Flavus.*

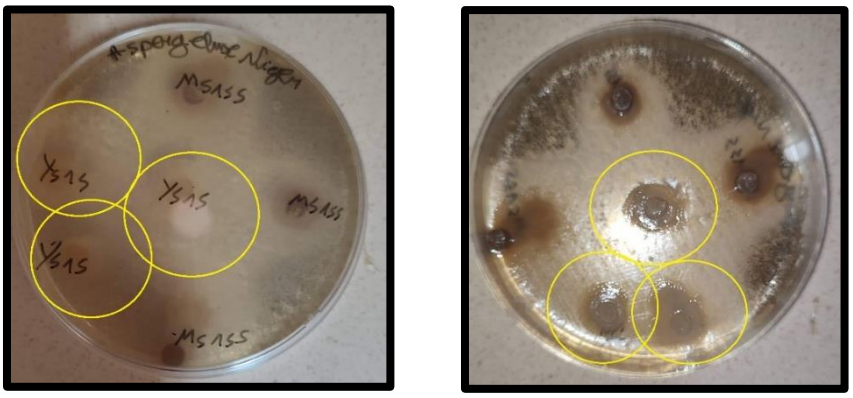


Figure 19: Antifungal activity of YS15 strain against *Aspergillus niger*

YS1 strain exhibited significant antifungal activity against *A. flavus* with total inhibition zone of 22 mm in diameter. YS155 strain showed significant activity against *A. flavus* with total inhibition zone of 16 mm diameter, and a particularly weak activity against *A .niger* and *Penicillium aeruginosa*. YS15 strain also presented the strongest activity against *A. niger* with inhibition zones of 26 mm diameter.

YS56 strain exhibited significant antifungal activity against *A. niger* and *Penicillium aeruginosa* with inhibition zones of 15 mm and 14 mm diameter respectively, and a particularly weak antibacterial activity against *A .flavus.*

According to the data presented in **(table 10)** YS1 strain presented the strongest activity against *A. flavus* followed by YS155 strain. YS15 exhibited the strongest antifungal potential against *A. niger*. While YS56 showed the strongest activity against *Penicillium aeruginosa*.

4. Biological activities evaluation of ethyl acetate crude extracts

In order to study the ability of crude extracts to inhibit the growth of target germs, an antagonism test based on the well method was carried out.

The antagonistic activity of the crude extracts against pathogenic germs was evaluated by the method of wells on Mueller Hinton medium previously seeded by the targeted germs mentioned above at a rate of 10^7 CFU / ml. Briefly, this technique consists of forming wells of 6mm in diameter using a cookie cutter and then a volume of 50µL of each extract as well as the solvent used (methanol) considered as a control, is introduced into the wells. The boxes are set at 4°C for 30min to allow the diffusion of active substances and then incubated at 37°C for 24h. The reading of the results is done by measuring the diameter of the inhibition zones expressed in millimeters around the wells.

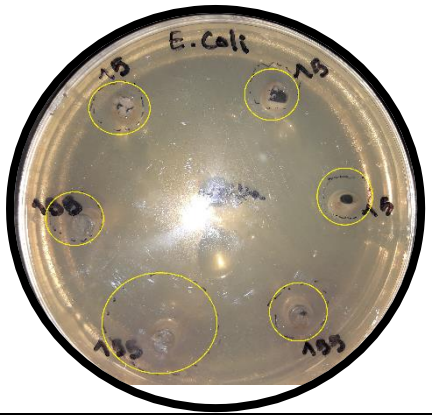
4.1. Antibacterial activity evaluation of the extracts

The antibacterial effects of the tested strains are reported in table 11, figure 21 below. Antibacterial activity of actinobacteria ethyl acetate crude extracts by agar well diffusion method.

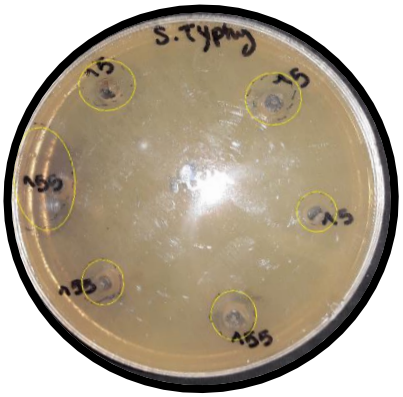
Table 11: Antibacterial evaluation of the strains against target germs.

	YS1	**YS15**	**YS56**	**YS155**
E.coli	-(6)	-(6)	+(7)	-(6)
S. typhi	-(6)	+(7)	-(6)	+(8)
B. cereus	+(7)	-(6)	-(6)	-(6)
B. subtilis	-(6)	+(9)	-(6)	+(7)
V. cholera	-(6)	+(8)	-(6)	-(6)
Acinetobacter	-(6)	+(7)	-(6)	-(6)
Enterobacter	-(6)	-(6)	-(6)	-(6)
S. aureus	-(6)	-(6)	-(6)	-(6)
Klebsiella	-(6)	-(6)	-(6)	-(6)
Pseudomonas	-(6)	+(10)	-(6)	-(6)
S.A.R.M	-(6)	-(6)	-(6)	-(6)

= 6mm: negative activity, > 6mm: positive activity



Antibacterial activity of YS15 and YS155 extracts against *E.coli* presenting the inhibition zones



Antibacterial activity of YS15 and YS155 extracts against *S.typhi* presenting the inhibition zones

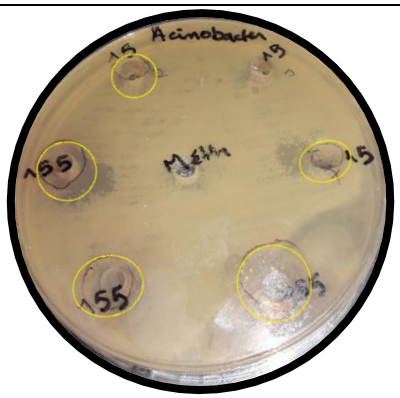
	
Antibacterial activity of YS15 and YS155 extracts against *Acinetobacter* presenting the inhibition zones	Antibacterial activity of YS15 and YS155 extracts against *B.subtilis* showing the inhibition zones

Figure 21: Some of antibacterial activity results with agar well diffusion method.

YS1 ethyl acetate crude extract showed the strongest activity against *S. aureus* with inhibition zone of 16mm in diameter and a weak antagonism activity against *B. cereus, Pseudomonas aeruginosa, and Acinetobacter baumanii*. with inhibition zones of 7mm diameter.

The crude extract of YS15 strain presented a moderate activity against *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* with inhibition zones of 11mm, 10mm, 11mm in diameter respectively, and a weak activity against *S. Typhi*, B. *subtilis*, *Acinetobacter baumanii*. and *Enterobacter* sp.

YS56 extract exhibited a remarkable activity against *S. aureus* with inhibition zone of 13mm, and a weak activity against *S. Typhi, B. cereus, Acinetobacter baumanii*. along with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

YS155 extract showed a strong-activity against *E. coli*, and *Acinetobacter baumanii*., and *S. aureus* with inhibition zones of 16mm, 12mm, 14mm respectively, and a weak effect against *S. Typhi, B.subtilis* and *MRSA* with inhibition zones of 7mm diameter.

4.2. Antifungal activity evaluation of the extracts

The antifungal effects of the tested strains are reported in table 12, figures 22 and 23 below.

Table 12: Evaluation of antifungal activity of the strains with agar well diffusion method.

	YS1	YS15	YS56	YS155
Penicillium aeruginosa	-	-	+	+
Aspergillus niger	-	+	+	+
Aspergillus flavus	+	-	+	+

(-) : no inhibition , (+) : presence of inhibition

Figure 22: Antifungal activity of YS15 and YS155 extracts against *A. niger*.

Figure 23: Antifungal activity of YS15 and YS155 extracts against *P. aeruginosa.*

YS1 extract presented an antifungal activity against *A. niger*, however no activity against *A.flavus* nor *Penicillium aeruginosa*. YS15 extract showed an activity against *Penicillium aeruginosa*, and *A. niger* and no activity against *A.flavus*. YS56 extract exhibited only an activity against *A.niger*.

YS155 extract showed activity against *Penicillium aeruginosa*. and *A. niger* and no activity against *A. flavus*.

According to the graph **(table 12)**, YS155 presented the strongest activity against *Penicillium aeruginosa*, and the strongest activity against *A. niger* followed by YS15, and no activity presented against *A. flavus*. In accord with the obtained results, the diameters of the inhibition zones differ from one bacterium to another and from one extract to another. This can be

explained by the variation in the chemical composition of the molecules produced by these four isolates studied (Boudjouref, 2011).

5. Total phenolics assay

The evaluation of the presence of a potential phenolic compounds in crude extracts of actinobacteria strains is estimated according to the method described by Kahkönen et al., (1999). A calibration curve has been established using gallic acid as a standard **(annex IV).**

In this assay and the next antioxidant evaluations each extract is diluted, 50µl of the extract added 4ml of solvent which is methanol, furthermore the concentrations of the four extracts will be as following: YS1 (1,04 µg/ml); YS15 (1,90 µg/ml); YS56 (1,58 µg/ml); YS155 (4,12 µg/ml). The total phenolic compounds content of the extracts is shown in the **figure 24** bellow.

The Student test revealed presence of a significant difference between the polyphenolics compounds of the four strains of a ($p<0.05$).

The highest phenolic compounds extracts are YS56 (27,46±1,28mg EAG /g) and YS1 (24,53±1,07mg EAG /g) and the lowest is recorded for YS15 and YS155 extracts (6,78±0,87mg EAG /g; 5,30±0,34mg EAG /g), respectively. The results of YS1 and YS56 are higher than those obtained by Avilala et al. (2013) who showed total phenol levels ranging from 13.62±1.12 to 14.37±1.47 mg EAG/g.

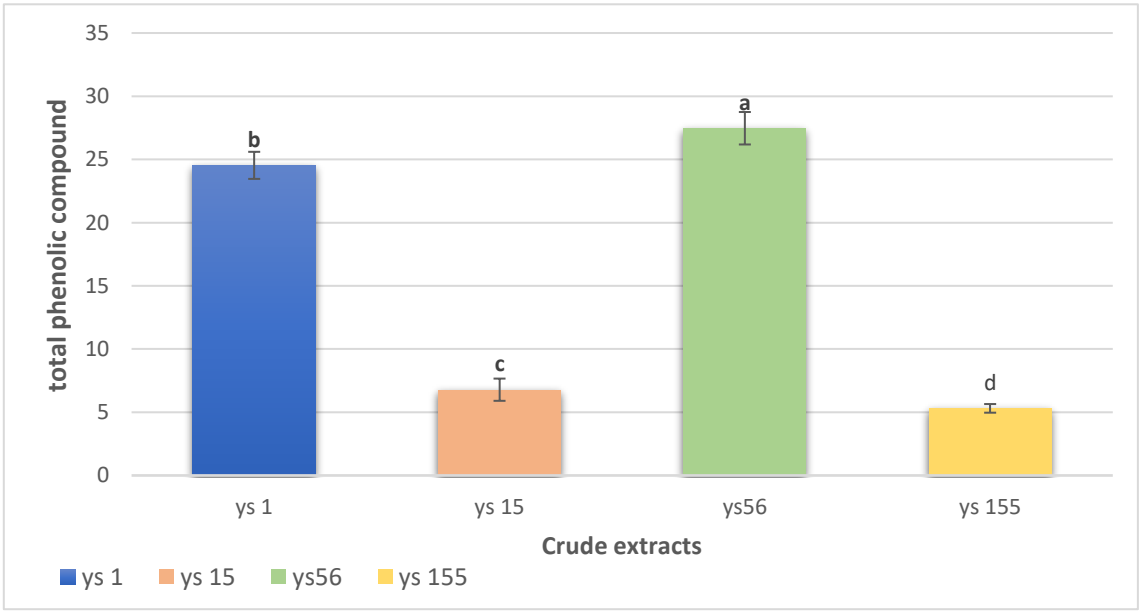


Figure 24 : Total phenolic compounds of crude extracts.

In a study conducted by Lertcanawanichakul et al. (2015), on a strain of *Streptomyces lydicus*, low levels of polyphenols were reported and estimated at 0.18± 0.01 mg EAG / g of ES which are much lower than those obtained with our crude extracts. These results prove and demonstrate the richness and diversity of the secondary metabolites produced by our four strains YS1, YS15, YS56 and YS155. The highest antioxidant capacity of bioactive compound could be attributed to the presence of high total polyphenol contents, since a positive correlation between phenolic composition and antioxidant activity was proved by Katalinic et al. (2006).

6. Antioxidant activity evaluation

The evaluation of the antioxidant properties of *Actinobacteria* crude extracts were evaluated using four different methods: radical trapping activity (DPPH) and iron reducing power (FRAP), metal chelating, which are chemical tests and their principle is based on a color change that was followed by a reading of the absorbance at specific wavelengths for each radical.

6.1. Free radical activity (DPPH)

The DPPH method is independent of the polarity of the substrate. This method is based on the reduction of an alcoholic solution of DPPH in the presence of an antioxidant that gives a hydrogen or an electron. The anti-radical activity of crude extracts is evaluated according to the Lesage-Meessen et al. (2001) method. The percentages of inhibitions of the four extracts and the standards are shown in **Figure 25** below. The results are obtained from from the ascorbic acid calibration curve comparison **(figure 2, annex VI).**

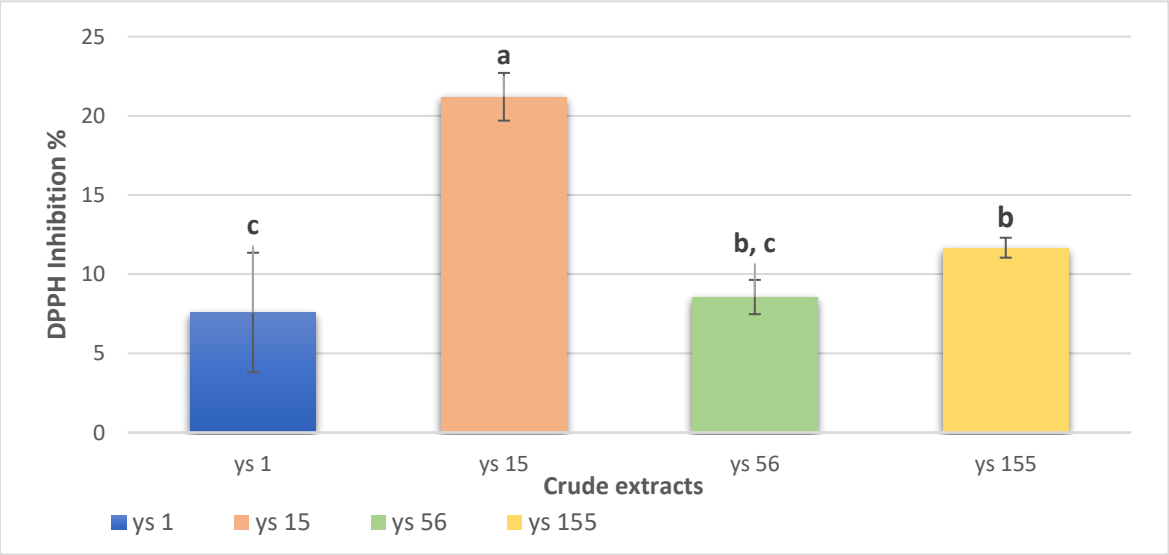


Figure 25: DPPH inhibition percentage.

The highest percentage of DPPH inhibition was obtained for YS15 extract with 21,19%±1,5 followed by YS155 with 11,66%±0.62 and lowest percentage of DPPH inhibition was obtained with YS56 and YS1 extracts with values of 8,5%±1,08; 7,57%±3,77 respectively. The percentages are lower compared to the study done by Kumar et al. (2021) which is 26.42% with a concentration of 25µg/µl of extract. This results are not in accordance with polyphenols contents in extracts. This may due to supplement activity of other components than polyphenols as well as to inadequate structure of phenolics compounds to scavenge DPPH radicals in these extracts (Aouachria, 2012).

6.2. Iron chelating assay

The chelating capacity of extracts is measured by following inhibition of Fe^{2+}–ferrozine complex formation after incubation of samples with divalent iron according to the method of Wang et al. (2008). The percentage inhibition of the formation of the $Fe2^{+}$–ferrozine complex of each crude extract tested is shown in **figure 26** below.

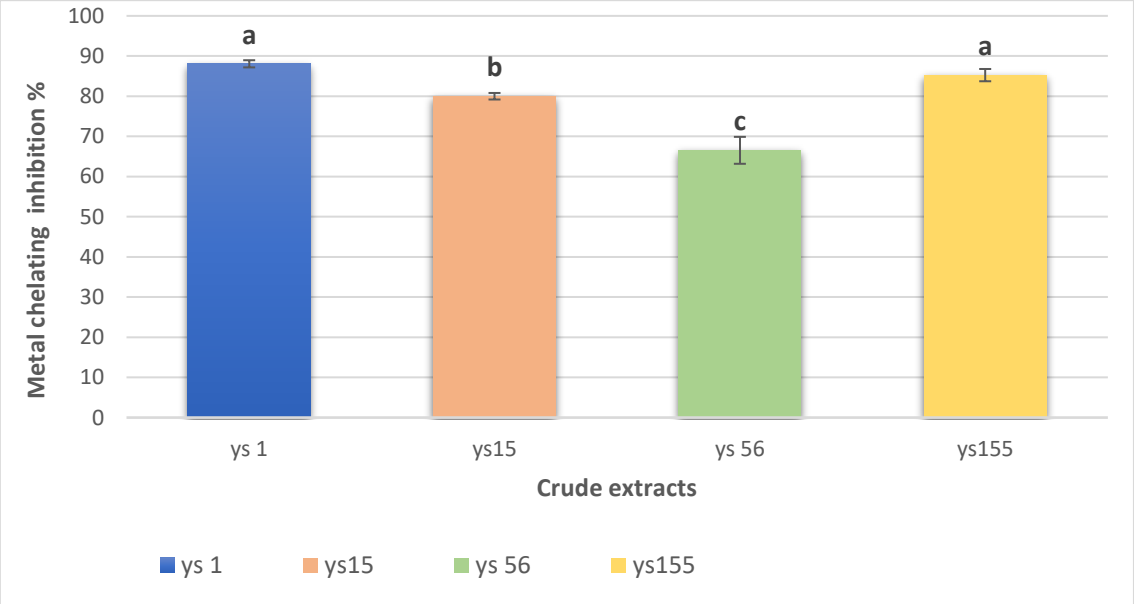


Figure 26 : Metal chelating inhibition percentage.

The YS1 and YS155 extracts gave a better percentage of inhibition of formation of the Fe^{2+}–ferrozine complex with a percentage of 88% ±0.88; 85%±1,52 followed by YS15 and YS56 extracts with 79%±0,81 and 66%±3,35, respectively.

The differences noted between the extracts studied may be related to the structural properties of the antioxidants present in the strains. This may explain the results obtained for extracts that contain a moderate content of phenolic compounds, but exert the highest chelating activity. This is consistent with the results of Wang et al. (2008) who explained that the chelating

capacity of metals depends on the number and position of hydroxyl groups present in the structure of phenolic compounds.

6.3. Ferric reducing antioxidant power assay (FRAP)

The reducing power can be evaluated by several tests, namely the reduction of ferric chloride (Sousa et al., 2008; Sahreen et al., 2010), which is the ability of the antioxidants present in the extract to reduce the ferric iron of the ferricyanide complex (Fe^{3+}) to ferrous iron (Fe^{2+}) in a medium acidified by TCA. The reduced form of this complex gives a green color that is proportional to the reducing power of the extract (Odabasoglu et al., 2004). The reducing power is estimated by the method of Gülçin et al. (2002).

The obtained results are presented in **Figure 27** below.

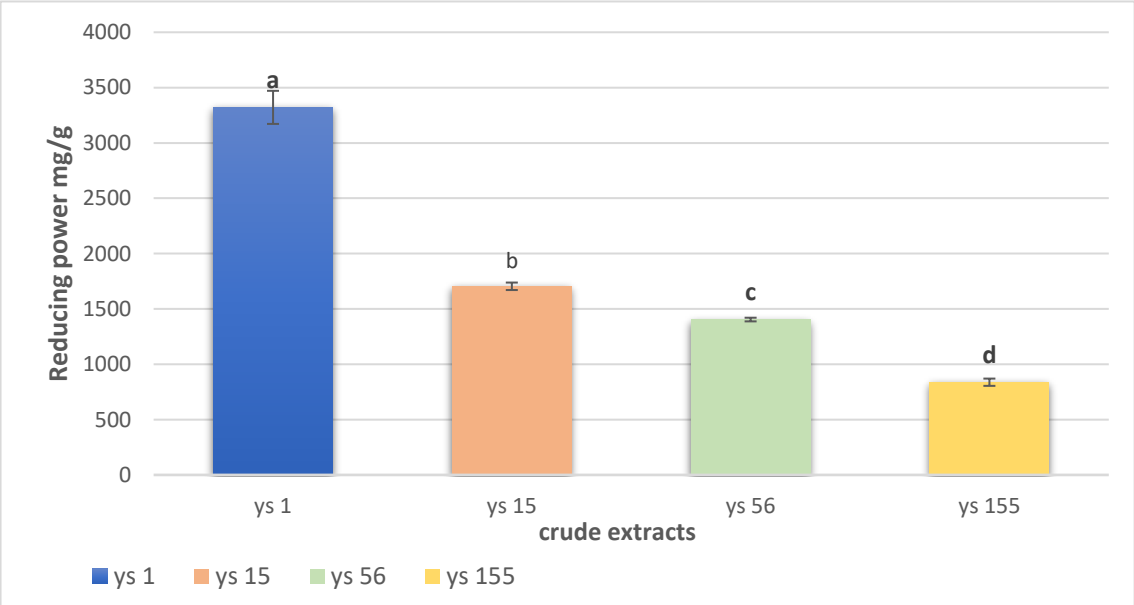


Figure 27: Reducing power of the extracts.

The highest power reducing concentration obtained is with YS1 extract 3321,48±194,55mg/g, followed by YS15 than YS56 extracts with 1704,58±33,57mg/g; 1404,04±16,46mg/g, respectively, the lowest power reducing concentration is YS155 with 837,66±32,77mg/g. These results are obtained from the gallic acid calibration curve comparison **(figure 1, annex VI).**

The differences found between the varieties analyzed could be related to the content of phenolic compounds given the role of these compounds as reducing agents.

7. Antibiofilm activity evaluation of actinobacteria crude extracts

7.1. Biofilm formation

The ability to form a biofilm has been tested for the bacterial strains: *Pseudomonas aeruginosa*, *Acinetobacter baumanii*. and *Staphylococcus aureus*. The absorbances indicating the formation of biofilms were measured, after staining with purple crystal, a microplate reader (BioTeK). The optical density absorbance of negatif control and bacterial strains are represented in **table 13** bellow.

Table 13 : Optical density absorbance in nm of negatif control and bacterial strains.

	Negative Control MHB	**Strains**		
OD (630nm)		***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Acinetobacter baumanii***
	0.116	0.328375	0.24825	0.15075

The ethyl acetate crude extracts obtained by maceration then recovered in methanol of YS1, YS15, YS56 and YS155 cultures were tested for their anti-biofilm effect against the three bacteria strains. The obtained results are illustrated in **figure 28** bellow.

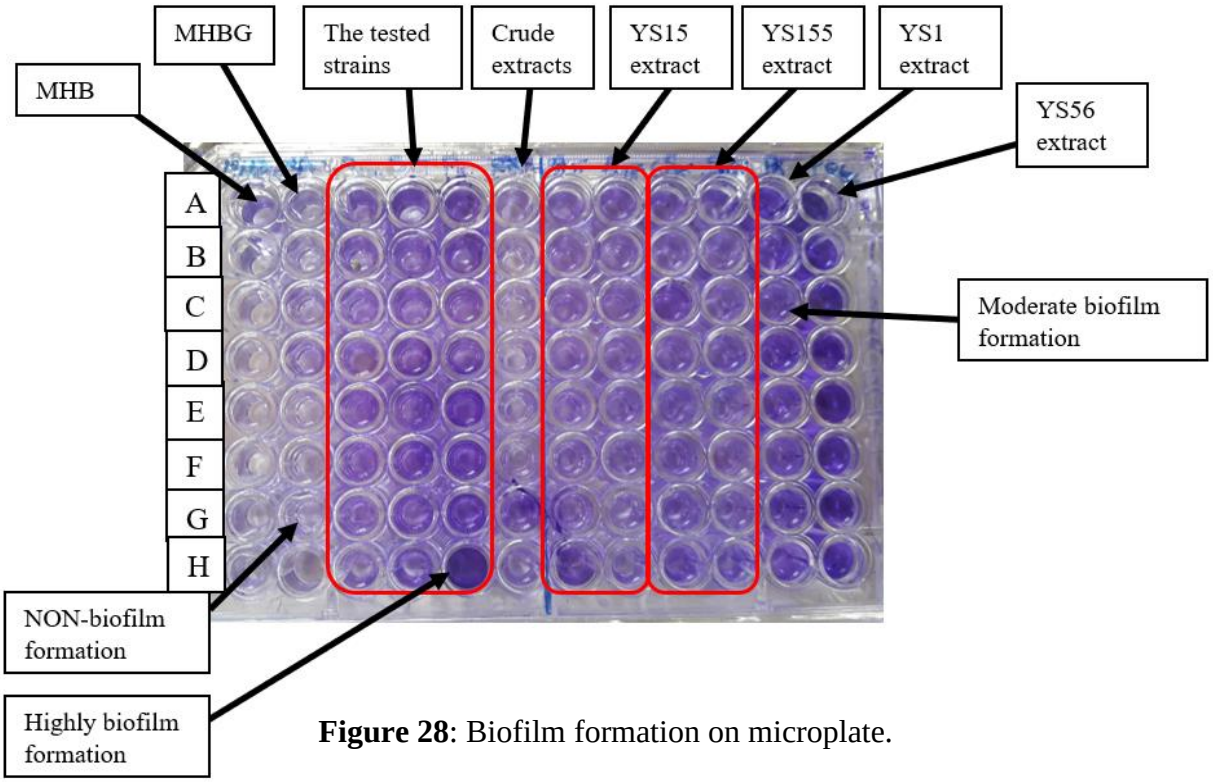


Figure 28: Biofilm formation on microplate.

7.2. Antibiofilm activity of YS1 and YS56 crude extracts

In this test, we used *Pseudomonas aeruginosa* as the target germ to evaluate the antibiofilm activity of the extracts YS1 and YS56.

The results obtained are represented in the figure 29 bellow.

From the obtained results, it is clearly noticed a significant decrease in biofilm formation by *Pseudomonas aeruginosa* by of YS1 extract with 47% and a slightly decrease of biofilm formation by *Pseudomonas aeruginosa* of YS56 crude extract with 4% **(figure 29).**

YS1 extract exerted the higher antibiofilm activity.

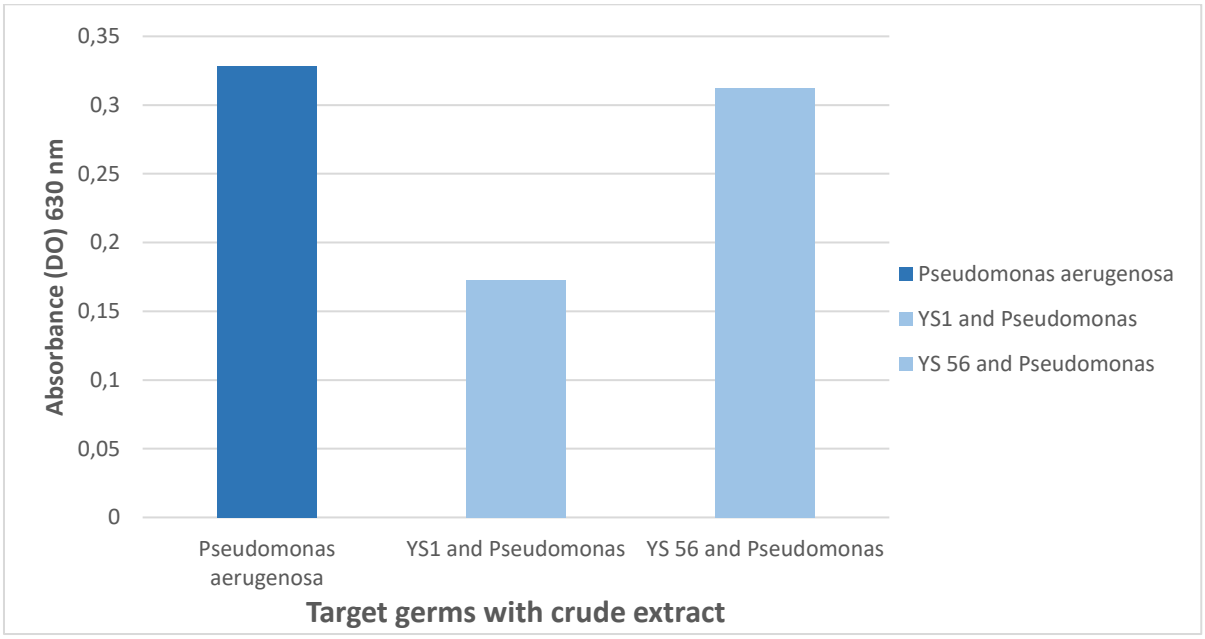


Figure 29: The effect of YS1 and YS56 on crude extract on biofilms formed by *Pseudomonas aeruginosa*

7.3. Antibiofilm activity of YS15 and YS155 crude extracts

In this test, we used two target germs, *Acinetobacter baumanii* and *Staphylococcus aureus* to evaluate the antibiofilm activity of the extracts YS15 and YS155.

The results obtained are represented in the **figures 30 and 31** bellow.

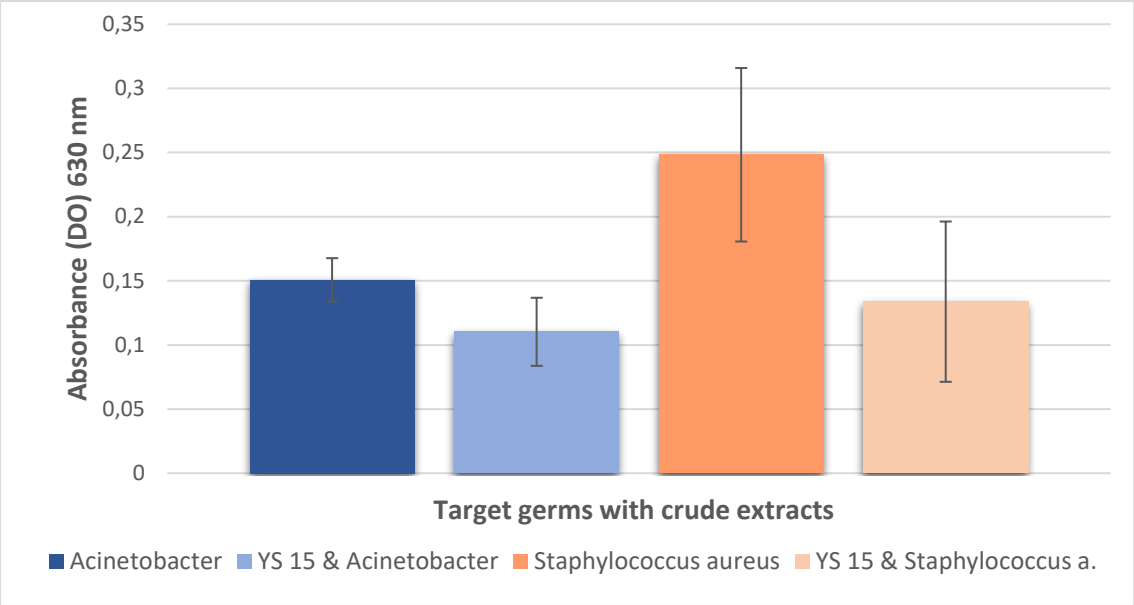


Figure 31: The effect of YS15 crude extract on biofilms formed by *Acinetobacter baumanii* and *Staphylococcus aureus.*

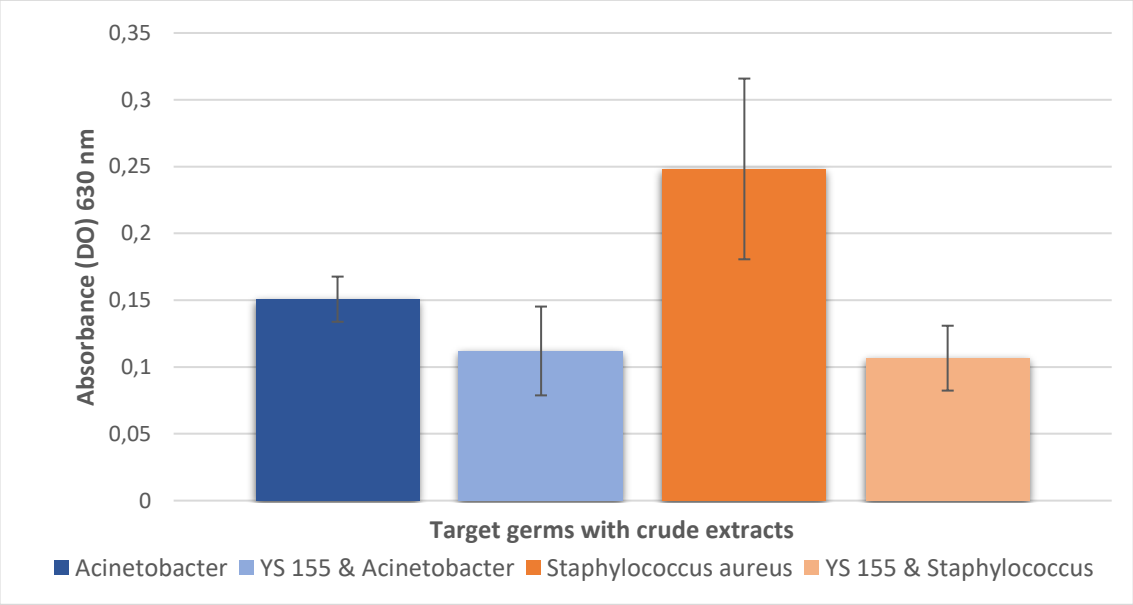


Figure 32: The effect of YS155 crude extract on biofilms formed by *Acinetobacter baumanii*-and *Staphylococcus aureus.*

From the obtained results **(figure 30, 31),** it is clearly noticed significant decrease in biofilm formation of *Staphylococcus aureus* by YS15 extract with 46% and YS155 extract with 59%, and a moderate decrease of *Acinetobacter baumanii* by YS15 and YS155 extracts with 27%.

This results lead us to conclude that YS15 and YS155 crude extracts present a better antibiofilm activity against multi-resistant strain of *Staphylococcus aureus.*

In addition, it should be noted that there is no antibiofilm activity of the broth used namely Muller Hinton Broth and Mueller Hinton Broth glucose added against the tested strains.

General Conclusion

Conclusion

Actinobacteria are microorganisms of significant industrial interest. These microorganisms are the most investigated for their ability to produce variety of secondary metabolites.

The present work was devoted to the evaluation of the pharmacological potential of *Actinobacteria* strains isolated from two different regions Boulimat and Sidi Ali labhar (Bejaia) by the laboratory team of Applied Microbiology (LMA) of Abderrahmane Mira University of Bejaia.

The evaluation of the pharmacological potential was conducted with an evaluation of antagonistic activity using bacteria and fungi as target germs, evaluation of the biological activities with active molecules extracted by ethyl acetate, along with the evaluation of antioxidant activity with polyphenolics compounds contents and anti-biofilm activity.

The evaluation of the antagonist potential of the isolates YS1, YS15, YS56 and YS155 showed an antibacterial and antifungal potential against the target germs used such as *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus,* MRSA, *Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, Enterobacter sp, Vibrio cholerae, Aspergillus flavus, Aspergillus niger, Penicillium sp.* However the evaluation of biological activity showed a better results.

The evaluation of the antioxidant activity with polyphenolics compounds content showed a moderate concentrations. The highest phenolic compounds extracts are YS56 (27,46±1,28mg EAG /g) and YS1 (24,53±1,07mg EAG /g).

DPPH inhibition showed also a moderate percentages, the best inhibition obtained are 21,19%±1,5 of YS15 extract followed by YS155 with 11,66%±0.62. The YS1 and YS155 extracts gave a better percentage of inhibition of formation of the Fe^{2+}–ferrozine complex with a percentage of 88% ±0.88; 85%±1,52 followed by YS15 and YS56 extracts with 79%±0,81 and 66%±3,35, respectively.

The power reducing activity showed a better concentration of 3321,48±194,55mg/g with YS1 extract, followed by YS15 than YS56 extracts with 1704,58±33,57mg/g; 1404,04±16,46mg/g.

The anti-biofilm potential evaluation showed a significant decrease in biofilm formation by *Pseudomonas aeruginosa* by of YS1 extract with 47%. YS15 extract showed a significant

decrease in biofilm formation of *Staphylococcus aureus* with 46% and YS155 extract with 59%, and a moderate decrease of *Pseudomonas aeruginosa* by YS15 and YS155 extracts with 27%.

YS15 and YS155 crude extracts present a better antibiofilm activity against multi-resistant strain of *Staphylococcus aureus.*

Finally, the various evaluations of these isolates differs between the target germs used and from one actinobacteria isolate to another.

It would be interesting to address the following points in the future in order to refine this present work:

- Characterization of the active molecules of the actinobacteria tested,
- Complete the molecular identification of the actinobacteria tested.
- Identify the molecules at the origin of antioxidant activity.

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Annex I

Table 1: Some examples of enzymes produced by *Actinobacteria*.

Enzyme	**Species**	**Application in biotechnology**	**Reference**
Cellulase	*Streptomyces craterifer*	The pulp and paper, textile, wine and brewing industries. The treatment of animal skins.	Gobalakrishnan et al., (2016).
Xylanase	*Streptomyces* sp.	Pulp and paper industry. Clarification of fruit juice.	Rosmine et al., (2017).
Amylase	*Streptomyces* sp.	Distillation industries. Saccharification of starch.	Chakraborty et al., (2009).
Lipase	*Actinomadura* sp.	The treatment of fats and oils. Cosmetic industries.	Taibi et al., (2012).
Protease	*Streptomyces carpaticus*	Textiles and detergents industry. Dairy and cheese industries.	Haritha et al., (2012).

Table 2: Some examples of bioactive compounds produced by bacteria of the order Actinomycetales.

Biological activity	**Substance**	**Species**	**Other pharmacological activities**	**References**
Antibacterial	N-acetyl-N-demethylmayamycin	*Streptomyces sp.*	Antitumorale	Liang et al., (2016)

Antibacterial	Curvularin-7-O- a-dGlucopyranoside	*Pseudonocardia sp.*	Anticancer	Ye et al., (2015)
Antifungal	Saadamycin	*Streptomyces sp.*	-	El-Gendy et El Bondkly, (2010)
Antiviral	Butenolides 1a, 1b, 2, 3, 4	*Streptomyces sp.*	Cytotoxic	Strand et al., (2014)
Antimalarial	Trioxacarcin A,B and C	*Streptomyces ochraceus*	Antibacterial Antitumor	Maskey et al., (2004)
Antimalarial	Cyclomarin A	*Streptomyces sp*	Anti-Tuberculosis	Bürstner et al., (2015)
Antibacterial	Diazepinomicin	*Micromonospora*	Anti-inflammatory Antitumor	Charan et al., (2004)
Antibacterial	2-Allyloxyphenol	*Streptomyces sp*	Oral disinfectant	Arumugam et al., (2009)
Antimalarial	Salinosporamide A	*Salinispora tropica*	Anticancer	Prudhomme et al., (2008)

Annex II

Matériel analytique

- Autoclave (ALFA-10-Plus).
- Bain-marie.
- Analytical balance (RADWAG).
- Etuve à 28°C, 37°C, 60°C.
- Optical microscope (Optika B-350).
- PH- meter.
- Stirring plate (VELP scientifica :AM4).
- Vortex (VELP scientifica : ZX 3).
- Centrifugal (HETTECH).
- Rotavapor (Bûchi Rotavapor R-114).
- Spectrophotometer (UV mini 1240 ; SHIMADZU) (UviLinde 9400).
- Bunsen burner.
- Beakers.
- Funnel.
- Erlenmeyers.
- Bottles.
- Microplates.
- Microplate Reader (BioTek ELx808).

Annex III

Composition of culture media

- **Medium ISP4 :**
  - Starch: 10 g;
  - K2HPO4: 1 g;
  - MgSO4, 7H2O: 1 g;
  - NaCl: 1 g;
  - (NH4)2SO4: 2 g;
  - CaCO3: 2 g;
  - Standard saline solution (see ISP3): 1 ml;
  - agar: 20 g;
  - Distilled water s.q.f. 1000 ml;

    pH 7.2.
- **Gauss medium** (Ivantiskaya et al., 1978)
  - Starch : .....10 g ;
  - K2HPO4 : .....0,5 g ;
  - KNO3 : .....1 g ;
  - MgSO4, 7H2O : .....0,5 g ;
  - FeSO4, 7H2O : .....0,01 g
  - Agar : .....18 g ;
  - Distilled water : .....1000 mL.

    pH : 7,4±0,2.
- **Muller-Hinton medium** (John Howard Mueller and Jane Hinton, 1941)
  - Box of 250g of dehydrated Muller Hinton (Pasteur Institut): 38g
  - Distilled water: 1000mL,

    pH: 7.3±0.2

Annex IV: Calibration curve used for antioxidant determination

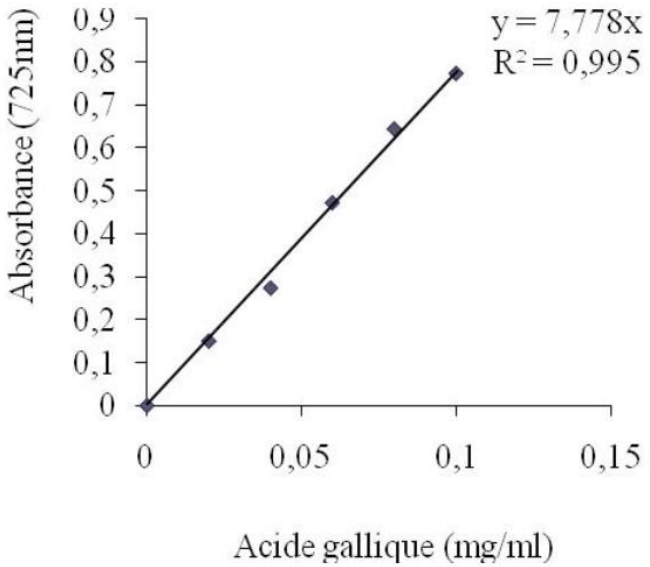

Figure 1: Calibration curve of polyphenols with gallic acid.

Calibration curve used for the determination of antioxidant activity

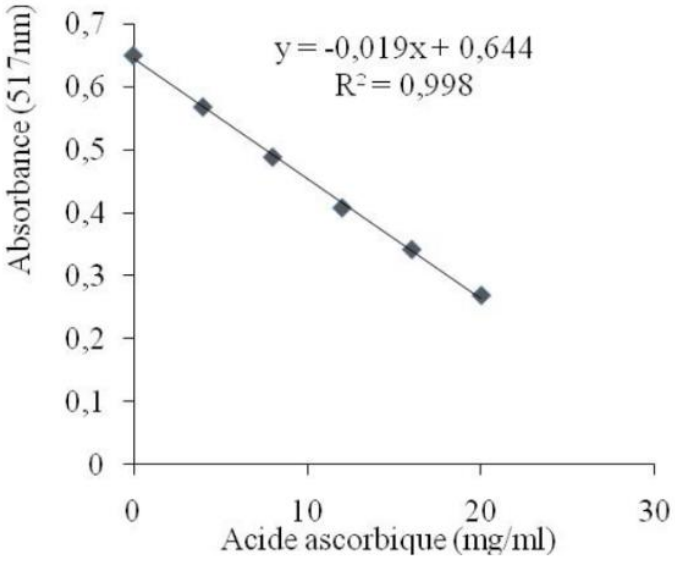

Figure 2: Calibration curve of DPPH with ascorbic acid.

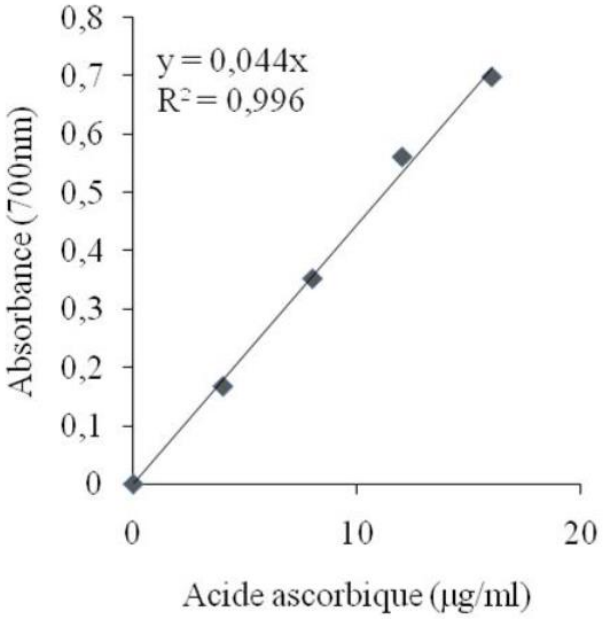

Figure 3: Calibration curve of reducing power with ascorbic acid.

Abstract

Actinobacteria constitute prolific sources of novel and vital bioactive metabolites for pharmaceutical utilization. In recent years, research has focused on exploring actinobacteria that thrive in extreme conditions to unearth their beneficial bioactive compounds for natural product drug discovery. This study focuses on the diversity and bioactive potentials/medically relevant biomolecules of four isolated actinobacteria found in discharged soil from two ecosystems around Bejaia region. Ethyl acetate extracts from isolates displayed strong antibiotic activities against a panel of important resistant clinical pathogens, including Gram-positive and Gram-negative bacteria, most strains have displayed substantial antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Bacillus cereus, Bacillus subtilis*, *Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, Enterobacter sp, Vibrio cholerae.* Several strains exhibited other prominent bioactivities such as antifungal potential against *Aspergillus flavus, Aspergillus niger, Penicillium sp,* and antioxidant activity using DPPH, Ferric reducing antioxidant power (FRAP), ferrozine assay and polyphenolics compounds content, most strains showed an anti-biofilm potential. By providing an overview of the four isolates and their important metabolites, we hope to enhance the understanding of their potential for the medical world.

Keywords: Actinobacteria, pharmacological potential, antibacterial, antifungals, antioxidant, anti-biofilm.

Résumé

Les actinobactéries constituent des sources prolifiques de métabolites bioactifs nouveaux et vitaux pour une utilisation pharmaceutique. Au cours des dernières années, la recherche s'est concentrée sur l'exploration des actinobactéries qui prospèrent dans des conditions extrêmes afin de découvrir leurs composés bioactifs bénéfiques pour la découverte de médicaments naturels. Cette étude se concentre sur la diversité et les potentiels bioactifs / biomolécules médicalement pertinentes de quatre actinobactéries isolées trouvées dans un sol de décharge de deux écosystèmes autour de la région de Bejaia. Les extraits d'acétate d'éthyle provenant d'isolats ont montré de fortes activités antibiotiques contre un panel d'agents pathogènes cliniques résistants importants, y compris les bactéries Gram positif et Gram négatif, la plupart des souches ont montré une activité antibactérienne substantielle contre *Staphylococcus aureus* résistant à la méthicilline (SARM), *Staphylococcus aureus*, *Bacillus cereus, Bacillus subtilis*, *Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, Enterobacter sp, Vibrio cholerae.* Plusieurs souches présentaient d'autres bioactivités importantes telles que le potentiel antifongique contre *Aspergillus flavus, Aspergillus niger, Penicillium sp,* et l'activité antioxydante en utilisant la DPPH, le pouvoir antioxydant réducteur ferrique (FRAP), le dosage de la ferrozine et la teneur en composés polyphénoliques, la plupart des souches ont montré un potentiel anti-biofilm. En donnant un aperçu des quatre isolats et de leurs métabolites importants, nous espérons améliorer la compréhension de leur potentiel pour le monde médical.

Mots-clés : Actinobactéries, potentiel pharmacologique, antibactérien, antifongiques, antioxydant, anti-biofilm.

ملخص

الأكتينوباكتيريا هي مصادر غزيرة الإنتاج للمستقلبات النشطة بيولوجيا الجديدة التي تعتبر حيوية للاستخدام الصيدلاني. في السنوات الأخيرة ، ركزت الأبحاث على استكشاف الأكتينوباكتيريا التي تزدهر في الظروف القاسية لاكتشاف مركباتها النشطة بيولوجيا المفيدة لاكتشاف الأدوية الطبيعية. تركز هذه الدراسة على التنوع وإمكانات الجزيئات الحيوية النشطة بيولوجيا / الجزيئية الحيوية ذات الصلة طبيا لأربعة بكتيريا أكتينوباكتيريا معزولة موجودة في تربة مكب النفايات في نظامين إيكولوجيين حول منطقة بجاية. أظهرت مستخلصات خلات الإيثيل من المعزولات نشاطا قويا للمضادات الحيوية ضد مجموعة من مسببات الأمراض السريرية المقاومة الهامة ، بما في ذلك البكتيريا إيجابية الجرام وسالبة الجرام ، وأظهرت معظم السلالات نشاطا كبيرا مضادا للبكتيريا ضد المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) ، *المكورات العنقودية* الذهبية ، *العصوية الدماغية* ، *العصوية الفرعية* ، *الإشريكية القولونية*، *السالمونيلا التيفي*، *الزائفة الزنجارية*، *كليبسيلا الرئوية*، *Enterobacter sp*، *Acinetobacter baumanii*، *ضمة الكوليرا*. أظهرت العديد من السلالات أنشطة حيوية مهمة أخرى مثل الإمكانات المضادة للفطريات ضد *flavus* Aspergillus و *Aspergillus niger* و *Penicillium sp* والنشاط المضاد للأكسدة باستخدام DPPH ، والقوة المضادة للأكسدة التي تقلل من الحديديك (FRAP) ، وفحص الفيروزين ومحتوى مركب البوليفينول ، وأظهرت معظم السلالات إمكانات مضادة للأغشية الحيوية. من خلال تقديم نظرة عامة على العزلات الأربع ومستقلباتها المهمة ، نأمل في تحسين فهم إمكاناتها للعالم الطبي.

الكلمات المفتاحية: أكتينوباكتيريا، إمكانات دوائية، مضاد للجراثيم، مضاد للفطريات، مضاد للأكسدة، مضاد للبيوفيلم.

Resumen

Las actinobacterias son fuentes prolíficas de nuevos metabolitos bioactivos que son vitales para el uso farmacéutico. En los últimos años, la investigación se ha centrado en explorar las actinobacterias que prosperan en condiciones extremas para descubrir sus compuestos bioactivos beneficiosos para el descubrimiento de fármacos naturales. Este estudio se centra en la diversidad y los potenciales bioactivos / biomoléculas médicamente relevantes de cuatro actinobacterias aisladas que se encuentran en un suelo de vertedero de dos ecosistemas alrededor de la región de Bejaia. Los extractos de acetato de etilo de aislados mostraron una fuerte actividad antibiótica contra un panel de importantes patógenos clínicos resistentes, incluidas las bacterias Gram-positivas y Gram-negativas, la mayoría de las cepas mostraron una actividad antibacteriana sustancial contra *Staphylococcus aureus* resistente a la meticilina (MRSA), *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, Enterobacter sp, Vibrio cholerae.* Varias cepas exhibieron otras bioactividades importantes, como el potencial antifúngico contra *Aspergillus flavus, Aspergillus niger, Penicillium sp* y la actividad antioxidante utilizando DPPH, poder antioxidante reductor férrico (FRAP), ensayo de ferrozina y contenido de compuestos polifenólicos, la mayoría de las cepas mostraron potencial anti-biopelícula. Al proporcionar una visión general de los cuatro aislados y sus metabolitos importantes, esperamos mejorar la comprensión de su potencial para el mundo médico.

Palabras clave: Actinobacterias, potencial farmacológico, antibacteriano, antifúngico, antioxidante, anti-biofilm.

Abstract
Actinobacteria constitute prolific sources of novel and vital bioactive metabolites for pharmaceutical utilization. In recent years, research has focused on exploring actinobacteria that thrive in extreme conditions to unearth their beneficial bioactive compounds for natural product drug discovery. This study focuses on the diversity and bioactive potentials/medically relevant biomolecules of four isolated actinobacteria found in discharged soil from two ecosystems around Bejaia region. Ethyl acetate extracts from isolates displayed strong antibiotic activities against a panel of important resistant clinical pathogens, including Gram-positive and Gram-negative bacteria, most strains have displayed substantial antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, Enterobacter sp, Vibrio cholerae.* Several strains exhibited other prominent bioactivities such as antifungal potential against *Aspergillus flavus, Aspergillus niger, Penicillium sp,* and antioxidant activity using DPPH, Ferric reducing antioxidant power (FRAP), ferrozine assay and polyphenolics compounds content, most strains showed an anti-biofilm potential. By providing an overview of the four isolates and their important metabolites, we hope to enhance the understanding of their potential for the medical world.

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Mots-clés : Actinobactéries, potentiel pharmacologique, antibactérien, antifongiques, antioxydant, anti-biofilm.

ملخص

الأكتينوباكتيريا هي مصادر غزيرة الإنتاج للمستقلبات النشطة بيولوجيا الجديدة التي تعتبر حيوية للاستخدام الصيدلاني. في السنوات الأخيرة ، ركزت الأبحاث على استكشاف الأكتينوباكتيريا التي تزدهر في الظروف القاسية لاكتشاف مركباتها النشطة بيولوجيا المفيدة لاكتشاف الأدوية الطبيعية. تركز هذه الدراسة على التنوع وإمكانات الجزيئات الحيوية النشطة بيولوجيا / الجزيئية الحيوية ذات الصلة طبيا لأربعة بكتيريا أكتينوباكتيريا معزولة موجودة في تربة مكب النفايات في نظامين إيكولوجيين حول منطقة بجاية. أظهرت مستخلصات خلات الإيثيل من المعزولات نشاطا قويا للمضادات الحيوية ضد مجموعة من مسببات الأمراض السريرية المقاومة الهامة ، بما في ذلك البكتيريا إيجابية الجرام وسالبة الجرام ، وأظهرت معظم السلالات نشاطا كبيرا مضادا للبكتيريا ضد المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) ، *المكورات العنقودية الذهبية ، العصوية الدماغية ، العصوية الفرعية ، الإشريكية القولونية، السالمونيلا التيفي، الزائفة الزنجارية، كليبسيلا الرئوية، Acinetobacter baumanii، Enterobacter sp، ضمة الكوليرا.* أظهرت العديد من السلالات أنشطة حيوية مهمة أخرى مثل الإمكانات المضادة للفطريات ضد Aspergillus *flavus* و *Aspergillus niger* و *Penicillium sp* والنشاط المضاد للأكسدة باستخدام DPPH ، والقوة المضادة للأكسدة التي تقلل من الحديديك (FRAP) ، وفحص الفيروزين ومحتوى مركب البوليفينول ، وأظهرت معظم السلالات إمكانات مضادة للأغشية الحيوية. من خلال تقديم نظرة عامة على العزلات الأربع ومستقلباتها المهمة ، نأمل في تحسين فهم إمكاناتها للعالم الطبي.

الكلمات المفتاحية: أكتينوباكتيريا، إمكانات دوائية، مضاد للجراثيم، مضاد للفطريات، مضاد للأكسدة، مضاد للبيوفيلم.

Resumen

Las actinobacterias son fuentes prolíficas de nuevos metabolitos bioactivos que son vitales para el uso farmacéutico. En los últimos años, la investigación se ha centrado en explorar las actinobacterias que prosperan en condiciones extremas para descubrir sus compuestos bioactivos beneficiosos para el descubrimiento de fármacos naturales. Este estudio se centra en la diversidad y los potenciales bioactivos / biomoléculas médicamente relevantes de cuatro actinobacterias aisladas que se encuentran en un suelo de vertedero de dos ecosistemas alrededor de la región de Bejaia. Los extractos de acetato de etilo de aislados mostraron una fuerte actividad antibiótica contra un panel de importantes patógenos clínicos resistentes, incluidas las bacterias Gram-positivas y Gram-negativas, la mayoría de las cepas mostraron una actividad antibacteriana sustancial contra *Staphylococcus aureus* resistente a la meticilina (MRSA), *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii, Enterobacter sp, Vibrio cholerae.* Varias cepas exhibieron otras bioactividades importantes, como el potencial antifúngico contra *Aspergillus flavus, Aspergillus niger, Penicillium sp* y la actividad antioxidante utilizando DPPH, poder antioxidante reductor férrico (FRAP), ensayo de ferrozina y contenido de compuestos polifenólicos, la mayoría de las cepas mostraron potencial anti-biopelícula. Al proporcionar una visión general de los cuatro aislados y sus metabolitos importantes, esperamos mejorar la comprensión de su potencial para el mundo médico.

Palabras clave: Actinobacterias, potencial farmacológico, antibacteriano, antifúngico, antioxidante, anti-biofilm.