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# MASTER'S THESIS

## Academic Master's Degree

**Field:** Natural and Life Sciences

**Program:** Biological Sciences

**Specialization:** Applied Microbiology

**Presented by:**

*Chaima ATMANE* *Mouna BERKANI*

## *Topic:*

# *Antimicrobial analysis of hydroalcoolic extracts of apple and banana peels against microbial strains*

***Thesis publicly defended on 19/06/2025 before a jury composed of:***

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# *Dedication*

I dedicate this project to:

To my dear mother, Nasira Dgoghlafi,

To my dear father, Zidane Atmane.

Who never stopped praying for me and supporting me.

And to help me achieve my goals.

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To all my family.

To all my other friends,

To all those I love and who love me.

*ATMANE CHAIMA*

# *Dedication*

I dedicate this modest work

**To my dear father,**

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This success belongs to you as much as to me.

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# Abstract

***Antimicrobial analysis of hydroalcoholic extracts of apple and banana peels against microbial strains***

***Abstract***

The present study contributes to the valorization of two fruit peels, Apple (*Malus domestica*) and banana (*Musa paradisiaca*) peels, which are typically considered agro-industrial waste. In this work, two organic solvents were used, ethanol and methanol, to investigate the antibacterial activities of these peel fruits at different concentrations: 200, 100, 50, and 25 mg/ml. The different extracts were tested against five pathogenic bacterial strains: *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, and four clinical stains of *E .coli*; using disk diffusion assays. The results showed that both fruit peel extracts were efficient against the tested strains. Furthermore, ethanolic banana peel extract exhibited significant antibacterial activity, with the highest efficiency of all extracts against *S. aureus* (mean of diameters of inhibition zone = 26 mm at 200 mg/mL), followed by *E. coli* with 25 mm inhibition diameter zone at 100 mg/mL with banana methanolic extract. Moreover, we assessed their minimal inhibitory concentration (MIC) using a broth microdilution method, with values ranging from 100 to 1.56 mg/mL. Furthermore, the plant extracts were subjected to phytochemical screening, which detected the presence of flavonoids, polyphenols, saponins, tannins, quinones, and terpenoids. Quantitative analysis revealed a notable richness in flavonoids, with values ranging from 22± 0.048 to 53 ± 0.86 mgCE/g. These findings validate the antimicrobial potential of these fruit by products and indicate their potential utility as therapeutic agents.

**Keywords:** Antimicrobial activity; banana peels, apple peels, phytochemical screening,

# Résumé

**Analyse antimicrobienne des extraits hydroalcooliques de pelures de pomme et de banane contre des souches microbiennes**

***Résumé***

La présente étude contribue à la valorisation de deux pelures de fruits, la pomme (*Malus domestica*) et la banane (*Musa paradisiaca*), qui sont généralement considérées comme des déchets agro-industriels. Dans ce travail, deux solvants organiques ont été utilisés, l'éthanol et le méthanol, pour étudier les activités antibactériennes de ces écorces de fruits à différentes concentrations : 200, 100, 50 et 25 mg/ml. Les différents extraits ont été testés contre cinq souches bactériennes pathogènes : *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, et quatre souches cliniques d'*E. coli* ; en utilisant des tests de diffusion sur disque. Les résultats ont montré que les deux extraits d'écorces de fruits étaient efficaces contre les souches testées. De plus, l'extrait éthanolique de pelure de banane a montré une activité antibactérienne significative, avec la plus grande efficacité de tous les extraits contre *S. aureus* (zone d'inhibition = 26 mm à 200 mg/mL), suivi par *E. coli* avec une zone d'inhibition de 25 mm à 100 mg/mL avec l'extrait méthanolique de banane. De plus, nous avons évalué leur concentration minimale inhibitrice (CMI) par microdilution, dont les valeurs vont de 100 à 1,56 mg/mL. De plus, les extraits de plantes ont été soumis à un criblage phytochimique, qui a détecté la présence de flavonoïdes, de polyphénols, de saponines, de tanins, de quinones et de terpénoïdes. L'analyse quantitative a révélé une richesse notable en flavonoïdes, avec des valeurs allant de 22± 0.048 to 53 ± 0.86 mg CE/g. Ces résultats valident le potentiel antimicrobien de ces sous-produits de fruits et indiquent leur utilité potentielle comme agents thérapeutiques.

**Mots-clés :** Activité antimicrobienne ; pelures de banane, pelures de pomme, criblage phytochimique.

**تحليل مضاد الميكروبات لمستخلصات الكحول المائي لقشور التفاح والموز ضد سلالات ميكروبية**

***ملخص***

تُساهم هذه الدراسة في تقييم قشور فاكهة، التفاح (*Malus domestica*) و الموز (*Musa paradisiaca*) ، واللذين يُعتبران عادةً نفايات زراعية صناعية. في هذا العمل، استُخدم مُذيبان عضويان، الإيثانول والميثانول، لدراسة الأنشطة المضادة للبكتيريا لهذه القشور بتركيزات مختلفة: 200، 100، 50، و25 ملغ/مل. اختُبرت المستخلصات المختلفة ضد خمس سلالات بكتيرية مُمرضة: *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853,؛ واربع سلالات كلينيكية من *E .coli* باستخدام اختبارات انتشار القرص. أظهرت النتائج فعالية لمستخلصي قشر الفاكهة ضد السلالات المختبرة. علاوة على ذلك، أظهر مستخلص قشر الموز الإيثانولي نشاطًا مضادًا للبكتيريا، بأعلى كفاءة بين جميع المستخلصات ضد *Staphylococcus aureus* (متوسط أقطار منطقة التثبيط = 26 مم عند 200 ملغم/مل)، تليها *E coli* مع قطر تثبيط 25 مم عند 100 ملغم/مل مع مستخلص الموز الميثانولي. علاوة على ذلك، قمنا بتقييم الحد الأدنى لتركيز التثبيط (MIC) القيم تراوحت بين 100 و1.56 ملغم/مل .كما خضعت المستخلصات النباتية لفحص كيميائي نباتي، والذي كشف عن وجود الفلافونويدات، والبوليفينولات، والسابونينات، ، والكينونات، والتربينويدات. كشف التحليل الكمي عن ثراء ملحوظ في الفلافونويدات، حيث تراوحت قيمها بين22 ± 0.048 CE/g من المادة الفعالة إلى 53 ± 0.86 CE/g من المادة الفعالة. تُثبت هذه النتائج الإمكانات المضادة للميكروبات لهذه المنتجات الثانوية من الفاكهة، وتشير إلى فائدتها المحتملة كعوامل علاجية.

**الكلمات المفتاحية:** النشاط المضاد للميكروبات؛ قشور الموز، قشور التفاح، الفحص الكيميائي النباتي.

## List of figures

## List of photographies

## List of tables

## List of abbreviations

- ✓ AE: Enzymatic Activity
- ✓ ATB: Antibiotic
- ✓ CFU: Colony Forming Unit
- ✓ CMB: Minimum Bactericidal Concentration
- ✓ CMI: Minimum Inhibitory Concentration
- ✓ CNS: National Statistics Center
- ✓ CSA: South Algerian Center
- ✓ CSP: Socio-Professional Category
- ✓ DDM: Diameter of Inhibition Zone
- ✓ EPS: Exopolysaccharides
- ✓ FAO: Food and Agriculture Organization
- ✓ GL: Glycolipids
- ✓ INSP: National Institute of Public Health
- ✓ INSPQ: National Institute of Public Health Quebec
- ✓ MIC: Minimum Inhibitory Concentration
- ✓ MBC: Minimum Bactericidal Concentration
- ✓ MS: Dry Matter
- ✓ MRSA: Methicillin resistant Staphylococcus aureus.
- ✓ PNNS: National Nutrition and Health Program
- ✓ SCA: South Constantinois of Algeria
- ✓ UV: Ultraviolet
- ✓ WHO: World Health Organization
- ✓ ZA: Zone of Action

# *Table of contents*

# Table of contents

# Table of contents

# Table of contents

# Table of contents

# Table of contents

# *Introduction*

## Introduction

Medicinal plants have been integral to human healthcare since antiquity. Across diverse cultures and geographies, plant-derived remedies have offered effective, natural alternatives to conventional pharmaceuticals. The therapeutic potential of these plants lies in the array of bioactive secondary metabolites they produce, including alkaloids, phenolics, flavonoids, terpenoids, and others, which can exhibit antioxidant, anti-inflammatory, and antimicrobial properties **(Roy *et al.,* 2022)**. In recent decades, scientific interest has intensified in exploring plant byproducts, such as peels, leaves, and seeds, as low-cost, sustainable sources of pharmaceutically active compounds. Many of these by products, often discarded as agricultural waste, represent underutilized reservoirs of antimicrobial agents that can help combat infectious diseases and reduce environmental pollution through waste valorization **(Sagar *et al.,* 2018).**

Among fruit by products, apple and banana peels have attracted attention due to their abundance, ease of collection, and rich phytochemical profiles **(Sagar *et al.,* 2018).**

Apple peels and banana peels, as abundant agro-industrial byproducts, offer rich phytochemical profiles and ease of collection, making them ideal candidates for antimicrobial studies: apple peels contain high levels of phenolic acids such as chlorogenic acid, flavonoids like quercetin glycosides, and other bioactive compounds (**Smetanska *et al.,* 2016**), attributes linked to reduced cardiovascular and neurodegenerative risks due to antioxidant and antimicrobial mechanisms **(Zielińska &Turemko, 2020)**, and have demonstrated inhibitory activity against pathogens including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* **(Bartolini *et al.,* 2022; Mehrabkhani *et al.,* 2023)**; similarly, banana peels often discarded despite being rich in flavonoids (e.g., catechins), phenolic compounds, and trace minerals have shown antibacterial potential against organisms such as *Bacillus subtilis* and *Staphylococcus aureus* **(Kapadia *et al.,* 2015)**, while their well-documented antioxidant properties suggest applications in natural food preservation and pharmaceutical formulations. Leveraging these extracts provides cost-effectiveness and sustainability by valorizing waste, broad-spectrum antimicrobial potential against Gram-positive, Gram-negative, and fungal pathogens, including multidrug-resistant strains **(Kapadia *et al.,* 2015; Sar *et al.,* 2023)** eco-friendly alternatives to synthetic preservatives **(Patocka *et al.,* 2020)**, and additional health promotion through antioxidant and anti-inflammatory effects.

# Introduction

The present study aims to evaluate and compare the antimicrobial efficacy of hydroalcoholic extracts derived from apple and banana peels against a panel of clinically relevant microbial strains. Hence, determine inhibition zone diameters at varying extract concentrations to assess dose-dependent effects. Moreover, compare the relative potency of apple versus banana peel extracts and evaluate which extract shows broader-spectrum or more potent antimicrobial activity. Also, quantify the total flavonoid contents of the two studied fruit peels by spectrophotometry and conduct a phytochemical analysis of these fruit peels.

# *Chapter01:*

# *Studied Fruits and Their Medicinal Importance*

## 1. Apple (*Malus domestica*)

### 1.1. Historical background

The well-known fruits, apples, are cherished by many and have been enriching and stimulating human civilization for thousands of years. They have been found in a wide variety of cultures and civilizations, providing not only nutrition but also presenting diversified scientific effects **(Malik *et al.,* 2024)**.

The apple originated in the Tianshan Mountains of Central Asia, where the main ancestor of modern apples still grows in its natural state **(Taskuzhina*et al.,* 2024)**. Known for its easy storage and long shelf life, the apple has traveled across continents and been cultivated in numerous countries, where the cultivation of various landraces has been a cornerstone of agriculture for many centuries **(Aydın *et al., 2022*).**

Originating from the Junggar Alatau and Terskey Alatau mountains, the reputable *M. sieversii* has spread to many places in Central Asia. In total, there are 80 *Malus* species in Central Asia, including three regions located within the borders of the present territory of the Republic of Armenia: the southern regions of Syunik, Vayots Dzor, and Aragatsotn mountains. From here, the genus *Malus* gradually spread throughout the territory of the Transcaucasia. In ancient Armenian territories, apples were consumed over 50,000 years ago **(Bina *et al., 2021*)**.

The absorption of the apple into the organism was of paramount importance due to its goods and ingredients, many of which provided for longevity and health. The medical properties of the apple were recognized by the wise men of antiquity and were mentioned in ancient medical texts. Apples have played a significant part in the daily life of country people throughout centuries **(Guo *et al.,* 2024)**.

Through various legends and tales, they achieved a high status as early as medieval times. Raising the standard of living for rural people can also be viewed as preserving the traditionally well-established cultivation method of apples, which dates back to the dawn of centuries **(Akparov*et al.,* 2021).**

## 2. Origin

The apple (*Malus domestica*), a member of the *Rosaceae* family, has a long and rich history spanning Europe and Asia. Its linguistic and cultural significance is profound, with

its name reflecting its widespread presence **(Cornille *et al.,* 2012)**. The apple's earliest mentions in historical documents were unrelated to orchards, demonstrating its deeply rooted symbolism **(Haxim*et al.,* 2022)**. Botanical studies of apples date back over 7,000 years, showing their importance across diverse cultures (Figure 1). Mythologically, the apple carries varied meanings, including love, temptation, wisdom, and bounty, influencing literature, art, and traditions throughout European history **(Lv*et al.,* 2023; Zhang *et al.,* 2023).**

**Figure 1.***Malus domestica* **(Plantsam,2019)**

### 3. Botanical Classification of Apple (*Malus domestica*)

*Malus domestica Borkh*, for most, is an apple, the object of a multi-million-dollar fruit crop industry. There are more than 7,000 known cultivars that are grown around the world **(Cornille *et al.,* 2012).** It belongs to the family *Rosaceae*, which is part of the *Magnoliophyta*, the Botanical Kingdom. The genus of the apple is *Malus*, and the species is distinctly '*domestica*.'

The apple tree (*Malus domestica*) belongs to the plant kingdom and is classified as follows:

**Table 1 :** Botanical classification of Malus domestic **(Kim et al., 2022).**

| | |
|---|---|
| **Kingdom** | Plantae |
| **Phylum** | Angiosperms (Flowering plants) |
| **Class** | Eudicots |
| **Order** | Rosales |
| **Family** | Rosaceae |
| **Genus** | Malus |
| **Species** | *Malus domestic* |

## 4. Geographic distribution

### 4.1. In Algeria

The geographic distribution of apple production (*Malus domestica*) in Algeria is primarily concentrated in the provinces of Batna and Khenchela (both in the Mama region), which are known for having climatic conditions most suitable for apple cultivation. The government has supported the expansion of apple farming in these provinces through various initiatives, including the 'Plan National de Development Agricole (PNDA),' which aims to enhance fruit production, particularly in arid and mountainous regions (**Abdessemed*et al.*, 2022)**.

As a result of these efforts, Batna and Khenchela have become the principal apple-producing regions in Algeria, with significant areas dedicated to apple orchards. The provinces together cover a total area of 4,819 ha in Batna and 6,518 ha in Khenchela, accounting for a substantial portion of Algeria's apple production area. Overall, these regions represent key centers for apple cultivation, significantly contributing to Algeria's agricultural economy (**Abdessemed *et al.*, 2022)**.

Apple cultivation was expanded to the regions of Batna, Khenchela, Mascara, Tiaret, Djelfa, and Sidi Bel Abbés. These regions now represent the principal region of apple production in Algeria (Figure 2) **(Sahraoui, 2014)**.


**Figure 2.** A geographical map showing the central apple-producing region in Eastern Algeria is the information source for this map **(Sahraoui, 2014)**

### 4.2. Worldwide

Apple (*Malus domestica*) cultivation is widespread across various regions worldwide, with significant production concentrated in several key countries. China stands out as the largest producer, with major apple-growing regions including Shaanxi, Shandong, and Xinjiang **(Tian *et al.*, 2022).** The United States follows suit, particularly in states like Washington, New York, and Michigan, where diverse cultivars thrive (**De la Peña-Armada & Mateos-Aparicio, 2022).** Turkey also plays a crucial role in global apple production, with favorable growing conditions in the Aegean and Marmara regions. In India, apple cultivation is primarily found in the northern states such as Himachal Pradesh and Jammu and Kashmir, benefiting from the suitable climate( Figure 3) (**Basannagar& Kala., 2013)**

European countries, such as Germany, Italy, and France, also significantly contribute to the global apple industry, with notable production areas in Bavaria, Trentino-Alto Adige, and Normandy, respectively. Additionally, Russia, Poland, Canada, and Iran are important players in apple production, each region cultivating varieties that cater to local tastes and market demands. Overall, the global apple industry is characterized by a blend of traditional orchards and modern agricultural practices, adapting to a range of climatic conditions and consumer preferences **(Weber &Børve *et al.*, 2021).**

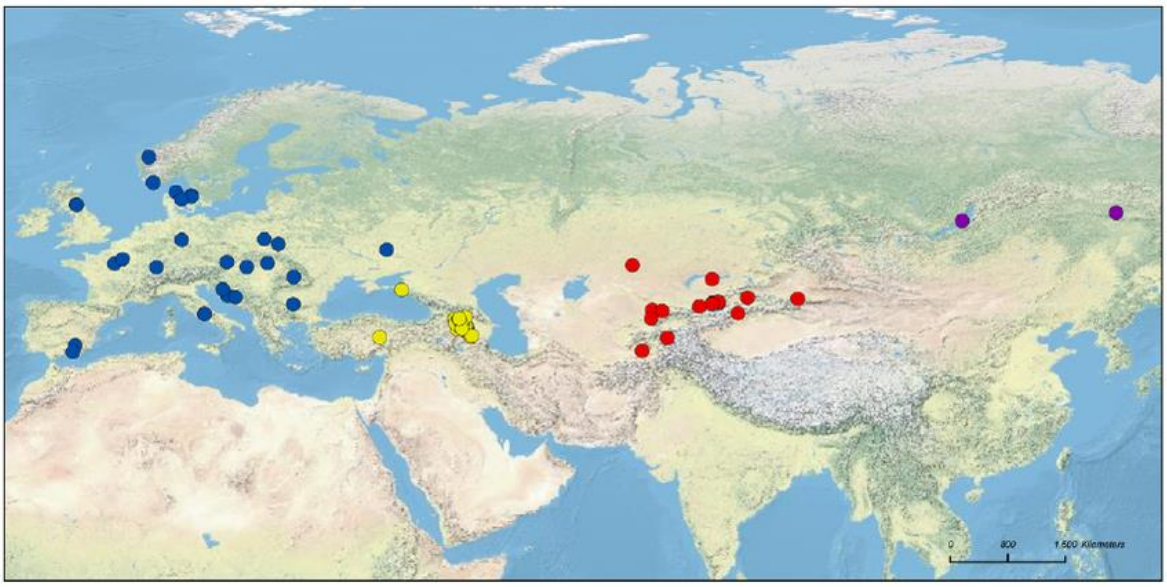


**Figure 3**. The geographic distribution of Malus species: *M. sylvestris* (blue), *M. orientalis* (yellow), *M. baccata* (purple), and *M. sieversii* (red) (**Cornille *et al.*, 2021)**

## 5. Botanical characteristics

The family *Rosaceae* includes a large number of important herbs, shrubs, ornamental plants, and fruit trees. The genus *Malus* belongs to the family *Rosaceae*. *Malus* consists of deciduous trees or shrubs. Trees grow up to 5–10 m tall. Seedling trees will grow about 10 years before they start producing fruit) **(Wang *et al.,* 2024)**.

Trees of several species have produced fruit for over 100 years. Apple trees have a pervasive root system. Appetitive trees and grapes are the most responsive of all the plants to mycorrhizae. Apple trees are planted about 3.7–4.6 m apart in rows of 6–8 trees, which provide a maximum of 1600 trees per hectare. Generally, in superfected gardens, apple trees are planted about 4–6 m apart. There are about 2500 different varieties of apples grown in the United States and 7500 varieties grown throughout the world **(Zuma, 2021)**

## 6. Biochemical composition

Apples (*Malus domestica Borkh*) are cultivated and consumed worldwide, thriving in over 100 countries, primarily in temperate climates. They provide essential vitamins, minerals, and fiber. Research on apple metabolites has progressed for over a century, initially focusing on primary metabolites, such as sugars. Since 1990, techniques such as GC/MS have enabled the detailed analysis of metabolite profiles, identifying approximately 100 peaks **(Van Lanen, 2022).** Metabolites are categorized as primary, which are essential for growth and environmental interactions, and secondary, which enhance nutrition and functionality without directly aiding growth or reproduction. Overall, they illustrate the interplay of genetics, environment, and biological processes**. (Patocka *et al.,* 2020)**

### 6.1. Primary metabolites

Carbohydrates, proteins, lipids, and organic acids are examples of primary metabolites, which are important substances required for plant growth and development. In apples (*Malus domestica*), these metabolites are important for fruit development and quality. **(Tijero *et al.,* 2021)**

**Acquavia *et al.* (2021)** presented a detailed assessment of various analytical methods for extracting and identifying primary and secondary metabolites in apple fruits. This study

highlights the importance of these chemicals in influencing the nutritional and sensory properties of apples.

Additionally, **Bagni *et al.* (1986)** investigated the absorption, transport, and metabolism of aliphatic polyamines in apple leaves and fruits. Polyamines, chemical molecules with numerous amino groups, play a role in cell division and growth, demonstrating their importance as key metabolites in apple development.

### 6.2. Secondary metabolites

Plants possess an array of secondary metabolites that are not directly essential for their growth but play a substantial role in plant defense and the quality of fruits and vegetables. Many of them make the fruit more attractive to organisms, playing a role in its dissemination and potentially representing important fitness advantages for the plants (**Roy*et al.,* 2022).**

Particularly in *Pomaceae*, the concentration of a specific subset of secondary metabolites is particularly high, including phenolics, flavonoids, and terpenes**(Bi *et al.,* 2024)**.

These compounds play crucial roles in the growth and development of the plant and, in particular, fruit, as well as defense against human pathogens. Thus, they have always been a relevant group of compounds of interest in the food industry due to the multitude of health-promoting effects attributed to it, such as cardiovascular health, anti-inflammatory properties, and the prevention of dementia and cancer (**Hilal*et al.,* 2024**).

## 7. Biological activities of apple peels

Apple peels are a rich source of phenolic compounds, including flavonoids and hydroxycinnamic acid derivatives, which possess significant biological activities. Several reports have highlighted the health-promoting, anti-aging, antimicrobial, anti-obesity, antiviral, and antioxidant properties of apple peels or their extracts, prompting the conversion of waste apple peels into high-value-added APIs (**Smetanska*et al.,* 2016)**.

### 7.1. Antimicrobial activity

Apple peels exhibit significant antimicrobial activity, as recent studies indicate, against various microbial pathogens, including *Vibrio*, *Escherichia*, *Salmonella*, *Staphylococcus*, and *Candida*. Much attention has been directed toward the effectiveness

of apple peel extracts in countering pathogens implicated in food spoilage and serious illnesses (**Rehab*et al.,* 2018; Suriyaprom*et al.,* 2022)**.

Compounds like polysaccharides, flavonoids, and phytosterols, identified in the phytochemical screening of apple peel, are likely linked to these antimicrobial effects. These insights lay the groundwork for investigating the use of apple peel materials as natural preservatives in food processing and health products. Research has focused on assessment techniques and understanding the antimicrobial mechanisms at play (**Bartolini *et al.,* 2022; Sar *et al.,* 2023).**

Various analytical methods, including large-scale studies, 96-well microplate assays, and microdilution approaches, have been employed to evaluate the effectiveness of apple peel extracts against diverse microorganisms (**Staš *et al.,* 2024)**.

### 7.1.1. Antimicrobial Mechanisms

The antimicrobial activity of apple peels is attributed to their high phytochemical content, including phenolic compounds, anthocyanins, and organic acids, which disrupt the microbial cell walls, leading to cell lysis (**Mehrabkhani *et al.,* 2023).** Mature apple peels, rich in flavonoids, exhibit strong antifungal, antioxidant, and antibacterial properties. This study found a dose-dependent inhibition of microbial growth by apple peel dichloromethane extracts, highlighting their potential as natural preservatives against food spoilage and postharvest diseases. The overuse of synthetic preservatives contributes to the rise of resistant pathogens, which in turn impacts agricultural exports. Exploring natural antibacterial and antifungal compounds from apple peels could enhance food safety while reducing harmful side effects **(Patocka *et al.,* 2020)**. Understanding their antimicrobial mechanisms is crucial for improving food preservation strategies **(Bartolini *et al.,* 2022).**

### 7.2. Antioxidant activity

Apple peels contain a high concentration of different classes of antioxidants, such as ascorbic acid (vitamin C), organic phenolic compounds in the forms of flavonoids, thiol compounds, carotenoids, and ascorbic acid, which contribute to more effectively preventing oxidative damage. The antioxidant activity and profile of apple peel from 50 different apple cultivars have already been reported. The results showed that the apple peel from a superior cultivar has a higher antioxidant capacity compared to the other tested cultivars (**Zielińska*et al.,* 2020)**.

Apple peel consists of more antioxidant compounds than apple flesh. It also contains a much higher total amount of such compounds than the amount inherent in the apple flesh based on an analysis of the entire apple (peel + flesh). Moreover, apple peel provides a significant portion of the health-promoting compounds contained in the entire apple (**Wolfe & Liu, 2002).**

Therefore, the consumption of the whole apple is more health-beneficial than consuming only apple flesh or undertaking the common practice of peeling the skin before eating the apple. In studies of nutritional health related to cancer and cardiovascular disease, the potential benefits of antioxidants are believed to outweigh the risks posed by pesticides used **(Boyer & Hai Liu., 2004).** Apples contain potent antioxidants that contribute significantly to various health benefits. Various reports on health benefits could be derived from apple peel. During apple processing, the peel of 'Fuji' pre-washed apple may be consumed directly as a health-promoting natural drug **(Ko & Ku, 2022).**

### 7.2.1. Antioxidant Mechanisms

Apple peels are rich in phenolic compounds, such as flavonoids and phenolic acids, and exhibit strong antioxidant properties by neutralizing reactive oxygen species (ROS) and preventing oxidative damage to lipids, proteins, and DNA (**Zielińska & Turemko, 2020).**

These polyphenols scavenge free radicals through electron transfer and structural modifications, effectively protecting protein thiols from oxidative dysfunction. Research indicates that apple peel extracts, even in low concentrations, enhance the body's antioxidant defense via the Nrf-2/ARE signaling pathway, boosting the production of glutathione, catalase, and superoxide dismutase while inhibiting ROS-induced inflammation (**Denis *et al.,* 2013).** Additionally, apple peel compounds may contribute to delaying aging and preventing chronic diseases. Further studies are needed to assess how different cultivation conditions influence its antioxidant capacity, emphasizing its potential in health, food, and pharmaceutical applications **(Plos one, 2022).**

## 8. Banana (*Musa spp.*)

### 8.1. Historical background

Bananas (*Musa spp.*) is one of the world's oldest cultivated crops, with their origins traced back to Southeast Asia, particularly in regions of modern-day Malaysia, Indonesia, and the Philippines, around 5000–8000 BCE **(Perrier *et al.,* 2011)**. The domestication process was facilitated by vegetative propagation and the selection of plants that produced fruit with edible pulp and few seeds. The mounding cultivation system for bananas began approximately 6,450 years ago, alongside the domestication of other staple crops like wheat and rice. Early domestication efforts led to the development of seedless bananas through the hybridization of wild species like *Musa acuminata* and *Musa balbisiana* **(De Langhe *et al.*,2009).**

During the Islamic expansion (7th–10th century CE), bananas were transported to the Middle East and North Africa. By the 15th century, Portuguese explorers carried them to the Canary Islands and later to the Americas, where they flourished in tropical regions **(Al-Busaidi, 2013).**

The 19th and 20th centuries witnessed the commercialization of bananas, particularly in Central and South America, driven by companies such as the United Fruit Company (now Chiquita) and Standard Fruit Company (now Dole). This period marked the dominance of the *Gros Michel* variety until the 1950s when Panama disease (caused by *Fusarium oxysporum* f. sp. *cubense*) led to its replacement by the *Cavendish* variety, which remains the most widely consumed banana today **(Coleman, 2020).**

### 8.2. Origin

The genus *Musa* comprises plants native to tropical Southeast Asia, which mainly include populations in Malaysia and Indonesia, as well as the coastal areas of the western tropical Pacific. However, some *Musaceae* species can be found in various regions, ranging from northern India to southwestern Hawaii and from New Guinea to northern Australia. Plants belonging to the *Musa* species generally grow well in a hot and humid climate with much rainfall **(Jenny *et al.,* 2007).**

Bananas (*Musa spp*.) are thought to have originated in the tropical regions of Southeast Asia and the Indo-Malay Archipelago, specifically in what is now Malaysia, Indonesia,

and the Philippines **(EFSA *et al.*,2021).** Their wild ancestors, *Musa acuminata* and *Musa balbisiana*, were naturally distributed across these areas, and early domestication led to the emergence of edible, seedless varieties **(Sardos *et al.*, 2021).**

From Southeast Asia, bananas gradually spread westward to the Indian subcontinent, where they were mentioned in ancient Sanskrit texts as early as 500 BCE. The fruit was later introduced to the Middle East and North Africa through Islamic trade networks between the 7th and 10th centuries CE (**Salas-Pascual & Cáceres-Lorenzo, 2022)**. During the 15th century, Portuguese explorers transported bananas to the Canary Islands, which eventually facilitated their introduction to the Americas during the colonial era **(Román-Busto *et al.*, 2012).**

## 9. Botanical classification

Bananas and plantains belong to the genus *Musa*, which is part of the *Musaceae* family. The classification of *Musa spp.* is as follows:

**Table 2 :** Botanical classification of musa spp. **(GRIN, 2009).**

| | |
|---|---|
| **Kingdom** | Plantae |
| **Phylum** | Tracheophyta (Vascular plants) |
| **Class** | Liliopsida (Monocotyledons) |
| **Order** | Zingiberales |
| **Family** | Musaceae |
| **Genus** | Musa |

## 10. Geographic distribution

### 10.1. In Algeria

Banana (*Musa spp*.) cultivation is not generally associated with Algeria. Alas, few other fruits are better adapted to the dry, arid conditions and poor, salty soils of the higher steppes, and the bananas have proved tractable enough to produce in the environs of Guelma and Skikda in the guise of the Chiquita banana. However, by the early 1980s, fewer than 200 ha of Musa spp. were cultivated in Algeria as a whole (**Chabi *et al.*, 2018).**

The success of this modest experiment led to the launch of a national plan, resulting in the plantation of 220 hectares of banana trees in several coastal wilayas, where heat and humidity levels are high. As a result of this initiative, Algeria produced four banana varieties: *Petite Naine*, *Grande Naine*, *Williams*, and *Poyo*. The taste was considered good,

and the yield was significant, with the weight of banana bunches ranging from 20 to 50 kg and a planting density of 2,500 plants per hectare. However, the resumption of banana imports from abroad led to a complete halt of this activity in 2002 **(Bourihane, 2017).**

In recent years, banana production has gradually resumed in a few coastal wilayas, including Jijel, Algiers, and Tipaza **(APS, 2018).**

In Jijel, according to the Director of Agricultural Services, the wilaya experienced significant banana production in the 1980s. Today, two types of production are identified: the first was carried out in greenhouses in 2019, while the second, in multi-span greenhouses, was planned for November 2020 **(APS, 2020).**

### 10.2. Worldwide

Around the world, bananas *(Musa spp*.) are grown in tropical and subtropical areas, mostly between 30°N and 30°S latitude, where warm temperatures, high humidity, and enough rainfall provide ideal growing conditions. Asia, Latin America, Africa, and Oceania are the primary regions for banana production. Asia is the world's largest producer, with India accounting for over 26% of global production. China, the Philippines, Indonesia, and Thailand are next in line **(FAO, 2023).** The world market for banana exports is dominated by Latin America and the Caribbean, with Ecuador, Colombia, Costa Rica, and Guatemala serving as the leading suppliers, especially for the Cavendish variety. **(Tripathi *et al.,* 2019).** In Africa, countries such as Uganda, Nigeria, Ghana, and Cameroon are significant producers, with Uganda having one of the highest per capita banana consumption rates, particularly for cooking bananas (*Matoke*) ( Figure 4) **(Ploetz, 2020).**

Bananas rank among the most internationally traded fruits, with key importing areas comprising the European Union (EU), the United States, China, Japan, and Russia. The EU constitutes one of the largest banana markets, importing bananas from Latin America and Africa, whereas the U.S. predominantly relies on imports from Ecuador, Costa Rica, and Colombia **(FAO, 2023).** Demand has risen in China and Japan for bananas sourced from the Philippines and Ecuador. For more than 400 million people residing in tropical areas, bananas serve as a vital staple food. However, their production is under considerable threat due to climate change, Panama disease (Fusarium TR4), and

disruptions in the supply chain **(Ploetz, 2020).** Through genetic research and enhanced farming practices, efforts are ongoing to bolster disease resistance and sustainability in global banana cultivation **(Tripathi *et al.*, 2019).**


**Figure 4.** Apple evolutionary in the world (**Duan, 2017)**

## 11. Botanical characteristics

Banana plants can adapt to non-tropical growing conditions beyond their original environment, allowing them to be cultivated in many regions worldwide, with Antarctica being the only exception. Grasses' giants are generally plants that reach a height of 1.8–3.0 m. Nevertheless, depending on factors such as the type and growth conditions, a banana plant can grow to heights ranging from under 1.0 m to as much as 9.0 m **(OluwatomideOyeyinka& Jide Afolayan, 2020)**. The trunk, which has no secondary growth, consists of tightly clasped leaf sheaths. Even though the leaves are situated entirely above the ground, they are called a false stem or pseudostem and can endure powerful winds from typhoons or agricultural areas. The vegetation is green **(Christophe *et al.*, 2024)**. In cooler areas, though, the pseudostem may redden. Each node represents the position of a leaf, while aerial roots emerge from the bottom side of the internode **(Martin *et al.*, 2023)**. The majority of *Musa* species exhibit a fan-like growth habit, categorizing them as fan plants. Conversely, ensetes exhibit a growth habit resembling that of a pseudomato tree. This genus, which originates from Ethiopia and the adjacent plateau region, comprises enseteventricalosum. On the other hand, there are mountain bananas

native to the Gili area of Nepal and the Himalayas, which have a fan-like dwarf growth habit and a height of less than 1.8 **m (Figure 5) (Mertens *et al.,* 2021).**

**Figure 5.** Banana plant with ripening fruit bunch **(Mertens *et al.,* 2021)**

## 12. Biochemical Composition

Bananas are a highly nutritious fruit that offers vitamins, minerals, carbohydrates, protein, and lipids. They also contain important secondary metabolites, such as vitamins and polyphenols **(Narwal *et al.*,2024)**. Understanding their biochemical composition can enhance health benefits. While the nutritional profile of bananas is not as well-known as other fruits, recent studies have detailed the chemical makeup of bananas, including the fruit, flower, roots, and other parts. This has highlighted the potential of bananas for developing functional foods with superior health benefits **(Kumari *et al.,* 2023; De Souza *et al.,* 2024).**

A variety of bioactive compounds like phenolics, carotenoids, terpenoids, alkaloids, and vitamins in fruiting bodies and stems have diverse pharmacological properties. These compounds enhance endogenous antioxidant systems and immunological responses by inducing cytoprotective enzymes **(Amiri *et al.,*2024)**. Many secondary metabolites at low

concentrations are absent from official food composition databases due to their complex nature. However, non-essential compounds significantly affect fruit and leaf health, promoting redox balance, radical scavenging, and neuropharmacological activities **(Chen *et al.,* 2022).** Flavonoids are particularly noted for their neuroprotective effects against oxidative stress-related damage **(Jazvinšćak *et al.,* 2023)**. Rat studies showed that rutin and luteolin effectively counteract oxidative insults in brain homogenates, with high degradation of the pentose phosphate pathway preventing cell apoptosis. Additionally, flavonoids and polyphenolic compounds influence the gene expression of monoamine oxidase isoforms **(Das *et al.,* 2024; Faysal *et al.,* 2024).**

### 12.1. Primary metabolites

Primary metabolites are essential biomolecules that play fundamental roles in the growth, development, and metabolism of plants. In bananas (*Musa spp*.), the primary metabolites include carbohydrates, proteins, lipids, amino acids, and organic acids, which contribute to the fruit's nutritional and physiological properties **(Drapal *et al.,* 2019).**

Carbohydrates are essential for energy and structure, comprising simple and complex molecules. Key sugars include glucose, fructose, sucrose, and xylose, with glucose and fructose increasing during banana ripening while sucrose decreases (**Holesh*et al.,* 2023)**. Tropical bananas tend to have higher sucrose content. ATP-derived glucose boosts sugar phosphate and starch levels, with key processes involving pentose phosphate formation and starch synthesis **(Aggarwal, 2021).**

### 12.2. Secondary metabolites

Bananas have a relatively large array of secondary metabolites, including simple molecules such as terpenes, sterols, and other lipids, as well as alkaloids and phenolic compounds such as phenylpropanoids, flavonoids, and aminophenoliccompounds **(Mondal *et al.,*2021)**. The chemical diversity of secondary metabolites can vary based on various factors, starting with species; for example, the phenylpropanoids present in pineapples are not the same as those found in apples or grapes. Similarly, the banana is a complex of species **(Krishnamurthy *et al.,* 2023)**

### 12.2.1. Alkaloids

Alkaloids are defined as nitrogen-containing secondary metabolites with potent physiological and toxicological effects. Accordingly, quinolone alkaloids are known to have antiprotozoal activities **(Pereira *et al.,* 2023)**. They can also act as photosystem II electron diverts, which is lethal to the involved plant cells. Although the complete structure of cinchonains has not been resolved, they represent two cyclic architectures that have been modified at various positions, resulting in their structural diversity **(Rajput *et al.,*2022).** One of the cinchona alkaloids that has shown promise as a preventive treatment in animal models is quinolinic acid **(Dhruv *et al.,* 2022).**

### 12.2.2. Phenolic Compounds

Phenolic compounds are also involved in the defense mechanisms of plants. They exhibit in vitro antioxidant and radical scavenging activities under different experimental conditions. Phenolics possess health-promoting effects since the antioxidant defense system in living organisms can be significantly enhanced **(Al Mamari, 2021)**. Diets enriched with phenolic compounds may reduce the risk of chronic diseases in humans **(Yu *et al.,* 2024)**. The main structural characteristics of bananas are phenolic compounds, which are mostly derivatives of cinnamic acid **(Kaczmarek-Szczepańska *et al.,* 2023)**. Flavonoids have a skeleton with a C6-C3-C6 structure. Anthocyanins are a class of flavonoids involved in the sensory attributes of fruits. Leucoanthocyanidins are compounds belonging to the class of flavanols having a flavan core **(Ben Alaya *et al.,* 2024).**

Anthocyanins are key water-soluble **pigments** in plants, serving as antioxidants against oxidative damage. The biosynthetic pathways of flavonols indicate that 2-oxoglutarate-dependent dioxygenase is crucial for banana skin synthesis, while H3-CoA-ligase converts 4-coumarin-CoA into early flavonoids such as naringenin chalcone **(Méndez-Galarraga *et al.,* 2025)**. Tartaric and citric acids contribute to banana fruit color stability during storage. Additionally, bananas have phenolic compounds that influence color and aid in anthocyanin synthesis. Studies reveal that phenolic levels in bananas decline due to environmental effects on polyphenols and antioxidants, although late harvesting can enhance total phenolic content (**Jongrattanavit *et al.,*2024)**. Variations in phenolic content and activity in bananas stem from cultivation methods, ripening stages, shelf-life, and processing. These compounds are vital for protecting plants from stress and pathogens and

possess therapeutic potential for human diseases influenced by genetic and environmental factors. **(Manzoor&Ahmad, 2021).**

## 13. Biological activities of banana peels

### 13.1.1. Antimicrobial activity

Banana fruit has a large number of bioactive compounds. The peels of banana fruit, which constitute about 30% of the weight of the fruit, are a significant component of the fruit discard. In recent years, the pharmaceutical, food, and cosmetic industries have been emphasizing the use of banana peels due to their potential for waste recycling and reduced health risks. The bioactive compounds found in the peels are characterized as potent antimicrobial agent**s (Gupta *et al.*,2022).**

Several studies suggest that banana peels possess antibiotic properties and could serve as an alternative to common antibiotics. Research indicates that these peels contain bioactive compounds like polyphenols, flavonoids, tannins, and macrosimpsonellin. Hydroacetone extracts of both unripe and ripe banana peels showed similar bioactive chemicals, while unripe peels uniquely contained macarthurin, simpterol, ampelopsin H, and β-arabanase. Additional tests of water extracts from different banana cultivars demonstrated effective antibiotic activity against pathogenic E. coli strains, K12 and O157:H7**(Chaudhry *et al.*,2022)**. Further research is needed to investigate the potential of banana peels in combating bacterial infections and extending product shelf life. Overall, these findings underscore the importance of banana peels' antibiotic properties in daily life (**Kanedi, 2023).**On the other hand, the antimicrobial property of banana peel extracts has been shown to inhibit the majority of pathogenic bacteria, including *Staphylococcousacareus, Bacillus subtilis, Staphylococcus faecalis*, and *Escherichia coli* (**Singh *et al.*, 2022)**.

### 13.1.2. Mechanisms of Action

The antimicrobial properties of banana sap and both unripe and ripe peel extracts were assessed using the agar well diffusion method, with comparisons made to kanamycin as a positive control for bacterial inhibition and nystatin for fungal inhibition. The sap extracts are derived from *Musa acuminata* var. Tanduk exhibited a broad-spectrum antimicrobial effect, achieving a mean inhibition zone of 8 mm against the various microorganisms tested. Both unripe and ripe peels displayed considerable antimicrobial

activity, with a mean inhibition zone of 15 mm. Additionally, the ripe peel and sap of *M. acuminata*, when extracted using ethanol, exhibited a notably significant inhibitory effect on bacterial growth **(Gupta *et al.,* 2022; Mihai *et al.,* 2024).**

### 13.2. Antioxidant activity

Banana peel, a by-product of the fruit industry, exhibits numerous bioactivities, including antioxidant properties. Free radicals can harm or benefit the body, and antioxidants neutralize these free radicals to counteract their harmful effects. Research is ongoing to explore the role of natural antioxidants in reducing the effects of aging and chronic diseases, with reviews highlighting their potential benefits **(Sundaram *et al.,* 2011).** Bananas (Musa spp.), a widely popular fruit, are rich in dietary nutrients, with the peel making up about 6% of their weight. Due to bioactive compounds, banana peels demonstrate various biological activities, including potent antioxidant effects **(Bashmil *et al.,* 2021).**

Banana peel extracts exhibit significant antioxidant and antimicrobial activities due to their rich composition of bioactive compounds, including polyphenols, flavonoids, tannins, and alkaloids. The antioxidant activity primarily functions through free radical scavenging, where these compounds donate electrons to neutralize reactive oxygen species (ROS), preventing oxidative damage (**Mihai *et al.,* 2024)**. Additionally, banana peel antioxidants chelate metal ions, such as $Fe^{2+}$ and $Cu^{2+}$, reduce their ability to generate free radicals through Fenton reactions. They also enhance endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), which further contribute to cellular protection against oxidative stress. Another key mechanism is the inhibition of lipid peroxidation, which helps protect cell membranes from oxidative degradation, playing a crucial role in neuroprotection and cardiovascular health (**González-Montelongo *et al.*,2010).**

# *Chapter 02:*

## *Study of Targeted Microorganisms*

## 1. General Aspects of Studied Microbial Strains

Different microorganisms have demonstrated antimicrobial properties through various compound types for several decades. The strains exhibit distinct metabolic characteristics, as well as special growth patterns and unique gene regulation systems, which contain antibiotic resistance resulting from horizontal gene transfer. The range of microbial strains enhances research on antimicrobial mechanisms using principles from complex systems **(Urbaniak *et al.*, 2021**).

The text investigates how co-cultures adapt through their species interactions and the development of constraints, as well as their environmental effects. Research in this field is promisingly creating new antimicrobials that medical practitioners require to combat pathogens and biofilms. Researchers are focusing increasingly on identifying antimicrobial agents that originate from microbes, as well as chemically produced substances. Research organizations have demonstrated the impact strain variability has on growth behavior and antagonistic activities in their efforts to strengthen natural immune responses **(Salazar & Mitri., 2025).**

Research progress on understanding complex structural and pattern aspects of microorganisms remains limited to this point. Different test results obtained from multiple species limit scientists' grasp of multi-level ecological relations. The investigation of these processes is conducted through simple model ecosystems because existing experimental conditions present significant issues, as noted by **(Touzani *et al.*, 2021).**

## 2. Microorganisms

### 2.1. *Escherichia coli* (*E. coli*)

#### 2.1.1. Definition

*Escherichia coli* express rod morphology as a Gram-negative facultative anaerobic bacterium that exists in the *Enterobacteriaceae* family from the *Proteobacteria* phylum. Wild populations of *E. coli* exist within the digestive systems of endotherms, which encompass the majority of birds and mammals **(Ahmed, 2021)**.

The microbial genus contains numerous species, among which *E. coli* represents one particular species formerly known as *Bacterium coli* commune. The bacterial species *E. coli* exists in various physical forms, revealing its capacity to cause harm. Pathogenic

strains develop from non-pathogenic strains that exist within *E. coli*, although their initial state does not cause illness. Researchers have conducted thorough investigations on *E. coli* due to its dual value as both a model organism in the laboratory and a valuable organism in environmental and medical contexts. **(Balkrishna *et al.*, 2021)**


**Figure 6.** *Escherichia coli* (*E. coli*) **(Encyclopædia Britannica,2025).**

### 2.1.2. Classification

Classification of *Escherichia coli (*E. coli) is presented in the table 3:

**Table 3**: Taxonomic Classification of *Escherichia coli (E. coli*) (**Nwabunwanne, 2022)**

| **Domain** | ***Bacteria*** |
|---|---|
| **Phylum** | Proteobacteria |
| **Class** | Gammaproteobacteria |
| **Order** | Enterobacterales |
| **Family** | Enterobacteriaceae |
| **Genus** | Escherichia |
| **Species** | *Escherichia coli* |

### 2.1.3. Habitat

*E. coli* exists as a gram-negative facultative anaerobic bacterium, which naturally finds its residence within the gastrointestinal regions of warm-blooded animals, including humans while facilitating digestion along with vitamin production. Many strains of *E. Coli*

do not harm the body, but pathogenic variants predispose people to critical infections **(Makvana& Krilov, 2015; Mueller & Tainter, 2023)**

*E. coli* inhabits both water environments and the guts of animals, serving as a vital microorganism for assessing water contamination during quality assessments. The bacterium primarily resides in water bodies that contain sewage effluents and agricultural runoff. *E. coli* establishes survival mechanisms in soil and sediments, particularly in wet and nutrient-rich environments where it develops biofilm colonies (**Navab-Daneshmand*et al.,* 2018)**.

Who (2017) describes *E. coli* as a common bacterium detected in food products that risk causing illnesses through raw meat, unpasteurized milk, and unclean vegetables. Healthcare facilities must be concerned about antibiotic-resistant *E. coli* strains, including that expressing extended-spectrum beta-lactamase (ESBL), as they frequently cause healthcare-associated infections. Public health monitoring requires continued attention to *E. coli* due to its widespread distribution across many ecological environments **(Conway & Cohen, 2015).**

### 2.1.4. Pathogenic potential

The pathogenic *E. coli* strain *Escherichia coli* poses a high risk for infections due to its virulent qualities, which can produce serious intestinal and additional external infections affecting humans as well as animals. Pathogenic *E. coli* exists in two main varieties: diarrheagenic strains and extraintestinal pathogenic *E. coli* (ExPEC) (**Sora *et al.,*2021)**.

ETEC modes of infection produce heat-labile (LT) and heat-stable (ST) enterotoxins, which primarily induce watery diarrhea in developing countries. Enterohemorrhagic *E. coli* (EHEC), as one of its strains, is composed of *E. coli* O157:H7 that releases Shiga toxins (stx1 and stx2), resulting in hemorrhagic colitis with the potential development of fatal hemolytic uremic syndrome (HUS) **(Joffré*et al.,* 2023).**

The two important diarrheagenic *E. coli* strains include Enteropathogenic *E. coli* (EPEC), which destroys intestinal microvilli, and Enteroaggregative *E. coli* (EAEC), which creates biofilms and releases cytotoxins to cause extended diarrhea (**Pakbin *et al.,* 2021).**

## 2.2. *Staphylococcus aureus*

### 2.2.1. Definition

The Gram-positive bacterium *Staphylococcus aureus* comes from the *Staphylococcaceae* family and shows facultative anaerobic coccus characteristics with grape cluster formation habits but also exists as paired and chained cells. The bacteria reside in the facial skin and mucous membranes of all domestic mammals, including birds, with urinary tract infections ranking as the second most common infection **(Taylor & Unakal, 2017; Becker, 2018).**

Medical scientists discovered this bacterium in pus and named it from Greek terms meaning berry and a bunch of grapes. Within the population of healthy humans, *S. aureus* exists as a typical resident bacterium, as around 30% of individuals fall under this category. This population distribution varies according to gender identity, geographical location, and age demographics. Factors like temperature, salinity, and nutrient concentration also influence their growth (Figure 7) **(Yamazaki *et al.,* 2024).**

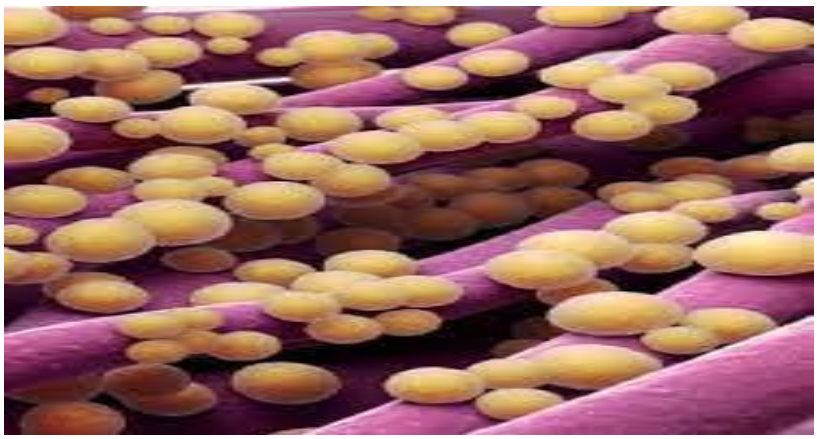


**Figure 7**. *Staphylococcus aureus* **(Yamazaki*et al.*,2024)**

### 2.2.2. Classification

According to Denis *et al*. (2016), the classification of *S. aureus* is represented as follows in table 4:

**Table 4 .** Taxonomic Classification of *S. aureus* **(Denis *et al.,* 2016)**

| **Kingdom** | ***Bacteria/Eubacteria*** |
|---|---|
| **Phylum** | Firmicutes |
| **Class** | Bacilli |
| **Order** | Bacillales |
| **Family** | Staphylococcaceae |
| **Genus** | Staphylococcus |
| **Species** | *Staphylococcus aureus* |

### 2.2.3. Habitat

The bacteria *Staphylococcus aureus* naturally resides on human skin and mucosal surfaces while showing significant and shared heritability between nasal and perianal bacterial isolates from individual persons. Multiple forms of microorganisms inhabit the skin environment, along with potential pathogens. Nasal colonization factors among individuals depend on their age and sex profile, in combination with blood glucose measurements, smoking history, contraceptive usage, renal dialysis treatment, and the presence of skin ailments such as dermatitis and Wegener's granulomatosis **(Burian *et al.,* 2022)**.

The primary population that harbors *S. aureus* consists of humans, although the bacteria maintain resilience in diverse ecological environments, including soil, salt, and non-human skin and mucosal surfaces. The increase in antibiotic medications has led to the development of resistant bacterial strains, while inadequate hygiene measures contribute to the spread of these strains. *Staphylococcal* species from pets, as well as livestock, can invade human bodies through animal contact **(Johannessen *et al.,* 2012)**

### 2.2.4. Pathogenic potential

The pathogenic bacterium *Staphylococcus aureus* exhibits high adaptability, causing severe skin infections, as well as systemic diseases such as impetigo, cellulitis, abscesses, and staphylococcal scalded skin syndrome (SSSS) (**Del Giudice, 2020)**.

The toxin TSST-1, produced by *Staphylococcus aureus*, causes toxic shock syndrome (TSS) and is associated with various complications, including pulmonary conditions, bloodstream infections, heart valve infections, and bone tissue infections. Heating does not

destroy the enterotoxins responsible for food poisoning caused by *Staphylococci* (**Ahmad-Mansour *et al.,* 2021)**.

MRSA infection represents a global public health threat because it is resistant to β-lactam antibiotics, leading to treatment-resistant infections in hospital facilities and community treatment centers. The bacterium poses an international health concern because it effectively colonizes human hosts while maintaining its survival in healthcare areas and developing resistance to antibiotics (**Abebe& Birhanu, 2023).**

## 2.3. *Pseudomonas aeruginosa*

### 2.3.1. Definition

*Pseudomonas aeruginosa* (*P. aeruginosa*) functions as an important pathogen that results in different types of acute and persistent infectious diseases **(Qin *et al.,* 2022).** This bacterium belongs to the *Pseudomonadaceae* family, exhibiting characteristics of aerobic, gram-negative, rod-shaped, motile forms **(Diggle & Whiteley, 2019).**

*P. aeruginosa* demonstrates exceptional versatility in metabolic activity while retaining robust adaptive abilities to handle unfavorable settings, as it is widely found in various locations, including soil, water, sewage, and hospital facilities. Many researchers find the issue of its metabolic substrate selection preferences to be significant. Proof of *P. aeruginosa* occurrence in clinical samples indicates it is necessary to conduct investigations about its environmental origins (**Figure 8)** (**Morin*et al.,* 2021).**

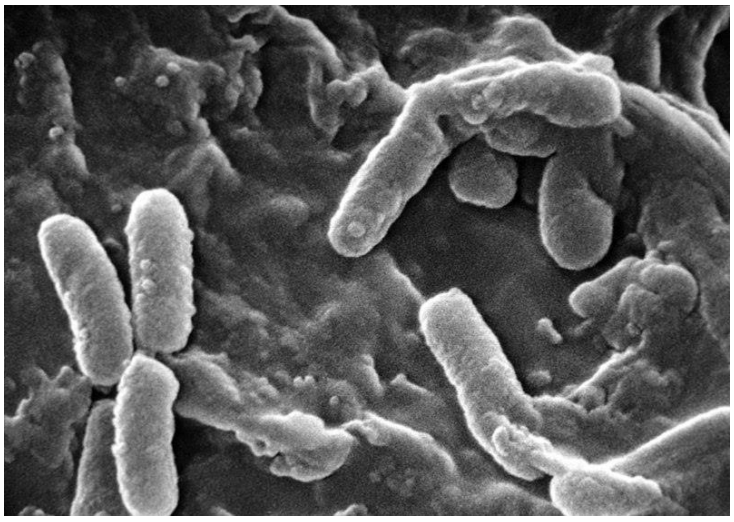


**Figure 8.***Pseudomonas aeruginosa* under scanning electron microscope **(Microbiology in Pictures, 2015).**

### 2.3.2. Classification

The classification of *Pseudomonas aeruginosa*is represented as follows in table 5:

**Table 5.** Taxonomic Classification of *Pseudomonas aeruginosa* (**Benabid,2009)**

| **Kingdom** | ***Bacteria*** |
|---|---|
| **Phylum** | Prokaryota |
| **Division** | Proteobacteria |
| **Class** | Gammaproteobacteria |
| **Order** | Pseudomonadales |
| **Family** | Pseudomonadaceae |
| **Genus** | Pseudomonas |
| **Species** | *Pseudomonas aeruginosa* |

### 2.3.3. Habitat

*Pseudomonas aeruginosa* appears as a flexible, free-living Gram-negative bacterium that continuously inhabits various ecological places, from soils to aquatic ecosystems. The bacterium *Pseudomonas aeruginosa* inhabits a wide range of locations, from plants to wet surfaces, and also exists within extreme habitats such as ice sheets, hot thermal waters, petroleum deposits, and acidic mineral runoff **(Morin *et al.,* 2021)**.

*Pseudomonas aeruginosa* tolerates lengthy osmotic stress, as well as cares for scarce nutrients, harsh temperature extremes, and acidity through its virulence factors and adaptable metabolic abilities. Biofilms formed by this organism develop structural resistance to physical and chemical threats, which hinders their competitive interference with other biological entities. **(Bédard *et al.,* 2016)**

### 2.3.4. Pathogenic potential

The pathogenicity of *Pseudomonas aeruginosa* depends on numerous virulence factors, which makes this bacterium responsible for frequent hospital-acquired infections. Most strains become multidrug-resistant due to their strong antibiotic-resistance capabilities. The biofilms created by *P. aeruginosa* enable effective surface colonization because they protect against environmental stressors, immune responses, and antibiotic exposures. The biofilm matrix consists of primary components, including exopolysaccharides, DNA, and proteins. *P. aeruginosa* maintains colonization through

multiple diverse survival approaches that assist its settling process (**Yin*et al.,* 2022; Tuon*et al.*,2021**).

The microorganism cultivates toxic substances along with enzymes, which destroy host tissues through pathway signaling disruption and membrane disruption. Through its specialized export systems (**Qin *et al.,* 2022**), the bacteria transport critical factors from the cytoplasm into target host cells using channels, such as the type III secretion system and release these factors into the extracellular environment. **(Morin *et al.,* 2021)**

### 2.4. *Bacillus subtilis*

#### 2.4.1. Definition

*Bacillus subtilis* exists as a Gram-positive aerobic bacterium that displays fast growth patterns while maintaining rods as its cell shape. Its cell dimensions range from 2 to 6 µm in length and 1 µm in diameter. Under optimal growth conditions, *Bacillus subtilis* reproduces at a rate of 20 minutes to double its population. The cells establish lengthy chains that remain connected via unfinished septal wall material under certain growth conditions (Figure 9) **(Errington & Aart, 2020).**

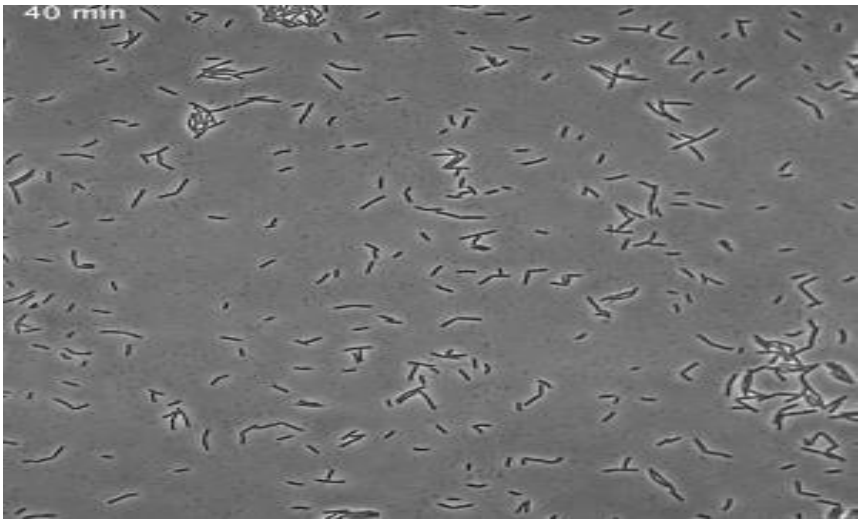



**Figure 9.** *Bacillus subtilis* under electronic microscope (**Płoszaj-Pyrek*et al.,* 2014)**

### 2.4.2. Classification

The classification of *Bacillus subtilis*is represented as follows:

**Table 6 .** Taxonomic Classification of *Bacillus subtilis* **(Barber,2010)**

| **Domain** | ***Bacteria*** |
|---|---|
| **Phylum** | Firmicutes |
| **Class** | Bacillus |
| **Order** | Baciliales |
| **Family** | Bacillaceae |
| **Genus** | Bacillus |
| **Species** | *Bacillus subtilis* |

### 2.4.3. Habitat

The Gram-positive bacterium *Bacillus subtilis* appears rod-shaped and spreads throughout many natural environmental areas. The natural habitat of *Bacillus subtilis* exists in soil because it functions as a critical agent that decomposes organic matter and rotates nutritional substances **(Mahapatra *et al.,* 2022)**. In addition to soil, it is also found in plant rhizosphere areas because it develops protective biofilms that simultaneously enhance plant growth and function as antimicrobial agents against pathogens **(Mageshwaran *et al.,* 2022).**

The commensal organism *B. subtilis* lives in the gastrointestinal tract of animals, including humans, which shows potential benefits for maintaining microbial equilibrium throughout the gut **(Hong *et al.,* 2009).** *B. subtilis* shows a remarkable environmental versatility because it thrives in surface and groundwater aquatic systems, according to **Earl *et al.* (2008).***B.subtilis* maintains survival through endospores capable of enduring harsh environmental conditions, which allows it to inhabit air and dust particles along with specific fermented foods **(Ulrich *et al.,* 2018).**

### 2.4.4. Pathogenic potential

As a bacterium occurring naturally in soil ecosystems, *Bacillus subtilis* shows effectiveness against many fungal and bacterial infections.

- Through the synthesis of fencing together with other lipopeptides, *B. subtilis* inhibits fungal growth by damaging fungal mycelium and spores (**Geissler *et al.*, 2019)**.
- *B. subtilis* establishes a physical occupation by colonizing plant surfaces, which subsequently depletes nutrients and saturates the pathogen ecosystem. This combination hinders the settlement and growth of pathogens. (**Hashem *et al.*, 2019)**.
- Through its presence, *B. subtilis* activates induced systemic resistance (ISR) in plants, resulting in enhanced innate defense functions against pathogens (**Hashem *et al.*, 2019)**.

## 2.5. *Clostridium perfringens*

### 2.5.1. Definition

Belonging to the *Bacillaceae* family, it is a Gram-positive bacillus, a strict anaerobe, non-motile, and spore-forming, classified within the group of sulfite-reducing anaerobes **(Bourgeois &Leveau, 1980)**.

The bacterial organism occurs frequently on Earth, in water, and is often associated with spoiled vegetation, as well as throughout the digestive systems of people and animals. The bacteria produce numerous toxins that enable it to become a leading cause of foodborne illnesses, gas gangrene infections, and enteric infections. *C. perfringens* demonstrates rapid growth under anaerobic conditions, producing heat-resistant endospores as its primary feature, which maintains environmental and food product persistence (Figure 10) **(Rood & Cole, 1991).**

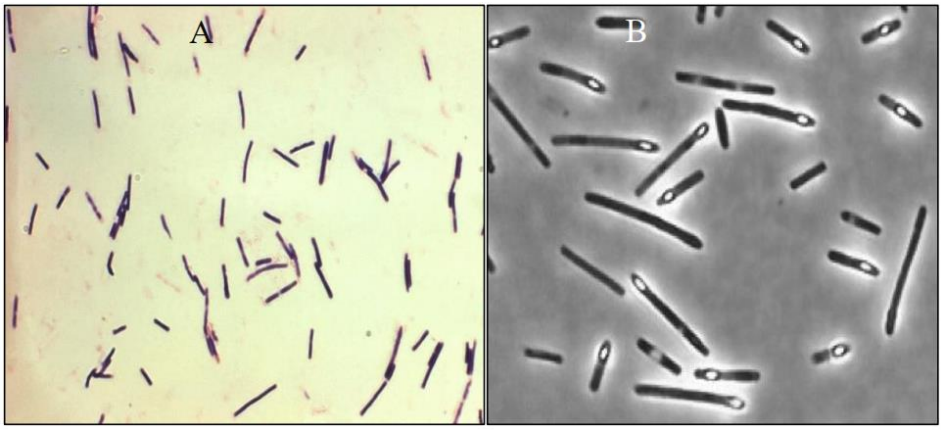



**Figure10**. Different forms of *C. perfringens* spores under a light microscope

**A:** Vegetative cells**- B:** Spores **(Meyer, 2021)**

### 2.5.2. Classification

The taxonomy of *Clostridium perfringens* is as follows in table 7:

**Table 7.** Taxonomic Classification of *Clostridium perfringens*(**NCBI,2020)**

| **Kingdom** | ***Bacteria*** |
|---|---|
| **Phylum** | Bacillota (formerly Firmicutes) |
| **Class** | Clostridia |
| **Order** | Clostri |
| **Thatprimarily** | Clostridae |
| **Genus** | Clostridium |
| **Species** | *Costridium perfringens* |

### 2.5.3. Habitat

*C. perfringens* is a pathogenic agent whose primary targets are humans and animals **(Kiu & Hall, 2018).**This bacterium can be found in various habitats, including the normal flora of the human gastrointestinal (GI) tract and the environment, such as wastewater and soil **(Yao & Annamaraju, 2023).**

### 2.5.4. Pathogenic potential

Several common diseases are associated with *C. perfringens*, including food poisoning, gas gangrene, and numerous veterinary diseases. *C. perfringens* enterotoxin (CPE) is the main virulent factor responsible for triggering several critical GI diseases (Figure 11) **(Uzal *et al.*, 2014).**

This bacterium is also classified into five serotypes, A, B, C, D, and E, based on the extracellular toxins it secretes: alpha, beta, epsilon, and iota **(Grenda *et al.*, 2023).**

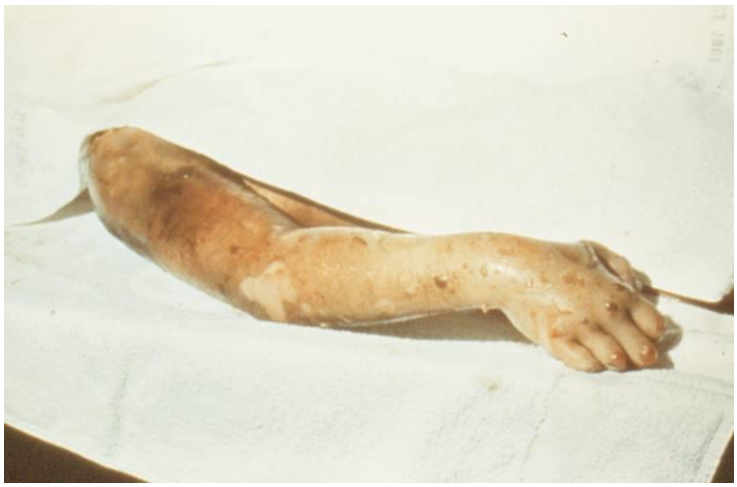


**Figure 11.** Gas gangrene **(Larry &Bush, 2020)**

## 2.6. *Candida albicans*

### 2.6.1. Definition

*Candida albicans* is a cosmopolitan yeast and a commensal of the oropharyngeal, gastrointestinal, and genitourinary mucosae. It can occasionally colonize the skin **(Talapko*et al.*, 2021).**

Morphologically, *C. albicans* always appear as small, round, or oval yeast cells (Coulibaly, 2003). It is characterized by its ability to form chlamydospores and true filaments or hyphae, which play a significant role in its virulence **(Mayer*et al.*, 2013).**

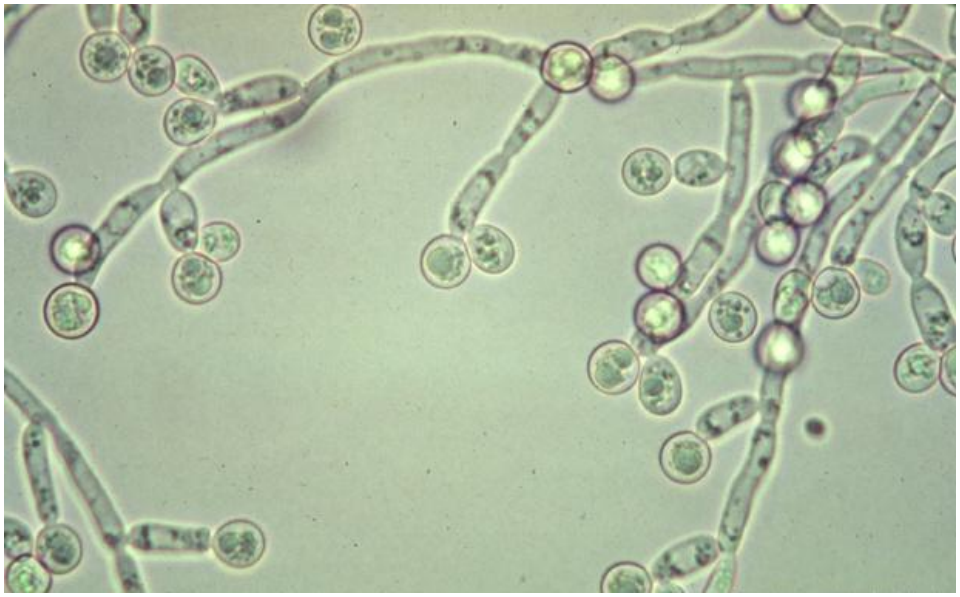


**Figure 12.** *Candida albicans* **(Pierquin, 2010)**

### 2.6.2. Classification

Botanical Classification of *Candida albicans* are presented in table 8**:**

**Table 8.** Taxonomic Classification of *Candida albicans* **(Department of Agriculture, 2021).**

| **Kingdom** | ***Fungi*** |
|---|---|
| **Phylum** | Deuteromycotina |
| **Class** | Blastomycetes (Asexualyeasts) |
| **Order** | Moniliales |
| **Family** | Moniliaceae |
| **Genus** | Candida |
| **Species** | *Candida albicans* |

### 2.6.3. Habitat

*Candida albicans* is a yeast with a variable shape, ranging from round to elongated. It is a commensal organism in the digestive tract of humans, mammals, and birds**(Neville*et al.,* 2015).**

It is not typically found in the environment unless there is contamination by humans or animals. This yeast is an opportunistic pathogen that becomes harmful under the influence of general or local predisposing factors **(Kulesza *et al.,* 2021).**

### 2.6.4. Pathogenic potential

*Candida albicans* is a saprophytic organism that becomes pathogenic when present in large quantities. It causes a burning erythema, sometimes covered with creamy white deposits.On the skin, *Candida* acts as a pathogen, leading to pruritic, erythematous plaques with a scaly appearance **(Rafiq, 2023).**

## 2.7. *Aspergillus niger*

### 2.7.1. Definition

In 1867, Philippe Édouard Léon van Tieghem described the black mold *Aspergillus niger* as the first detailed description of the species **(Dijksterhuis & Wösten, 2013).** A black mold fungus from the Eurotiales order, *Aspergillus niger*, appears on fruits and vegetables **(Raper & Fennel, 1977; Zakaria, 2024).**

The fungus *Aspergillus niger* displays its conidial heads through two radial groups that assemble into numerous brown and black arrayed columns (Figure 13) **(Zulkifli & Zakaria, 2017)**. Agricultural production of cell wall products depends on *A. niger* because of its genetic stability together with its high yields and ability to process low-cost materials **(Książek, 2023)**

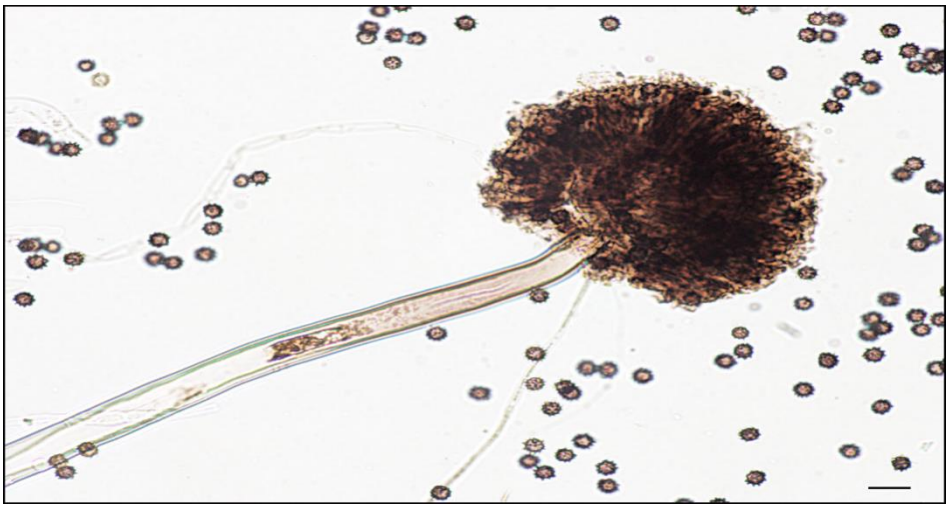


**Figure 13.** *Aspergillus niger* **(INSPQ,2021).**

### 2.7.2. Classification

The taxonomy of *Aspergillusniger*is as follows in table 9**:**

**Table 9.** Taxonomic Classification of *Aspergillus niger* **(Abd Mallick, 2019)**

| **Kingdom** | ***Fungi (mycetes)*** |
|---|---|
| **Division** | Eumycota |
| **Class** | Hyphomycetes |
| **Order** | Moniliales |
| **Family** | Trichocomaceae |
| **Genus** | Aspergillus |
| **Species** | *Aspergillus niger* |

### 2.7.3. Habitat

*A. niger* is common in many temperate and tropical habitats. It is a prevalent species found on various organic substrates, such as cereals and their derivatives **(Abdel-Azeem *et al.*, 2019)**

These fungi thrive on organic matter under aerobic conditions and can also grow on other substrates, including cultivated or polluted soil and grasslands. They can even be found in frozen soils and marine environments, although they typically prefer dry and warm soils (**Šimonovičová*et al.,* 2021**; **Erdel*et al.,* 2023)**.

### 2.7.4. Pathogenic potential

The microorganism *Aspergillus niger* has both industrial importance and the potential to disrupt human health through diseases it causes in humans and plants (**Zakaria, 2024)**. Scientists have proven that infections of the respiratory system originate from this fungus, and studies show that *A. niger* commonly causes hypersensitivity pneumonitis in people **(Mousavi *et al.,* 2016)**.

Its pathogenic properties include mycotoxin formation, together with allergic responses, as well as actions of enzymes and immune system avoidance by conidia. The health of the respiratory system faces additional risks due to exposure to contaminated surroundings **(Heinekamp *et al.,* 2015)**.

# *Chapter 03:*

# *Material and Methods*

## 1. Vegetal material preparation

### 1.1. Obtaining the fruit

We have selected two fruits for our work. For the present study, Banna and apples were purchased from a local market in the city of Khenchela (Algeria) in December 2024.

This research was conducted at the Khenchela University Educational Laboratory.

### 1.2. Cleaning

**This is a crucial step to remove**

- Dirt and debris from handling and transportation.
- Potential pesticide residues.
- Microorganisms that could cause spoilage

### 1.3. Peeling

- This step separates the peels, which are the desired material for the next steps, from the rest of the fruit.
- The method of peeling is not specified (e.g., by hand with a knife or with a peeler).

### 1.4. Drying

This step is essential to:

- Reduce moisture content, which prevents microbial growth (mold, bacteria) and spoilage.
- Concentrate the compounds present in the peels.
- Make the peels brittle for easier grinding.
- This process is achieved away from Sunlight because Sunlight can degrade some heat-sensitive compounds in the peels.

### 1.5. Grinding

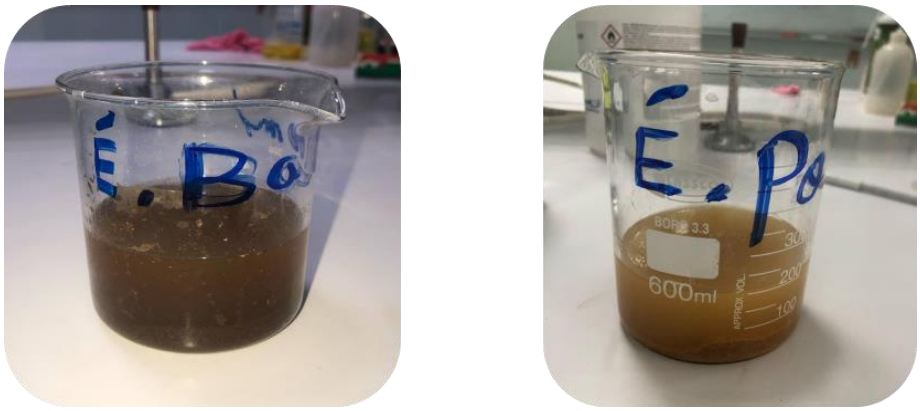
Once the peels were dried, we ground them using a grain mill into smaller particles

## 2. Extract preparation

A mixture was prepared using 60g of both apple and banana peel powder, along with a solvent mixture of 600 ml of 80% ethanol or 80% methanol **(Cowan, 1999).** The mixture

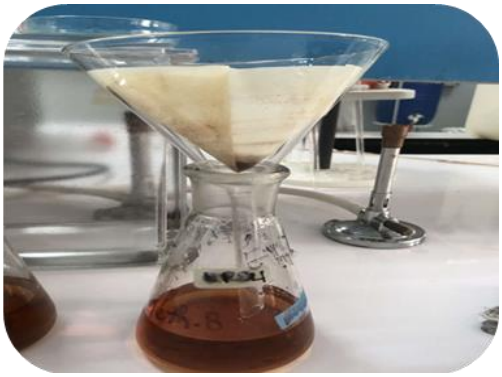
was agitated for 30 to 60 minutes and covered with aluminum foil and allowed to macerate for 24 hours at room temperature, protected from light **(Mau *et al.*, 2001; Abhary& AL-Hazmi, 2015)**

**Photograph 1.**60 g of banana powder on the left and 60 g of apple powder on the right




**Photograph 2.**The ethanolic maceration of apple and banana peels

a. **Filtration:** The resulting extracts were filtered to remove any particulate matter. This was achieved using a gas compressor and filter paper (e.g., Whatman filter paper) to obtain a clear liquid extract.



**Photograph 3.** Filtration due to Whatman paper


**Photograph 4.** Filtration due to gas compressor

**b. Solvent Removal:** The solvents (ethanol and methanol) were removed from the extracts using a rotary evaporator under reduced pressure and controlled temperature (e.g., 40°C). This process concentrates the extracted compounds **(Mostafa *et al.*, 2018).**

After using the rotary evaporator to remove the bulk of the solvent, the remaining solvent extract was placed in an oven at 40°C to ensure that any last traces of the solvent were evaporated entirely. The obtained extracts were stored in the refrigerator at 4 °C for further experiments.

### 2.1. Yield determination

The following equation determines the extraction yield:

Extraction yield (%) = W1/W2×100; W1 is the mass of crude extract (g), and W2 is the sample (g) mass. **(Mohammedi, 2014).**

## 3. Phytochemical analysis

### 3.1. Phytochemical Screening

Photochemical tests were carried out on the aqueous and hydro alcoholic extracts of both plants. Detection of any compounds was carried out using the methods described by **Khaldi *et al.* (2012) and Vijayalakshmi *et al.* (2012).**

**A. Polyphenols**

Place 2 ml of aqueous or hydro alcoholic extract in a test tube and add a drop of 2% alcoholic ferric chloride solution. A positive test is revealed by the appearance of a more or less dark blue-black or green coloration. **(Talukdar & Chaudhary., 2010).**

**B. Flavonoids**

The presence of flavonoids is revealed by a rapid and straightforward test called the "Shinoda reaction" **(Soulama *et al.,* 2014).** The test involves adding a few drops of concentrated HCl and around 0.5 g of metallic magnesium to 1 ml of extract. Leave to react for 3 min. The appearance of a red, orange, pinkish, or purplish-red coloration indicates the presence of flavonoids.

**C. Tannins**

In a test tube, add 1 ml extract with 2 ml H 2 O and 1 ml 2% aqueous FeCl3 (ferric chloride). The presence of tannins is indicated by a greenish (catechic tannins) or blue-blackish (gallic tannins) coloration. (The appearance of a dark green or blue-green coloration indicates the presence of tannins). **(Talukdar & Chaudhary., 2010).**

**D. Terpenoids**

Detection involves treating 0.5 ml of the aqueous extract with 2 ml of chloroform and a few drops of concentrated sulfuric acid. In the event of a positive reaction, a brownish-red ring is formed where the two liquids come into contact. **(Alamzed *et al.,* 2013).**

**E. Saponosides**

We have taken 1 ml of the extract and added 3 ml of distilled water. The mixture is vortexed for 30 seconds and left to stand for 15 seconds. If foaming persists during this period, saponosides are present**. (Alamzed *et al.,* 2013).**

**F. Quinones**

1 ml of concentrated sulfuric acid is added to 1 ml of our extract. The formation of a red color reveals a positive test. **(Thusa & Mulmi., 2017).**

**G. Anthraquinones**

Anthraquinones are detected by adding a few drops of HCL (hydrochloric acid) to 0.5 mL extract. The appearance of a red precipitate indicates the presence of anthraquinones **(Bruneton, J., 1999)**

**H. Anthocyanins**

The flavonoid detection reaction involves treating 2 mL of extract with 2 mL of sulfuric acid ($H_2SO_4$) and 2 mL of ammonia (NH3). If the coloration increases with acidification and then turns blue in a basic medium, we can conclude that anthocyanins are present. **(Paris & Moyes., 1969).**

### 3.2. Flavonoid content determination

The application of the aluminum trichloride (AlCl3) method is frequently used to estimate the flavonoid content in extracts of *Thymus vulgaris* L. and *Mentha spicata*L.. The protocol involves placing 500 µL of each extract or standard (with suitable dilution) in a test tube and adding 500 µL of the AlCl3 solution (2% in methanol). After 10 minutes of incubation in the dark and at room temperature, the absorbance is read at 430 nm **(Baghiani*et al.,* 2012).** The total flavonoid content of the extracts is expressed in mg of quercitin equivalent per g of extract (mg CE/g).

## 4. Antibacterial activity

### 4.1. Microbial strains

The antibacterial activity of peel extracts was tested using nine microorganisms. These test microbes include three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, and *Klebsiella pneumonia* ATCC *4352*) and two Gram-positive bacteria (*Bacillus cereus* ATCC11778 and *Staphylococcus aureus* ATCC25923). Furthermore, 4 different strains of *E coli* from clinical source (isolated from human urines) were also tested.

### 4.2. Preparation of the inoculum

From a young culture obtained after (18 to 24h), 4 to 5 isolated and perfectly identical colonies were taken using a platinum loop, then placed in 9ml of sterilized physiological water, and the bacterial suspension was mixed using a vortex. The suspension is adjusted to a DO between 0.08 and 0.1 (read at 625 nm), which will

correspond to a suspension containing 108 CFU (or misadjusted to McFarland Standard 0.5), for spore solution is prepared at a rate of $10^7$ spores/ml.

**1. Seeding**

Petri dishes poured with the Muller-Hinton agar are sown by swabbing: the swab is soaked in the bacterial suspension prepared after diluting to 1/10 (about 107 CFU/ml), seeded over the entire surface of the agar from top to bottom in parallel and tight streaks, the operation is repeated three times by turning the 60° box each time without forgetting to rotate the swab at the gel rim **(Karou *et al*., 2005).**

**2. Preparation of dilutions**

The extracts were dissolved in the solvent used for the initial extraction (methanol or ethanol) to prepare four concentrations for each extract: 200, 100, 50, and 25 mg/mL. Therefore, we will weigh 200 mg, 100 mg, 50 mg, and 25 mg of the extracts. Then, each weighed extract was solubilized with 1 mL of DMSO in a sterile tube and mixed well until the total solubilization was achieved **(Finegold and Martin, 1982; Lino and Deogracious, 2012).**

**3. Solid media disc diffusion method**

Whatman paper discs (1,6mm diameter) pre-sterilize at 120°C for 30min in the Pasteur oven; they are then impregnated with 20 µl of each extract (reconstituted to the desired concentration) and deposited at equal distances from each other (4 or 6 discs per box) in such a way as to avoid overlapping of the inhibition zones on the seed with the test strain, a slight pressure shall be exerted on each disc to achieve good adhesion. A disc saturated with pure DMSO (100%) was utilized as a negative control. After diffusion at 4ºC for 3 hours, the Petri dishes were placed in an incubator at 37 C° for 24 hours **(Yazdani *et al.,* 2012).** The diameter of the zone of inhibition was measured in millimeters (mm) after incubation. The experiments were conducted in triplicate.

### 4.3. Determination of the minimum inhibitory concentration (MIC)

#### 4.3.1. Preparation of the Concentration Range

The concentration ranges were prepared in a series of 7 test tubes (T) numbered T1 to T7, using the double dilution method in liquid medium. These concentrations range from 200 mg/ml to 3.125 mg/ml. To do this, 10 ml of methanol or ethanol was placed in

tube T1 and 5 ml in all other tubes. A mass of 2 g of plant extract was dissolved in tube T1 and then thoroughly homogenized to give a 200 mg/ml concentration. Half the volume of tube T1 (5 ml) was transferred to tube T2 and then homogenized. This process was repeated with tube T7, after which half the volume was discarded. This resulted in concentrations C1 = 200 mg/ml; C2=100 mg/ml; C3=50 mg/ml; C4=25 mg/ml; C5=12.5 mg/ml; C6=6.25mg/ml; C7=3.125 **(Kouamé *et al.,* 2008; Ouattara *et al.,* 2013).**

### 4.3.2. Preparation of the inoculum for the liquid medium study

Two bacterial colonies were collected using a Pasteur pipette and emulsified in a test tube containing 10 ml of sterile Muller-Hinton broth (MHB) to prepare the inoculum. The mixture was incubated at 37°C for 3 hours. After the incubation, 0.3 ml of the bacterial pre-culture was taken out, added to 10 ml of sterile Muller-Hinton broth, and mixed well with a vortex mixer. The bacterial load of this bacterial inoculum was $10^6$ CFU **(Yapo Yomeh Cynthia Viviane *et al.,* 2020).**

### 4.3.3. Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was determined using the method reported by **Yapo Yomeh Cynthia Viviane *et al* .(2020)** in 96-well microplates arranged in 8 rows of 12 columns. In the last column (12) of each microplate, 200 µL of MH broth was placed, which was used to monitor the sterility of the culture medium (Ts). In column (n° 11) of each microplate, 200 µL of bacterial inoculum was added, which was used to monitor the growth of the germs (Tc). Each other column received 100 µl of bacterial inoculum at a dilution of 100. Then, 100 µl of each concentration of the prepared plant extract was added to each of the wells containing the 100 µl of inoculum. All wells' final volume (inoculum + extract) was 200 µl. The final concentrations of the plant extract dilution range thus generated were between 100 and 1.56 g/ml. The plates were covered and incubated at 37 °C for 18 to 24 hours. Following incubation, the Minimum Inhibiting Concentration (MIC) corresponded to the lowest concentration that did not exhibit turbidity.

# *Chapter 04:*

# *Results and discussion*

## 1. Yield determination

The weight of the dry Extract is determined by the difference between the weight of the full flask (after evaporation) and the weight of the empty flask (before evaporation) **(Mohammedi, 2014).** The percentage yield of the Extract by maceration by dividing the weight of the extracts by the weight of the samples and multiplying by 100 (Table 10) **(Safdar *et al.*, 2016).**

The results are shown in the Table below:

**Table 10.** The yield of the extracts

| Extract | Solvent | Extract Weight (g) |
|---|---|---|
| **Apple Peel** | Ethanol | 113.79 |
| **Apple Peel** | Methanol | 17.8 |
| **Banana Peel** | Ethanol | 9.84 |
| **Banana Peel** | Methanol | 12.98 |

**Table 11 .** Appearance, color and yield of hydroalcoholic extracts from the two fruits studied

| The fruit | Extract | Appearance | Color | Yield |
|---|---|---|---|---|
| **Apple** | Ethanol+ | Viscous | Brownishyellow | 29,63 % |
| **Apple** | Methanol | Viscous | Brownishyellow | 28,5 % |
| **Banana** | Ethanol | Viscous | Darkbrown | 16,4% |
| **Banana** | Methanol | Viscous | Darkbrown | 21,6 % |

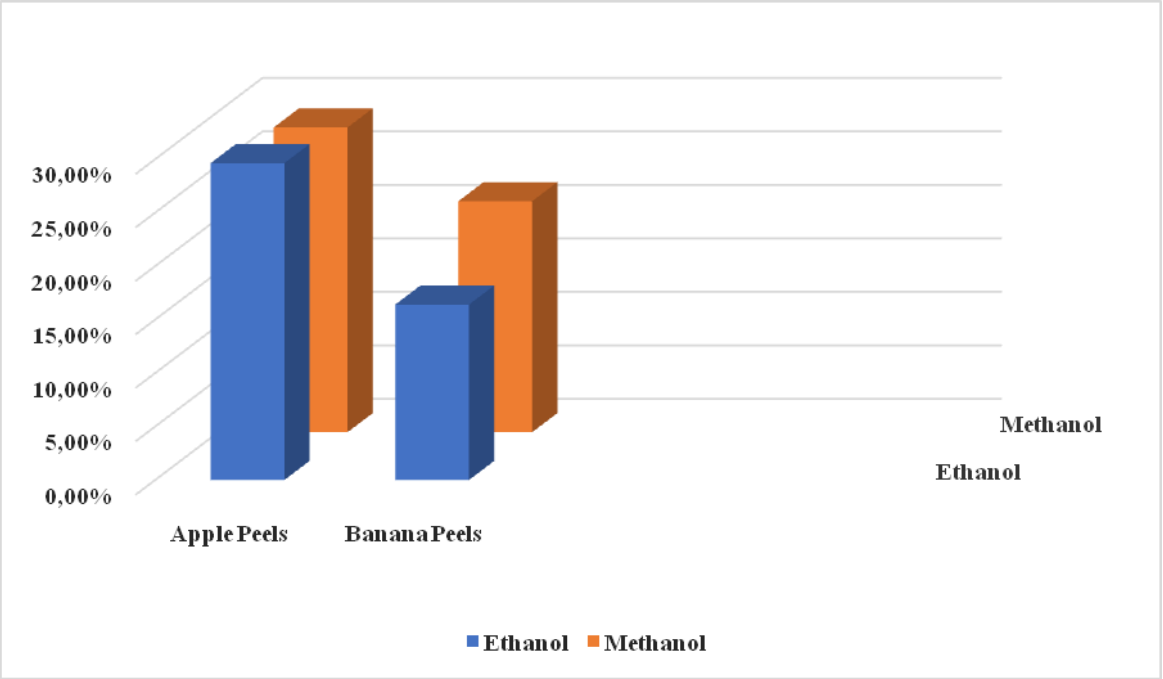



**Figure 6.** Comparison of apple and banana peel extract yields

According to the results obtained (Table 9), the yield of apple fruit with methanol and ethanol extract is higher than that of banana fruit with both hydro alcoolic extracts.

The ethanol extract of apple fruit is higher than that of banana fruit, while the methanol extract of both fruit types is almost the same, but the ethanol extract of apple fruit is higher than that of banana fruit.

Yields vary depending on the type of plant, the extraction substance, the drying conditions, the number of metabolites in each species, the type of solvent used for extraction or fractionation, and its polarity **(Daoudi *et al.*, 2015).** The primary reason why the results of one Extract differ from those of another is the extraction solvent used; for example, polar solvents exhibit superior extraction performance compared to less polar solvents. A wide variety of secondary metabolites can be extracted thanks to the polarity differences of the solvents used **(Green, 2004).**

## 2. Phytochemicals Screening

According to Table n°10, terpenoids, tannins, and quinones are strongly present in all extracts, which can be explained by the fruits' high concentration of these three metabolites.

**Table 12 .**Phytochemical Screening Results

| Compound | Solvent | Plant | Results |
|---|---|---|---|
| **Polyphenols** | **Ethanol** | **Apple** | + |
| | | **Banana** | + |
| | **Methanol** | **Apple** | - |
| | | **Banana** | + |
| **Flavonoids** | **Ethanol** | **Apple** | + |
| | | **Banana** | + |
| | **Methanol** | **Apple** | + |
| | | **Banana** | - |
| **Tannins** | **Ethanol** | **Apple** | + |
| | | **Banana** | + |
| | **Methanol** | **Apple** | + |
| | | **Banana** | + |
| **Terpenoids** | **Ethanol** | **Apple** | + |
| | | **Banana** | + |
| | **Methanol** | **Apple** | + |
| | | **Banana** | + |
| **Saponosides** | **Ethanol** | **Apple** | + |
| | | **Banana** | - |
| | **Methanol** | **Apple** | + |
| | | **Banana** | - |
| **Quinones** | **Ethanol** | **Apple** | + |
| | | **Banana** | + |
| | **Methanol** | **Apple** | + |
| | | **Banana** | + |
| **Anthraquinones** | **Ethanol** | **Apple** | + |
| | | **Banana** | - |
| | **Methanol** | **Apple** | + |
| | | **Banana** | - |
| **Anthocyanins** | **Ethanol** | **Apple** | + |
| | | **Banana** | + |
| | **Methanol** | **Apple** | - |
| | | **Banana** | - |

Additionally, there is a complete absence of saponosides and anthraquinones in the methanolic and ethanolic extracts of bananas, as well as a strong presence of this metabolite in the two solvents for the apple . Additionally, it is noted that the anthocyanins are only present in the ethanolic Extract of the two fruits. In contrast, the methanolic Extract shows a complete absence of this metabolite in the two fruits.

The high concentration of the polyphenolic metabolite in the ethanolic Extract of the two fruits suggests that they are rich in this metabolite. In contrast, the methanolic Extract only contains the banana and shows a complete absence of it in the apple, which may indicate that the fruit is deficient in this metabolite.

The phytochemical screening of banana (*Musa spp.*) and apple (*Malus domestica*) extracts reveals significant variation in the distribution of secondary metabolites depending on the solvent used, highlighting the importance of solvent polarity in phytochemical extraction. Both methanolic and ethanolic extracts of the two fruits exhibited a strong presence of terpenoids, tannins, and quinones. These compounds are well-documented for their biological activities, such as antioxidant, antimicrobial, and anti-inflammatory properties **(Agarwal *et al.,* 2016; Bakar *et al.,* 2015)**. The extraction efficiency of these metabolites is likely attributed to the polar nature of both methanol and ethanol, which are effective solvents for recovering a broad spectrum of phytoconstituents **(Nawaz *et al.,* 2022).**

Interestingly, saponins and anthraquinones were completely absent in the methanolic and ethanolic extracts of bananas, suggesting their negligible presence in this fruit or the possibility of matrix interference inhibiting their extraction. This absence is consistent with findings by **Likittrakulwong *et al.* (2023),** who reported low to non-detectable levels of anthraquinones in banana peel extracts. In contrast, these two classes of metabolites were strongly present in both solvent extracts of apple, indicating a richer phytochemical profile in this regard. Several studies corroborate this result, noting the abundance of saponins and anthraquinones in apple peels and their potential bioactivities **(Saeed *etal.,* 2023)**

Anthocyanins were found exclusively in the ethanolic extracts of both fruits, underscoring ethanol's superiority in extracting these water-soluble pigments. This result aligns with research by **Araújo *et al.* (2023),** which demonstrated that acidified ethanol

enhances anthocyanin recovery due to its optimal polarity and pH sensitivity, particularly in colored fruits like apples **(Dowlath*et al.,* 2020).** The complete absence of anthocyanins in methanolic extracts could reflect either the low solubility of these pigments in methanol or degradation during the extraction process.

Moreover, polyphenolic compounds were highly concentrated in the ethanolic extracts of both fruits, with methanolic extraction being effective only for bananas. This suggests relatively lower polyphenolic content in apples when extracted with methanol, possibly due to fruit tissue structure or metabolite compartmentalization. Ethanol has been widely reported as a preferred solvent for polyphenol extraction, especially from fruits, because it ensures better penetration and solubilization of phenolic acids and flavonoids **(Talmaciu *et al.,* 2015).** These findings underscore the crucial role of ethanol in enhancing the yield of functional phytochemicals with health-promoting properties.

This comparative screening affirms the differential affinity of methanol and ethanol in extracting specific classes of secondary metabolites. While both solvents efficiently extract terpenoids, tannins, and quinones, ethanol is more effective for anthocyanins and polyphenols. The apple fruit demonstrates a broader spectrum of phytochemicals, particularly in the ethanolic extracts, compared to bananas. These observations are consistent with recent literature and demonstrate the potential of using fruit extracts as sources of bioactive compounds with nutraceutical applications**. (Kibria *et al.,*2019).**

### 3. Dosage of flavonoid

The results of the flavonoid spectrophotometric analysis are based on the absorbance values of the extract solutions. In this study, the flavonoid contents showed relatively high values. The mean concentrations of flavonoids in the fruit peel extracts ranged from 22 to 53 mg CE/ g sample **(Table 13).**

**Table 13.** Flavonoids content

| **Extract** | **Ethanolic Apple** | **Methanolic Apple** | **Ethanolic Banana** | **Methanolic Banana** |
|---|---|---|---|---|
| ***Flavonoids*** mgCE/ g | 32±0.38 | 22±0.048 | 50 ± 09.46 | 53 ± 0.86 |

The extracts were relatively rich in flavonoids. Hence, flavonoids are among the major groups of phenolic compounds, with a broad spectrum of chemical and biological activities, particularly radical scavenging and antimicrobial activities **(Ayele *et al.,* 2022).**

The difference in the content of flavonoids can be explained by several parameters influencing the extraction of these compounds, such as the chemical nature of the compounds, the extraction method used the size of the sample particles, and the extraction time **(Naczk& Shahidi, 2006).** The main reason for choosing to measure this class of secondary metabolites is that flavonoids are one of the classes of secondary metabolites responsible for the biological activities of vegetal **(Osman *et al.*,2013).** They are ubiquitously found in plants and have beneficial health effects. Studies on flavonoid derivatives have revealed antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities **(Ayele *et al.,* 2022).** Therefore, extracts richest in flavonoid compounds can also be considered the most potent regarding biological activities **(Gulcin*et al.,* 2010).**

**4. Antimicrobial activity**

The antimicrobial activities of the fruit peel extracts against multi-drug-resistant bacterial strains are presented in Table 02. The inhibition zone diameters ranged from 08 to 36 mm at four concentrations: 25, 50, 100, and 200 mg/ml **(Table 12)**. The data found indicated that most tested strains were susceptible to the extracts.

**Photograph 5.** The methanolic and ethanolic extract of banana tested on *E. coli* 2.

**Photograph 6.**The methanolic and ethanolic extract of banana tested on *E. coli 3*

**Table 14 .**Antimicrobial activity results.

| ***Bacterialstrains*** | **Concentration (mg)** | **Apple Ethanolic Extract (mm)** | **Apple Methanolic Extract (mm)** | **Banana Ethanolic Extract (mm)** | **Banana Methanolic Extract (mm)** |
|---|---|---|---|---|---|
| *B.cereus* | 200 | 17 | 22 | 16 | 20 |
| | 100 | 15 | 18 | 17 | 16 |
| | 50 | 13 | 16 | 17 | 13 |
| | 25 | 8 | 8 | 8 | 8 |
| *S. aureus* | 200 | 23 | 21 | 26 | 15 |
| | 100 | 21 | 20 | 18 | 13 |
| | 50 | 20 | 18 | 13 | 14 |
| | 25 | 8 | 11 | 8 | 8 |
| *P.aeroginosa* | 200 | 16 | 18.5 | 18 | 15.5 |
| | 100 | 18 | 19.5 | 15 | 15.5 |
| | 50 | 14 | 13.5 | 12 | 14 |
| | 25 | 8 | 13 | 8 | 8 |
| *K. pneumoniae* | 200 | 13 | 13 | 10 | 10.5 |
| | 100 | 12 | 11.5 | 12.6 | 11.5 |
| | 50 | 11 | 12.5 | 11 | 11.5 |
| | 25 | 8 | 12 | 8 | 8 |
| *E. coli R* | 200 | 19 | 23.5 | 16.5 | 22 |
| | 100 | 19.25 | 19 | 14.5 | 25 |
| | 50 | 14.5 | 15.5 | 12.5 | 16.5 |
| | 25 | 8 | 12 | 10 | 8 |
| *E. coli 1* | 200 | 16.5 | 21 | 14 | 14.5 |
| | 100 | 17.5 | 17.25 | 11.5 | 11 |

| | | | | | |
|---|---|---|---|---|---|
| | 50 | 16 | 18 | 11.25 | 12 |
| | 25 | 8 | 8 | 8 | 8 |
| *E. coli 2* | 200 | 13.5 | 17 | 14 | 14 |
| | 100 | 14.5 | 17.5 | 13.5 | 15.5 |
| | 50 | 11.5 | 17.5 | 14.5 | 13.5 |
| | 25 | 8 | 8 | 8 | 12 |
| *E.coli 3* | 200 | 16.5 | 16.5 | 14 | 16.5 |
| | 100 | 15.5 | 18 | 17 | 18 |
| | 50 | 19 | 13 | 19 | 15 |
| | 25 | 8 | 10 | 8 | 8 |
| *E.coli 4* | 200 | 13.5 | 19 | 15 | 14.33 |
| | 100 | 16 | 15 | 14.5 | 16.5 |
| | 50 | 14 | 17 | 14.5 | 17.5 |
| | 25 | 8 | 11 | 8 | 8 |

Due to variations in their modes of action and their chemical composition, different plant extracts have different antibacterial activities **(Masot *et al.,* 2024)**. Numerous circumstances can influence the antibacterial activity of various fruit peel extracts. These variables the extraction technique and solvent, the time the plant was cultivated, and the freshness of the used peels **(Chaiwarit *et al.,* 2021).**

Methanolic extracts from apples and bananas show more potent antibacterial activity than ethanolic extracts. Apple extracts show much stronger antibacterial activity than banana extracts.

***Bacillus cereus***

Methanolic extracts of apple and banana exhibited significant antimicrobial activity against *Bacillus cereus*, showing inhibition zones of 20–22 mm at a concentration of 200 mg/ml, notably stronger than ethanolic extracts (13–18 mm). However, at a lower concentration (25 mg/ml), activity significantly decreased (8 mm diameter). These results correlate with previous findings by Hanafy *et al*. (2021), who reported elevated antibacterial activity of methanolic banana peel extracts, with inhibition zones ranging between 20.3 and 25 mm against *Bacillus cereus,* supporting the potential of banana peel extracts as antimicrobial agents **(Hanafy*et al.,* 2021).**

***Staphylococcus aureus***

The current results demonstrated the potent antimicrobial activity of banana and apple extracts against *Staphylococcus aureus*. Specifically, ethanolic banana extracts

showed an inhibition zone of 26 mm at 200 mg/ml, surpassing the ethanolic apple extracts with 23 mm. At concentrations of 100 and 50 mg/ml, inhibition zones ranged between 13–21 mm. Comparable studies reinforce these observations; Shaukat *et al.* (2023) documented substantial antibacterial efficacy of ethanolic banana peel extracts against *S. aureus*, showing an inhibition zone of approximately 12.4 mm, confirming the effectiveness of banana extracts against this strain **(Shaukat *et al.,* 2023).** Additionally, Ousaaid*et al.* (2021) reported substantial antimicrobial properties of apple-derived products against *S. aureus*, further corroborating the results obtained in this study **(Ousaaid*et al.,* 2021).**

***Pseudomonas aeruginosa***

Methanolic apple extracts displayed superior antibacterial activity against *Pseudomonas aeruginosa*, producing inhibition zones of 19.5 mm and 18.5 mm at 100 mg/ml and 200 mg/ml, respectively. In comparison, other tested extracts exhibited moderate efficacy, with zones ranging from 12 to 16 mm at concentrations of 200 mg/mL and 50 mg/mL. These results align with the findings of Jelodarian *et al.* (2013), who reported moderate inhibitory activity of apple peel extracts against *P. aeruginosa*, with inhibition zones of approximately 11 mm. This suggests variability in effectiveness based on extraction methods and apple varieties **(Jelodarian*et al.,* 2014).**

***Klebsiella pneumoniae***

The antimicrobial activity of apple extracts (methanolic and ethanolic) against *Klebsiella pneumonia* demonstrated inhibition zones of approximately 13 mm at 200 mg/mL, with smaller zones (11–12 mm) at lower concentrations. These findings suggest moderate efficacy of apple extracts against this strain. While Liya *et al.* (2018) indicated limited or negligible antimicrobial effects of apple extracts against *K. pneumoniae*, emphasizing variability influenced by extraction solvents and methods, this study confirms the modest antibacterial potential of apple extracts, highlighting the importance of extraction methodologies and apple variety **(Liya*et al.,* 2018).**

***E. coli* (reference Strain):**

The methanolic extracts of apple and banana at 100 mg/ml demonstrated inhibition zones of 25 mm, decreasing to 19–23.5 mm at 200 mg/ml. Other extracts showed zones ranging from 10 mm to 16.5 mm across various concentrations. These findings align with

previous research where apple peel extracts exhibited inhibition zones of 15 mm against E. coli, suggesting moderate antibacterial activity. Similarly, banana peel extracts have shown inhibition zones of approximately 13 mm against *E. coli*, indicating comparable efficacy.

**Clinical strains of *E. coli***

**E. coli 1**

The results show that methanolic and ethanolic apple extracts produced inhibition zones between 16 and 21 mm across concentrations of 200, 100, and 50 mg/ml, outperforming banana extracts, which ranged from 11.5 to 14 mm. This suggests that apple extracts have a more potent antibacterial effect against this strain, consistent with studies reporting inhibition zones of 15 mm for apple peel extracts against *E. coli*.**(Mehrabkhani*et al.,* 2018).**

***E. coli 2***

The methanolic apple extract exhibited a consistent inhibition zone of 17.5 mm across all concentrations. However, at a concentration of 25 mg, other extracts showed zones ranging from 11.5 to 15.5 mm in diameter. These results are in line with previous findings where apple extracts demonstrated inhibition zones of 15 mm against *E. coli*.**(Nazar *et al.,* 2019).**

***E. coli 3***

Methanolic apple extracts at concentrations of 200 and 100 mg/ml showed inhibition zones between 16.5 and 18 mm. Other extracts at 50 mg/ml exhibited zones ranging from 13 to 19 mm. These findings are comparable to earlier studies where banana peel extracts showed inhibition zones of approximately 13 mm against *E. coli* **(Likittrakulwong *et al.,* 2023).**

***E. coli 4***

Methanolic extracts from apples and bananas demonstrated inhibition zones ranged from 15 to 19 mm at concentrations of 200, 100, and 50 mg/ml. These results are consistent with previous research indicating that banana peel extracts have inhibition zones around 13 mm against *E. coli* **(Likittrakulwong *et al.,* 2023).**

**5. Determination of the minimum inhibitory concentration (MIC)**

The results were read in daylight and with the naked eye. The medium's clarity indicates the tested Extract's antimicrobial effect, while the presence of cloudiness indicates its ineffectiveness (a sign of bacterial growth). The minimum inhibitory concentration (MIC) of the plant extracts against multi-drug-resistant bacteria strains is presented in **Table 13.** The minimal inhibition concentration of tested bacteria, corresponding to the lowest concentration of plant extracts that completely inhibited the organism's growth in the broth medium, ranged from 1.56 to 100 mg/ml against all tested bacteria.

**Table 15.** Minimum inhibitory concentrations of the studied extracts

| Extract / Strains | Ethanolic Apple peels | Methanolic Apple peels | Ethanolic Banana peels | Methanolic Banana peels |
|---|---|---|---|---|
| ***Pseudomonas aeruginosa*** | 1,56 mg/ml | 1,56mg/ml | 6,25mg/ml | 1,56mg/ml |
| ***Bacillus cereus*** | 12,5mg/ml | 6,25mg/ml | 6,25mg/ml | 1,56mg/ml |
| ***Klebsiella pneumoniae*** | 1,56mg/ml | 1,56mg/ml | 3,12mg/ml | 25mg/ml |
| ***E. coli R*** | 1 ,56mg/ml | 1,56mg/ml | 1,56mg/ml | 1,56mg/ml |
| ***Staphylococcus aureus*** | 1,56mg /ml | 1,56mg/ml | 1,56mg/ml | 1,56mg/ml |
| ***E. coli 1*** | 1,56mg/ml | / | 100mg/ml | / |
| ***E. coli 2*** | 3,12mg/ml | 1,56mg/ml | 50mg/ml | 100mg/ml |
| ***E. coli 3*** | 1,56mg/ml | 1,56mg/ml | 6,25mg/ml | 1,56mg/ml |
| ***E. coli 4*** | 3,12mg/ml | 6,25mg/ml | 12,5mg/ml | 25mg/ml |

Based on the table results, it can be observed that all four extracts have a potent inhibitory effect on *E. Coli R* and *Staphylococcus* (with MIC values of 1.56 mg/ml).

Additionally, the ethanolic and methanolic Extract of the apple exhibits a MIC of 1.56 mg/ml against *Pseudomonas*, *Klebsiella*, and *E. coli 3*.

The average inhibitory effect of the ethanolic banana extract was 3.12 mg/ml against *Klebsiella* and, for the apple extract, against *E. Coli 2* and *E. Coli* 4.

For, *Pseudomonas, Bacillus cereus,* and *E. coli* 3 the MIC values were 6.25 mg/ml with the ethanolic banana extract, whereas *Bacillus* and *E. coli* 4 showed the exact values with the apple methanolic extract.

For *Bacillus cereus*, the MIC of the ethanolic apple extract was 12.5 mg/ml, while similar value was detected for banana extract against *E. coli* 4.

The determination of the minimum inhibitory concentration (MIC) of banana (*Musa spp.*) and apple (*Malus domestica*) extracts against various bacterial strains revealed significant insights into their antimicrobial potential. When compared to other plant-based studies, these findings underscore the efficacy of these common fruits peels as potential sources of antibacterial agents.

In the current study, both methanolic and ethanolic extracts of banana and apple exhibited potent inhibitory effects against *Escherichia coli* R and *Staphylococcus* species, with MIC values as low as 1.56 mg/mL. Hence, previous studies indicated that *Staphylococcus aureus* and *Escherichia coli* were among the most targeted bacterial species, emphasizing the relevance of the current study's focus (**Chassagne *et al.*,2021).**

The ethanolic and methanolic extracts of apple demonstrated MICs of 1.56 mg/mL against *Pseudomonas*, *Klebsiella*, and *E. coli* 3, indicating a broad-spectrum antibacterial activity. This aligns with findings from other studies, where plant extracts exhibited varying minimum inhibitory concentration (MIC) values against these pathogens. In a study evaluating the antibacterial activity of various plant extracts, the minimum inhibitory concentration (MIC) values against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* ranged from 0.08 to 1.65 mg/mL (**Eloff, 1998).**

Interestingly, higher MIC values were observed for specific strains. For instance, *Pseudomonas*, *Bacillus*, and *E. coli* 3 were inhibited at 6.25 mg/mL for the ethanolic banana extract, whereas *Bacillus* and *E. coli* 4 required similar concentrations for the methanolic apple extract. These variations highlight the importance of selecting suitable solvents to optimize the extraction of bioactive compounds. In a study focusing on the antimicrobial activity of banana peel extracts, it was noted that the minimum inhibitory concentration (MIC) is dose- and solvent–dependent, highlighting the significance of extraction methods **(Mostafa, 2021).**

Notably, the ethanolic Extract of the apple achieved a MIC of 1.56 mg/mL, while the banana extract required a significantly higher concentration of 100 mg/mL. This disparity highlights the superior efficacy of apple extracts against certain strains, possibly due to a richer composition of antimicrobial phytochemicals.

In the case of *Bacillus cereus*, the MIC of the ethanolic apple extract was 12.5 mg/mL, whereas the banana extract exhibited a similar value against *E. coli* 4. These results suggest that while both fruits have antibacterial properties, their effectiveness is strain-specific.

The ethanolic banana extract showed a relatively low inhibitory effect on *E. coli* 2, with an MIC of 50 mg/mL. Conversely, its methanolic Extract inhibited *Klebsiella* and *E. coli* 4 at 25 mg/mL. These findings highlight the impact of extraction solvents on the antimicrobial activity of plant extracts. These findings are consistent with existing literature. For instance, a study by **Hanafy *et al.* (2021)** reported that methanolic extracts of banana peels exhibited MIC values ranging from 75 to 300 mg/mL against various pathogens, including *Klebsiella pneumoniae* and *E. coli*, highlighting the solvent-dependent variability in antimicrobial activity.

According to **Howaida *et al.* (2002);** the impact on bacterial growth was caused by the discharge of compounds from the extracts into the medium upon mixing. Additionally, the extract's pH level could influence its antibacterial activity. Hence, the finding of antibacterial activity against the test bacterium suggests that the plants contain alternative antibiotic compounds that could be utilized in the future to produce novel antibacterial substances. The low MIC values found against the tested bacteria indicate that the plant extracts could efficiently cure diseases resulting from bacterial infections.

# *Conclusion*

**Conclusion**

The present study reveals that methanol and ethanol extracts derived from apple and banana peels possess antibacterial properties against pathogenic strains: *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, and *against* four strain of clinical *E. coli*. Additionally, the majority of fruit peels extract noteworthy antibacterial activity against all pathogenic strains tested in this study. Based on the MIC values, the extracts of apple and banana peels exhibited high effectiveness. This suggests that the studied fruit peels could be a valuable source for discovering antimicrobial products. Moreover, additional investigation is required to elucidate the structure of bioactive compounds.

The scientific significance of these findings lies in validating low-cost, sustainable sources of antimicrobial agents, which could reduce antibiotic resistance pressures and offer natural alternatives for food and pharmaceutical industries. Future work may involve characterizing specific phytochemical molecules responsible for the biological activity.

Likewise, it is essential to conduct a comprehensive investigation that includes fractionation studies using a range of polar and non-polar solvents, elucidating the mechanisms of microbial inhibition, and formulating optimized delivery systems for real-world applications. Overall, valorizing apple and banana peels aligns with circular economy principles and contributes to the development of eco-friendly antimicrobial strategies.

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# *Appendices*

**Appendices**

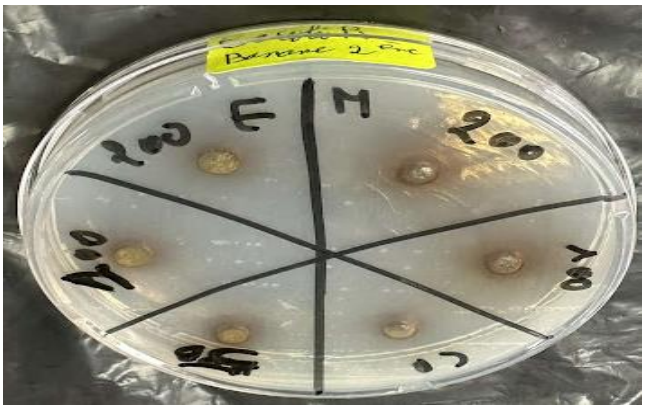
**Appendix 1. Nutrient agar preparation:**

Suspended 23 grams of the medium in one liter in distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes.

**Preparation of Muller Hinton medium:**

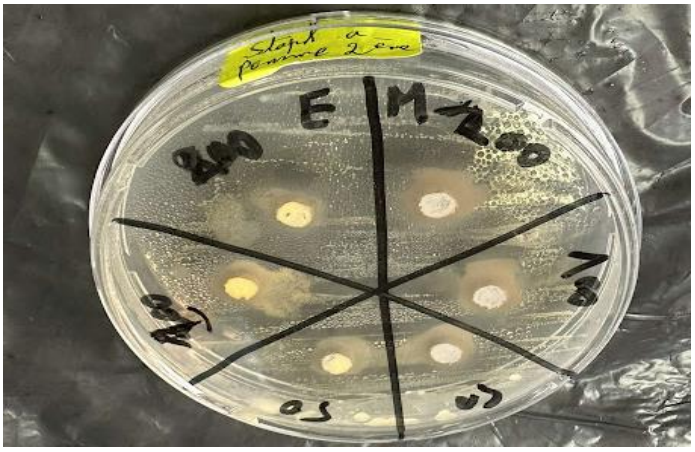
- Dissolve 38g in 1litre of purified water (mixture of 17.50g of peptone and 2g of meatextract and 1.50g of starch and 17g of agar)
- Heat underfrequentstirring and boil for 1 minute to completely dissolve the suspension
- Bottle
- Autoclaving 15 minutes at 121°C

**Appendix2. Antimicrobial activity results**

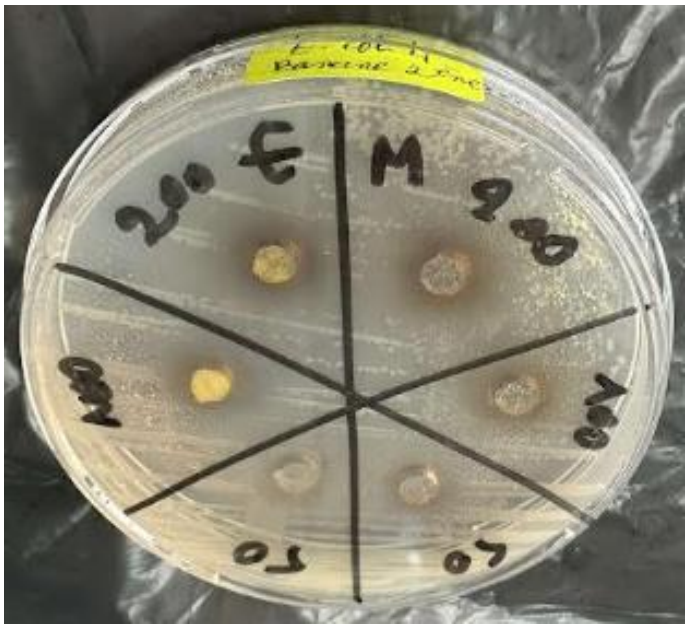



**A.**The methanolic and ethanolic extract of banana tested on *E.coli* 4


**B.** The methanolic and ethanolic extract of appel tested on *E.coli* 1

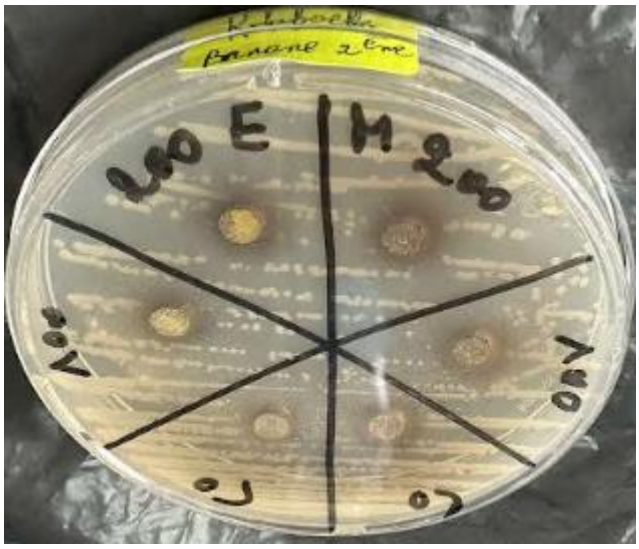



**C.** The methanolic and ethanolic extract of appel tested on *Staphylococcus aureus*




**D.** The methanolic and ethanolic extract of banana tested on *E.coli* 3


**E.** The methanolic and ethanolic extract of appel tested on *Bacillus cereus*




**F.** The methanolic and ethanolic extract of banana tested on *Klebsiella pneumonie*

**Photograph 7.** Results of the antibacterial activity.