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Phytochemical analysis and Antibacterial activity of two selected fruit peels cultivated in Algeria against bacterial strains.

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Dedication

*To my beloved parents, whose unwavering faith and boundless love have
guided me through every challenge.*

*To my dear brothers, whose camaraderie and encouragement have been a
constant source of strength.*

*To my precious sister, whose kindness and wisdom have inspired me to reach
for greater heights this work is dedicated with all my heart.*

NORA

Dedication

To my beloved parents,

*For your endless sacrifices, your quiet prayers, and the love that shaped me.
May Allah (swt) reward you with Jannat al-firdaws and grant you ease in
dunya and akhira*

To my siblings

*Sohaib & Ibrahim, my protectors and my wings.
Iness, my compass and Quit guide & Nour elyakine , the little heart with the
greatest light.*

To my entire family,

*You are my roots and my strength , my cousins, through smiles and storms you
never left thank you for being one soul with mine*

To soulmates from the cradle, Roumaissa and dounia, my forever reflection

*My dearest friend Dounia the one who shared my path but now lives oceans
away, this graduation is also yours.*

And finally,

The girl who doubted, feared, and still kept going.

*You laid the foundation for the woman I am becoming. Thank you for surviving
Aya melek Brahimi*

Abstract

Phytochemical analysis and Antibacterial activity of two selected fruit peels cultivated in Algeria against bacterial strains.

Abstract

The present study examined the antimicrobial properties and phytochemical composition of hydro-methanolic and hydro-ethanolic peel extracts from *Punica granatum* (pomegranate) and *Citrus sinensis* (orange). 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*; at different concentrations: 100, 50, and 25 mg/ml, using disk diffusion assays. The results showed that both fruit peel extracts were efficient against the tested strains. Furthermore, ethanolic and methanolic extracts of pomegranate had notably stronger inhibitory effects, especially against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. In contrast, orange peel extracts exhibited moderate activity, with methanolic extracts outperforming ethanolic ones. Furthermore, the minimum inhibitory concentration tests confirmed the superior antibacterial potency of pomegranate peel extracts, which were effective at much lower concentrations compared to orange extracts. Moreover, 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 byproducts and indicate their potential utility as therapeutic agents.

Keywords: Antimicrobial activity; Orange peels, Phytochemical screening, Pomegranate peels.

Résumé

Analyse phytochimique et activité antibactérienne de deux écorces de fruits sélectionnées cultivées en Algérie.

Résumé

La présente étude a examiné les propriétés antimicrobiennes et la composition phytochimique des extraits de pelures hydro-méthanoliques et hydro-éthanoliques de *Punica granatum* (grenade) et de *Citrus sinensis* (orange). 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* ; à différentes concentrations : 100, 50 et 25 mg/ml, grâce à des tests de diffusion sur disque. Les résultats ont montré que les extraits des écorces des deux fruits étaient efficaces contre les souches testées. De plus, les extraits éthanoliques et méthanoliques de grenade avaient des effets inhibiteurs nettement plus puissants, en particulier contre *Staphylococcus aureus*, *Escherichia coli* et *Pseudomonas aeruginosa*, tandis que les extraits de pelure d'orange présentaient une activité modérée ; les extraits méthanoliques surpassant les extraits éthanoliques. De plus, les tests de concentration minimale inhibitrice ont confirmé la puissance antibactérienne supérieure des extraits de pelure de grenade, qui étaient efficaces à des concentrations beaucoup plus faibles que les extraits d'orange. De plus, les extraits de plantes ont été soumis à une analyse phytochimique, qui a détecté la présence de flavonoïdes, de polyphénols, de saponines, de tanins, de quinones et de terpènes. L'analyse quantitative a révélé une richesse notable en flavonoïdes, avec des valeurs allant de $22 \pm 0,048$ à $53 \pm 0,86$ mgCE/g. Ces résultats valident le potentiel antimicrobien de ces sous-produits de fruits et indiquent leur utilité potentielle en tant qu'agents thérapeutiques.

Mots-clés : Activité antimicrobienne ; Ecorces d'orange, Analyse phytochimique, Ecorces de grenade.

التحليل الكيميائي النباتي والنشاط المضاد للبكتيريا لقشور فاكهتين مختارتين مزروعتين في الجزائر ضد السلالات البكتيرية.

ملخص

الدراسة الحالية فحصت الخصائص المضادة للميكروبات والتركيب الكيميائي النباتي لمستخلصات قشور الرمان (*Punica granatum*) والبرتقال (*Citrus sinensis*) المائية-الميثانولية والمائية-الإيثانولية. تم اختبار المستخلصات المختلفة ضد خمسة سلالات بكتيرية ممرضة *Bacillus cereus* ATCC 11778، *Staphylococcus aureus* ATCC 25923، *Escherichia coli* ATCC 25922، *Klebsiella pneumoniae* ATCC 4352، و *Pseudomonas aeruginosa* ATCC 27853، وأربع سلالات سريرية من *E. coli*؛ بتركيزات مختلفة: 100، 50، و 25 ملغ/مل، باستخدام اختبارات انتشار الأقراص. أظهرت النتائج أن مستخلصات قشور الفاكهة كانت فعالة ضد السلالات المختبرة. علاوة على ذلك، كانت المستخلصات الإيثانولية والميثانولية لقشر الرمان ذات تأثيرات مثبتة أقوى بشكل ملحوظ، خاصة ضد الميكروبات *Escherichia coli*، *Staphylococcus aureus*، و *Pseudomonas aeruginosa*، في حين أظهرت مستخلصات قشر البرتقال نشاطاً معتدلاً؛ حيث تفوقت المستخلصات الميثانولية على المستخلصات الإيثانولية. علاوة على ذلك، أكدت اختبارات الحد الأدنى من التركيز المثبط الفعالية المضادة للبكتيريا لمستخلصات قشر الرمان، والتي كانت فعالة عند تركيزات أقل بكثير مقارنة بمستخلصات البرتقال. علاوة على ذلك، تم إخضاع مستخلصات النباتات للفحص الكيميائي النباتي، الذي كشف عن وجود الفلافونويدات، والبوليفينولات، والصابونينات، والتانينات، والكينونات، والتربينويدات. أ revealed التحليل الكمي غنى ملحوظاً في الفلافونويدات، بقيم تتراوح بين 0.048 ± 22 إلى 0.86 ± 53 ملغ/CE غ. تؤكد هذه النتائج الإمكانيات المضادة للميكروبات لهذه المنتجات الثانوية من الفاكهة وتشير إلى إمكانية استخدامها كعوامل علاجية.

الكلمات المفتاحية: النشاط المضاد للميكروبات؛ قشور البرتقال، الفحص الكيميائي النباتي، قشور الرمان.

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- AE: Enzymatic Activity
- AlCl_3 : Aluminum chloride (reagent for flavonoid assay)
- ATB: Antibiotic
- CFU : Colony-forming unit
- CNS: National Statistics Center
- CS : *Citrus sinensis*
- DDM: Diameter of Inhibition Zone
- DMSO : Dimethyl sulfoxide
- DPPH : 2,2-Diphenyl-1-picrylhydrazyl (radical scavenging assay)
- EPS: Exopolysaccharides
- FAO: Food and Agriculture Organization
- GL: Glycolipids
- INSP: National Institute of Public Health
- MBC: Minimum Bactericidal Concentration
- MIC : Minimum inhibitory concentration
- PNNS: National Nutrition and Health Program
- SCA: South Constantinois of Algeria
- UV: Ultraviolet
- WHO: World Health Organization

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Introduction

Introduction

1. General introduction

Medicinal plants have been recognized for centuries as rich sources of bioactive compounds capable of addressing a wide range of health issues, from infections to chronic diseases. In recent years, attention has shifted toward valorizing agricultural byproducts—particularly fruit peels—as they often contain higher concentrations of secondary metabolites than edible portions. Studies have shown that fruit and vegetable peels can yield phenolics, flavonoids, tannins, and terpenoids with potent antimicrobial and antioxidant activities, making them promising candidates for combating antimicrobial resistance (**Saleem et al., 2020**).

Among the countless medicinal fruits, two species stand out due to their traditional use and phytochemical richness in Algerian cultivation: orange (*Citrus sinensis*) and pomegranate (*Punica granatum*). *Citrus sinensis* peel is particularly noted for its abundance of flavonoids (e.g., hesperidin, naringin), essential oils (limonene, linalool), and other phenolic compounds, which have demonstrated significant antioxidant and antibacterial properties (**Chen et al., 2020**). *Punica granatum* peel is similarly endowed with hydrolyzable tannins (ellagitannins), anthocyanins, and various phenolic acid compounds that confer strong antibacterial effects against a spectrum of pathogens (**Ranjha et al., 2021**).

The primary benefit of focusing on these two peels lies in two interconnected aims. First, extracting and characterizing their phytochemical profiles can identify natural compounds that inhibit bacterial growth offering alternatives to synthetic antibiotics in a context where resistance is rising. Second, valorizing fruit waste supports environmental sustainability and local agricultural economies by converting otherwise discarded biomass into value-added products. Previous research underscores that pomegranate peel extracts often outperform orange peel in inhibiting key pathogens, suggesting a promising avenue for developing new antimicrobial agents from locally available material.

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

Therefore, the overall goal of this work is to perform a comparative phytochemical analysis of *Citrus sinensis* and *Punica granatum* peels—using both methanolic and ethanolic solvents and to evaluate their antibacterial activity against clinically relevant bacteria.

This work was divided into four chapters: the first one was elaborated to highlight the importance of the studied fruits. Hence, the second chapter revisits some of the pathogenic bacteria associated with various pathological diseases. Moreover, the third chapter outlines the key procedures for conducting this research. Likewise, the fourth chapter highlights the results and their significance.

Chapter01

Studied Fruits and Their Medicinal Importance

1. Orange (*Citrus sinensis*)

1.1. Historical background

Citrus sinensis, commonly known as orange, is one of the ancestral species of the genus *Citrus*. Originating in Southeast Asia, particularly in southern China and Vietnam, the Tangerine played a pivotal role in the evolution and diversification of citrus fruits cultivated today (Figure 1). Orange is also widely recognized and appreciated for its medicinal properties, including its rich content of vitamin C, which makes it an essential food for health and well-being. Indeed, this fruit plays a significant role in the diet of many people around the world (Lado *et al.*, 2016).

This fascinating work aims to explore in depth the history, culture, various uses, and undeniable importance of the *Citrus sinensis* in the contemporary world and through the ages (Lado *et al.*, 2016).



Figure 1. Orange (*Citrus sinensis*) (Guellier, 2021)

2. Origin and etymology

2.1. Origine

Citrus sinensis (Orange) is one of the primary ancestral species of cultivated citrus fruits. It originates from Southeast Asia, particularly from southern China, Vietnam, and the Philippines, where wild populations have been recorded. The domestication of oranges dates back thousands of years, as they were selectively cultivated for their sweet taste and easy-

Chapter 1. Studied Fruits and Their Medicinal Importance

to-peel rind. Genetic studies confirm that oranges are among the original *Citrus* species, along with citron (*Citrus medica*) and pomelo (*Citrus maxima*). Many modern citrus varieties, including oranges (*Citrus sinensis*), tangerines, and hybrids like tangelos, have *Citrus sinensis* in their genetic background (Wu *et al.*, 2018)

Oranges spread to India and the Middle East through trade routes and later reached Europe via Portugal and Spain in the 19th century. They were introduced to the Americas in the early 1800s (Langgut, 2017).

2.2. Etymology of *Citrus sinensis*

The genus name "Citrus" comes from Latin, which in turn is derived from the Greek word *kedros* (κέδρος), referring to the cedar tree. Ancient Greek and Latin texts often confused citrus fruits with aromatic trees, such as cedars, due to their strong scents. The species name "reticulata" is derived from the Latin word *reticulum*, meaning "netted" or "reticulated." This refers to the characteristic reticulated (net-like) pattern of the orange's peel and the fine network of oil glands on the fruit's surface (Mabberley, 2004).

3. Botanical classification

Citrus reticulata belongs to the Rutacea family (Table 1).

Table 1. Botanical classification of *Citrus reticulata* (Angiosperm Phylogeny Group, 2016).

Royaume	Plantae
Clade	Angiospermes
Order	Sapindales
Family	Rutaceae
Genus	Citrus
Species	<i>Citrus sinensis</i>

4. Geographic distribution

4.1. In Algeria

Citrus sinensis is widely distributed in Algeria, with notable occurrences in Tizi Ouzou, Biskra, Jijel, Bouira, Bejaia, Skikda, and Boumerdes. This species thrives along the Mediterranean coast from Tlemcen to Annaba and from Oran to Bejaia. Overall, it is found

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in 19 of Algeria's 40 provinces, particularly in southern areas such as Béchar, Adrar, Naâma, Tamanrasset, and El Bayadh, where sightings are typically limited, with one to five per province. Notably, higher concentrations occur in specific provinces or areas (**John, Makris, 2022**).

Environmental factors influencing its distribution are analyzed based on province-specific data, including climate, altitude, and land type. In most provinces, annual rainfall ranges from 400 to 1200 mm, with normal to high humidity (**Shah et al., 2021**). These regions also enjoy at least 1750 hours of sunshine yearly, and average temperatures range from 16 to 21°C, with consistent patterns above 16°C after frost and below 21°C before colder days. These conditions resemble those in Nepal, indicating similar agricultural viability (**Attah et al., 2021**). However, some provinces experience lower rainfall (200 to 600 mm) and higher temperatures (21 to 26°C) near desert areas, impacting their microclimate. *Citrus sinensis* thrives in silty, loamy soils with an average pH of 6.5, while sandy loamy soils show a pH of around 7. Water availability decreases in regions farther from the coast, with humidity lasting 75 to 110 days a year. Research highlights humidity, altitude, and temperature as key factors influencing the distribution of *Citrus sinensis* across Algeria's diverse landscape (**Sager, Wiche, 2024**).

4.2. Worldwide

Citrus, the largest flowering plant species, is a very successful genus of the subtropical crop. Among them, sweet orange, orange orange, lemon, and grapefruit are the four most widely cultivated Citrus species, with a planting area of 40 million ha, of which the orange orange accounts for 24% (**Wang et al., 2021**). China is the largest producer of orange oranges, where *Citrus sinensis* is the major cultivar group. *Citrus sinensis* is considered one of the distinctive species of Citrus, which has been selected for cultivation in Oriental countries except for India. As the most popular Citrus species, the cultivation of *Citrus sinensis* can be found in different agroecological conditions, such as in topography from plains to hills and in climate from tropics of the south to subtropical and warm temperate of the central and northern parts of China. Consequently, more than 300 cultivars are obtained by various selections of this species. Therefore, the distribution of this species will reflect the adaptability of Citrus to various production environments and the differences in human utilization and manipulation of such developmental variation (**Wang et al., 2022**).

Citron, the believed original Citrus species, is cultivated in the warm temperate zone of the Mediterranean region, including countries such as Israel, Italy, Greece, Turkey, and parts of Nepal. Researchers have demonstrated the potential for wildness in *C. medica* through seedlings of a commercial cultivar (Shorbagiet *et al.*, 2022). Keraji, the Arabic name for Citrus, originated with Kedron, as used by the ancient Greeks, and was later adopted by the Romans as Citrus, which was widely spread through trade. Subsequently, following the expansion of the Roman Empire and the introduction of Citrus to several different countries, the name "citrus" was adopted by those European languages (Figure 2) (Costanzo *et al.*, 2022; Cai *et al.*, 2023). Groves of citron trees were frequently depicted in ancient temple inscriptions. The typical trait of oval fruit, as shown in those old paintings, maybe a form of the 'Buddha's hand' citron. Many citron rootstocks are grown commercially due to their advantages of dominance, seedlings retarding, and resistance to unfavorable soil. Due to its strong root vigor, severe plant-fruit unbalance results in most of the assimilates being allocated to any rooting sucker rather than flower and fruit, which makes it economically unfeasible (Fahmy *et al.*, 2022; Ahatiet *et al.*, 2022).



Figure 2. Region of origin, dispersion, and diversification areas of citrus fruit grown (Jacquemondet *et al.*, 2013)

5. Botanical characteristics

Subkingdom of Tracheobionta includes the Citrus Family and the Citrus Genus, notably *Citrus sinensis*, alongside *Citrus arguta*, *Citrus maxima*, *Citrus aurantiifolia*, and *Citrus aurantium*. These share similar genetic backgrounds, differing from species like *Citrus sinensis* and *Fortunella* sp. *Citrus sinensis*, or Tangerine, grows as a 400-500 cm tall shrub with a vegetation period of 6-11 months and a first harvest at 4-6 years. Its leaves exhibit a progressively growing apex with narrowly elliptical margins. The leaves alternate

Chapter 1. Studied Fruits and Their Medicinal Importance

on a large petiole, and the primary axis diameter can reach up to 1 mm, featuring thorns. The flowers have an ovary positioned above other parts, classifying them within the Rosidae subclass due to their 5-part floral grouping. The indefinite number of filiform stamens helps differentiate them from the Chloranti group (**Dorji et al., 2024**).

The solitary floral arrangement aligns with its solitary inflorescent species classification. The fanlike panicle structure supports flower bud attachment, with fruit diameters ranging from 50-90 mm, depending on orange species. Growth conditions include PL, which is not mandatory, while soil type and chemical composition factors, such as PTFO and pH ranges in PTFP and PTFT, are optional (**Dilip, 2024**). Deficiencies in these non-mandatory prerequisites may lead to plant withering or decay. Optimal growth varieties for *Citrus sinensis* include MONTAN. Oranges rank in popularity after oranges and lemons, celebrated as New Year gifts in KINOTAS with varying prices. ETTAN represents gift-ready ordinary tangors, while SCRAN denotes tallying with a generous fruit yield. UFOELs are premium tangerine oranges cultivated through artificial selection, and MICHAN yields fruit with a sugar content of 7-8% (**Pandey, Pandey 2023**).

6. Biochemical composition

6.1. Primary metabolites

Primary metabolites include essential cellular compounds, such as carbohydrates, proteins, and fats. In *Citrus sinensis*, carbohydrates serve as important dietary sources of bioactive compounds beneficial for health and play a substantial role in fruit growth. Proteins, composed of amino acids dictated by DNA sequences in the cells, are vital for various cellular processes (**Maqbool et al., 2023**).

Fats consist of dense macromolecules with hydrophobic properties formed from fatty acid prenyl groups. Sugars, the primary metabolites of carbohydrates, include glucose, fructose, sucrose, and xylose found in the fruit. Amino acids, primary metabolites of proteins in *Citrus sinensis*, include alanine, leucine, aspartic acid, and glutamic acid. Compound lipids, a class of primary metabolites for fats in *Citrus sinensis*, are abundant in seeds and include hexadecanoic acid and other related fatty acids (**Shorbagiet al., 2022**).

While secondary metabolites are more common in plants, the economic value of primary metabolites is significant. Carbohydrates from Citrus are commercially available in

fresh and liquid forms like sweet fruit juice. At the same time, dairy products like milk and yogurt utilize sugars and proteins for preservation and flavor enhancement. The protein content in fruit juice improves taste and aroma and helps maintain bioactive components. Fats are predominantly found in dairy products such as milk, butter, and cheese, with product characteristics influenced by environmental temperature. (Saini *et al.*, 2022; Heena *et al.*, 2022).

6.2. Secondary metabolites

Secondary metabolites are organic compounds from primary metabolites, not directly involved in growth but critical for plant survival. They primarily play a defensive role, deterring herbivores, fungi, bacteria, and competing flora. Most secondary metabolites are terpene derivatives, including alkaloids and indoles, aiding in reducing herbivore attraction and protecting against UV light (Arbona *et al.*, 2015). They also contribute to scents, flavors, and pigmentation, such as the characteristic flavor of *Citrus sinensis*, due to volatile secondary metabolites in its peel, which is rich in terpenoids. Citrus aurantium leaves, known for their alkaloid content, are traditionally used in Chinese medicine. The 'Dekopon' hybrida combination of *C. reticulata* and *C. Paradisi* has flavonoids as its primary, secondary metabolites, with its peel containing high amounts of tangeritin derivatives (flavanone-O-C-glycosides) (Wang *et al.*, 2023).

Flavonoids are associated with health benefits, including antioxidant and anti-inflammatory properties, yet their specific quantities in *C. Reticulata* remain undetermined. They typically form small bodies within vacuoles, observable under high light intensity, similar to the activation of pheomelanin in human skin. To comprehend the pharmacological effects of oranges and other Rutaceae fruits, it is vital to examine the in-situ types of secondary metabolites (Liu *et al.*, 2022; Liu *et al.*, 2024).

6.2.1. Flavonoids

Flavonoids, prominent secondary metabolites in *Citrus sinensis*, are diverse polyphenolic compounds categorized into subgroups like flavanones, flavones, flavonols, and catechins. In oranges, flavanones dominate the flavonoid content, contributing

significantly to their antioxidant properties, which surpass other antioxidants in orange juice (Bailly, 2021).

Flavonoids exhibit various health benefits, acting as free radical scavengers, reducing agents, inhibitors of pro-oxidant enzymes, and potential anti-inflammatory agents. They also enhance the color, taste, and aroma of oranges. The biosynthesis of flavonoids initiates from the phenylpropanoid pathway, using phenylalanine as the starting material (Oluwole *et al.*, 2022). The production of flavonoids is regulated by multiple enzymes, influenced by factors such as species, climate, harvest year, and maturation stage. Research into flavonoids has revealed their pharmacological potential, particularly in terms of antioxidant activity, marking them as vital nutritional components (Ciupeiet *al.*, 2024). However, vertebrates cannot convert flavonoids into aglycone forms, raising questions about their antioxidant benefits in animals. Studies also explore how environmental factors, such as osmotic treatments and light conditions, affect flavonoid levels (Chen *et al.*, 2023).

6.2.2. Tannins

Tannins are secondary metabolites primarily responsible for the astringent sensations affecting flavor and mouthfeel in humans. They serve as key defense agents in plants against herbivores and pathogens. The health benefits of tannins stem from their ability to interact with and precipitate proteins, thereby inhibiting enzymatic activities while providing antioxidant and antimicrobial properties (Wang *et al.*, 2017). Tannins are valuable in the pharmaceutical and cosmetic industries and play a significant role in human diets. Their potential as preservative agents highlight the importance of researching the agronomic and environmental factors that influence tannin production in *C. reticulata* Blanco (Sun *et al.*, 2010). Recent studies indicate that tannin levels can impact fruit quality and shelf life. This citrus species is globally recognized for its easy peeling and pleasant taste, even in less ripe fruit that undergoes degreening (Yang *et al.*, 2023). The peels are rich in tannins and are valued in Chinese culture for traditional remedies and as food additives, particularly in fruit candy production (Wu *et al.*, 2022).

6.2.3. Phenols

This review focuses on orange orange (*Citrus sinensis*), a significant source of phenolic compounds, which are important secondary metabolites. These compounds have diverse

chemical structures, typically featuring one or more benzene rings, and are classified into various categories, including simple phenols, phenolic acids, coumarins, flavonoids, tannins, and others. Flavonoids, the most diverse group, include subclasses like flavones, flavonols, and anthocyanins (Chen *et al.*, 2020).

They contribute to the health benefits of fresh agricultural products and influence the flavor, fragrance, and color of fruit. Oranges have garnered interest for their marketability and quality, with a limited timeframe for fresh consumption, making the harvesting of non-mature fruits relevant for export. Phenolic compounds can form astringent complexes with proteins, potentially enhancing the flavor of fresh Citrus (Chen *et al.*, 2021)

Understanding these compounds may help producers optimize harvesting times. However, research specifically on orange orange's phenolic content is limited, and the factors affecting it are not well-studied. Environmental factors and cultivation practices significantly influence the accumulation of these metabolites and overall yield (Saini *et al.*, 2022)

Furthermore, phenolic compounds are associated with various health benefits, including antiproliferative, anti-inflammatory, and antioxidant effects, which protect cells from oxidative damage and modulate important biological functions (Shorbagiet *al.*, 2022). This highlights the potential of phenolic compounds in functional foods and nutraceuticals, appealing to both researchers and consumers (Durmus *et al.*, 2024)

6.2.4. Other Secondary Metabolites

Orange (*Citrus sinensis*) contains various secondary metabolites beyond coumarins, terpenes, volatiles, and flavonoids, notably furoquinoline-type alkaloids, which have closely related structures. Terpenoids, comprising thousands of single compounds and diverse subgroups, can be found in free, esterified, or glycosidically bound forms. Their electrical excitation provides UV signals and influences interactions between tangerines on Madeira Island and the Silverleaf (SL) disease (Arbona *et al.*, 2015).

Other secondary metabolites, like low molecular mass amines and amino acids with methoxylated phenyl rings, present different odors from terpenes, while polyketides with keto- and hydroxyl groups show similar variations (Singh *et al.*, 2023). These compounds

have been overlooked in tangerines, yet this study identifies their specific patterns. It also evaluates the cumulative impact of these minority metabolites alongside major classes (volatiles, terpenes, phenolics, alkaloids), asserting that secondary metabolites can be treated as generic individuals, with rubes tangerines accumulating numerous unique compounds (Bureš *et al.*, 2023).

The accumulation of these substances, influenced by biotic and abiotic factors, leads to variations in biological functions. Therefore, examining large compound groups without considering the complexity of these metabolites is too simplistic for understanding the biochemical composition of plants like *Citrus sinensis* in terms of their secondary metabolites (Goh *et al.*, 2022).

7. Biological activities of orange peels

7.1. Antibacterial activity of Orange Peels

Orange peels (*Citrus sinensis*) have been utilized in traditional medicine and culinary practices for their antibacterial properties against various pathogens; however, the issue of current antibiotic resistance is a global concern, indicating that orange peels can serve as a valuable natural source (Alharthi *et al.*, 2021). Disk diffusion and minimum inhibitory concentration tests were performed against potential pathogens, including *Escherichia coli* O157: H7, *Salmonella Typhi*, *Salmonella Typhimurium*, *Listeria monocytogenes*, and *Staphylococcus aureus*. The results showed that both ethanol and water extracts exhibited observable zones of inhibition against all pathogens, particularly against *E. coli* O157:H7 and *S. Typhi* (Lee *et al.*, 2023).

Researchers found that orange peels contain bioactive compounds, such as terpenoids and hesperidin, which contribute to their antibacterial effects. Knowing the antibacterial properties of orange peels can help prevent foodborne illnesses and provide broader alternatives to other products (Khalid *et al.*, 2021). Additionally, understanding its bioactive compounds can elucidate the antibacterial behavior, facilitating commercialization. The use of natural sources, such as plants and herbs, has gained commercial interest in the discovery of novel compounds with a broad spectrum of antimicrobial activities. (Shin *et al.*, 2021) (Tshireletso *et al.*, 2021)

7.1.1. Mechanisms of Antibacterial Activity of Orange Peels

After sun drying, essential oil extraction follows. Volatile bioactive compounds in *Citrus sinensis* (orange) peel are extracted via steam distillation. The essential oil is tested for antibacterial activity against gram-negative and gram-positive bacteria. This exploration examines how these antibacterial compounds inhibit bacterial growth by disrupting essential cellular processes (**Caputo et al., 2018**).

Disruption of cellular integrity and membrane functions leads to bacterial cell death. Leaky bacterial cells with increased membrane permeability fail to maintain osmotic pressure and burst. Anti-enzyme compounds bind to enzyme surfaces, blocking substrate attachment and inhibiting catalytic reactions (**Narayanankutty et al., 2022**).

Microorganism growth and survival rely on synthesizing cytoplasmic and cellular components and enzymes. Disruption of cell growth and replication triggers an anti-starvation effect. For a bactericidal effect, metabolically active bacteria must be in the logarithmic growth phase. Disturbance in nutrient intake and protein synthesis significantly impacts active bacteria. Prolonged exposure to external stimuli or uncontrolled environmental factors easily stresses bacteria, leading to their death (**Andrade et al., 2022; Wang et al., 2023**).

7.2. Antioxidant Activity of Orange Peels

Efforts to utilize natural products in healthy foods, cosmetics, pharmaceuticals, and agrochemicals continue. Many natural products exhibit physiological activities against bacteria, fungi, cancer, and various diseases, including diabetes. Recent studies highlight the biological activities of citrus fruit peels—waste from the fruit processing industry showing excellent antibacterial and antioxidant properties. Antioxidant activity neutralizes free radicals, thereby preventing damage such as lipid oxidation in cell membranes (**Chen et al., 2020**).

Antioxidants, which help absorb reactive oxygen species (ROS) in the body, prevent oxidative stress related to chronic diseases such as cancer and cardiovascular diseases, leading causes of death globally. Consequently, there is a push to explore the beneficial biological activities of citrus peels. While the chemical constituents of citrus peels are well-documented, Korean studies primarily focus on their use as flavoring agents rather than their pharmaceutical applications (**Abdel-Naeem et al., 2022**). Korean oranges, a variety of

Tangerines, are utilized in traditional medicine due to their rich array of functional compounds. *Rhus verniciflua*, a plant with unique chemical properties, is also used for its decoction. The antioxidant activities of these extracts are anticipated to differ (**Abdel-Naeem *et al.*, 2022; Li *et al.*, 2022**).

This research involved measuring the contents of bioactive compounds and employing LC-MS analysis to explore these relationships. In summary, citrus peels from the fruit processing industry possess important biological activities, and enhanced extraction methods could lead to new commercial uses for these valuable substances. (**Malik *et al.*, 2021; Lin *et al.*, 2021**)

7.2.1. Mechanisms of Antioxidant Action

The essence of good health lies in maintaining an optimal state of being, not merely the absence of disease or pain. This requires proactive efforts to ensure efficient bodily functions, closely related to redox homeostasis, which balances oxidative stressors and antioxidants. At a molecular level, it involves preventing the oxidation of vital macromolecules while ensuring physiological processes that utilize reactive oxygen species (ROS) as secondary messengers remain optimal. Understanding ROS, identified as byproducts of aerobic metabolism, is crucial for preventing damage from oxidative stress due to a redox imbalance. Various antioxidative mechanisms must be employed to counteract this. ROS and free radicals arise from stimuli such as UV light, chemicals, and pollutants) (**Sajid *et al.*, 2023**).

The body has a complex network of antioxidants and enzymes acting as a first defense line by neutralizing these harmful agents or repairing their damage. ROS are highly reactive and able to harm DNA, proteins, and lipids. Citrus fruits provide significant phytochemicals known for their bio-activities that combat free radical formation, highlighting their potential against oxidative stress-related illnesses (**Sarangi *et al.*, 2024**)

Most research has focused on the bio-activities of citrus juice phytochemicals, with less attention given to the antioxidant properties of the peel, which is often discarded despite being a byproduct of the citrus juice industry. Finding new uses for these peels, particularly from oranges, oranges, and lemons, is essential (**Jide Afolayan, Oluwatomide Oyeyinka., 2020; Edo *et al.*, 2024**).

8. Pomegranate (*Punica granatum*)

8.1. Historical background of Pomegranate (*Punica granatum*)

Pomegranates (*Punica granatum*) have been cultivated and highly appreciated since ancient times in the Mediterranean region. Due to their appealing organoleptic features, pomegranates were adopted by cultivated societies and have since become a symbol of culture and tradition. A great diversity of pomegranates is found in a wide range of geographical conditions, different histories, and religious backgrounds (**Bar-Ya'akov *et al.*, 2019**). Many other plants and cultural aspects have been related to pomegranates in this environment over the years. This diverse cultural background has influenced the various botanical uses of the plant in these regions (**Meena, Vincent ., 2025**).

Pomegranates have different roles in various cultures, ranging from ornamentation and serving as a symbol of life, power, and fertility to being used as medicine, as described in ancient documents. Due to the growing contemporary interest in the fruit, recent scientific studies may offer insight into the potential beneficial effects of its consumption. In light of this diverse cultural background, the traditional uses of pomegranate in the Mediterranean Basin are described, and contemporary scientific studies are reviewed(Figure 3) (**Melgarejo-Sánchez *et al.*, 2021**).

Pomegranates (*Punica granatum*) are deciduous fruit trees found in regions of the Mediterranean basin, the Caucasus Mountains, and the Middle East. The pomegranate plant has been revered for centuries as a symbol of eternal life, love, and fertility around the world. Pomegranates are rich in specific phytochemicals due to their geographical regions of cultivation, unique growing conditions, and many edible parts such as the peels, seeds, and arils (seeds and fleshy coverings). The edible parts (seeds and peels) of the pomegranate fruit contain diverse phenolic metabolites, with some specific compounds, such as anthocyanins, hydrolyzable tannins, and ellagic acid (**Ranjha *et al.*, 2021; Türker *et al.*,2024**).



Figure 3. *Punica granatum* leaves, fruits, and flowers (Ochoraet *et al.*, 2023).

9. Origin and etymology

9.1. Origin

The pomegranate, *Punica granatum*, is a small tree that grows to a height of 5 to 8 meters. It is mainly found in Iran, the northern Indian Himalayas, China, the United States, and the Mediterranean region (Figure 4). Due to its ability to adapt to harsh environmental conditions, pg is one of Iran's most significant endemic plants, growing in the majority of the country's arid and semiarid regions. During a germplasm collection, more than 764 varieties of *Punica granatum* (Pg) were gathered and cultivated in the Iranian cities of Saveh and Yazd. Each cultivar has distinct fruit qualities, such as size, color, flavor, ripening period, and disease susceptibility (Razi *et al.*,2021).



Figure 4. Center of origin for Pomegranate (*Punica granatum* L.) (Stover, 2012).

9.1.1. Etymology

The term 'pomegranate' in English is thought to originate from the medieval Latin phrase 'pōmumgrānātum,' which translates to 'seeded apple.' In this context, the Latin component 'pōmum' refers to a round fruit or apple, while 'grānātum' indicates its defining feature—being filled with seeds (Chandra *et al.*, 2010). 'Grānātum' itself is derived from the Latin expression 'grānātummollē,' meaning 'apple with many seeds.' Over time, the word has been adapted in various languages, including Italian (pōmumgrānātum → Belgrano), Czech (granátovéjablko), Russian (гранатноеяблоко), Hungarian (gránátalma), German (Granatapfel), and Romanian (roadie) (Asgaryet *al.*, 2021; Noor *et al.*, 2022).

The term 'pōmumgrānātum' is found as early as in the work of ancient Roman scholar Pliny the Elder in his famous multi-volume study of natural history. The characteristic 'being seeded' in the pomegranate is already known to ancient Romans from the word 'pōmumgrānātum.' The term 'pōmumgrānātum berberis' refers to three large-seeded fruits: pomegranate, quince, and white mulberry. The term 'pōmumgrānātum' or 'grānātum' or 'punicum' or 'punicum malum' means 'seeded apple,' which points explicitly to the fruit full of seeds such as a pomegranate. Etymologically, the term 'Pune' from the pharaonic word of the 5th millennium BCE means pomegranate. Similarly, 'punicum' from the old Phoenician word also means pome (Asgaryet *al.*, 2021; Noor *et al.*, 2022).

10. Botanical classification

Punica granatum, belongs to the Lythraceae Family (Table2).

Table 2. Botanical classification of Pomegranate (*Punicagranatum*) (Kumari *et al.*, 2012)

Royaume	Plantae
Kingdom	Plantae (Angiosperms)
Order	Myrtales
Family	Lythraceae
Genus	Punica
Species	<i>Punicagranatum</i>

11. Geographic distribution *Punica granatum*

11.1. In Algeria

Punica granatum, commonly known as pomegranate, is widely cultivated in Algeria, particularly in the northeastern region around Constantine. The specific climate and soil conditions in this area are ideal for growing pomegranate trees, which thrive in warm, arid environments. The region's well-drained soils, ample sunlight, and relatively moderate temperatures create a suitable habitat for these fruit-bearing trees (Mouaset *et al.*, 2021)

Historically, pomegranates have played a crucial role in traditional agriculture and medicine in Algeria. They are valued not only for their nutrient-rich fruits but also for their various medicinal properties attributed to bioactive compounds found in all parts of the plant, including the peel, seeds, and juice. These components have been utilized in local remedies and dietary practices, underscoring the pomegranate's dual significance as both a food source and a natural therapeutic agent (Zouaoui *et al.*, 2019).

11.2. Worldwide

The pomegranate tree (*Punica granatum*) has a long history of cultivation, particularly in the Mediterranean region (Bar-Ya'ako, Holland., 2018), where its adaptability enables it to thrive. It has been cultivated since ancient times and holds cultural and nutritional significance. In the Middle East (Iran and Turkey), pomegranates are staple crops, with Iran being a top producer. South Asia (India and Pakistan) grows diverse varieties (Hassani Moghaddam, Sepahvand., 2020). widely used in cuisine and traditional medicine. California, located in North America, has developed a strong industry centered on

pomegranate production. In North Africa, including Algeria, the fruit plays a significant role in supporting local economies. Finally, Spain and Italy also cultivate pomegranates for both local consumption and export (**Ge *et al.*, 2021**).

12. Botanical characteristics

The pomegranate tree initially appears as a bush but transforms into a trunked form with natural pruning. Its bark is smooth, gray, and thorny, with many thorns fading over time. The small, narrow, deciduous leaves, measuring 2-8 cm, are glossy dark green on top and bright green underneath. They are lanceolate and can be confused with ornamental Myrtaceae, lacking the nightshade smell (**Melgarejo-Sánchez *et al.*, 2021**). The tree is self-pollinating, featuring red or bright orange flowers that grow singly or in clusters of 2 to 6, leading to fruit based on fertilization. Pomegranate juice ranges from red to purplish and varies in sweetness depending on the cultivar, gaining popularity due to its health benefits (**Ghasemi-Soloklue *et al.*, 2023**). The Mollar cultivar has a tender, sweet arils with few seeds. Pomegranates thrive in hot, humid, or dry climates, flowering in late spring and bearing fruit 5 to 7 months later. They are deciduous in frosty regions and prefer slightly alkaline soil, with pH levels of 7 to 7.5 (**Tinebraet *et al.*, 2021**).

A pomegranate tree typically lives around 40 years and begins bearing fruit at 4 to 5 years. Some trees can exceed 100 years when properly cared for with manure and drip irrigation. They have a complex root system, developing a fine reddish root early on, along with long, rigid radial roots that can extend 1.5 to 5 m and weigh between 0.1 to 1.5 kg (**Yilmaz *et al.*, 2021**). This robust root system enables them to withstand salinity, extending to depths of 10 m (**Joshi *et al.*, 2021**). Regular pruning is essential due to their vigorous growth and small fruit size; traditionally, they are pruned in a goblet shape for optimal lighting and flowering. Summer pruning is crucial to protect against whiteflies (**Ferrara *et al.*, 2023**).

13. Biochemical composition

Pomegranate is a traditional and historical fruit with high variability in different parameters, including major bioactive compounds. Significant attention is given to the health and other beneficial effects of this fruit (**Tarantino *et al.*, 2022**). The pomegranate fruit is unique in its internal anatomy. The arils part is visually, chemically, and nutritionally and

tastes different from the other fruit parts. Many pomegranate studies include nearly all the fruit; however, the focus is on arils, which comprise the majority of the fresh fruit weight (Ghasemi-Solokluei *et al.*, 2023; Farsi *et al.*, 2023).

13.1. Primary metabolites

Primary metabolites, including sugars, organic acids, amino acids, ammonium, and lipids, are essential for plant growth and development. They support normal physiological functions, while secondary metabolites contribute to plant defense, climate interactions, and pollination (Bar-Ya'akov *et al.*, 2019).

In pomegranates, different cultivars exhibit varying fruit characteristics. Sugars and lipids enhance flavor and texture, whereas organic acids contribute to acidity. The Anor cultivar has the highest sucrose levels in its arils, which are crucial for sugar transport during ripening. Similarly, orange juice reaches peak sucrose concentration in the eighth ripening stage. Amino acids, although essential for fruit development, can have anti-nutritive effects. Nitrogen is vital for plant health, and ammonium absorption is more efficient than protein uptake (Hooks *et al.*, 2021).

The pomegranate seed oil contains seven lipid types, with palmitoleic acid as the predominant fatty acid. It has antioxidant properties but is low in omega-3 fatty acids, and storing at low temperatures helps prevent rancidity (Tarantino *et al.*, 2022).

The fruit also contains anthocyanins and hydrolyzable tannins, which impact color and stability. Ruby concentrates are analyzed for soluble solids and stability at 25°C. Certain compounds, like gallagyl and punigluconin, degrade faster than gallagic acid. While aril concentrates are more stable than juice, prolonged room-temperature storage reduces anthocyanin content, especially at higher temperatures (Naser *et al.*, 2021).

13.2. Secondary metabolites

The secondary metabolites of pomegranate (*Punica granatum*) primarily include:

1. **Anthocyanins:** These are key color molecules found in various parts of the pomegranate, especially in the arils and peel. Pomegranate fruit is recognized as a rich source of anthocyanins, which produce several derivatives such as:

- Cyanidin (red pigments)
- Delphinidin (purple pigments)
- Pelargonidin (orange pigments) (Turkyilmaz, 2013).

Six anthocyanin molecules have been identified in pomegranate cultivars, with variability in their concentrations depending on both genetic and environmental factors.

1. **Hydrolyzable Tannins:** These are important for their antioxidant properties and are found in all parts of the fruit, contributing to both health benefits and bitter flavor profiles (Mo *et al.*,2022).
2. **Ellagitannins:** A specific type of hydrolyzable tannin, ellagitannins are noted for their health-promoting effects. They are predominantly found in the fruit peel and arils (Venatet *al.*,2021).
3. **Flavonoids:** Alongside anthocyanins, various flavonoid compounds are present throughout the pomegranate, contributing to its antioxidant capacity and health benefits (Venaet *al.*,2021).
4. **Other Phenolic Compounds:** Pomegranate contains numerous polyphenols that contribute to its antioxidant properties and various health benefits. This category includes various derivatives of flavonoids and other phenolic compounds (Saparbekovaet *al.*,2023).

14. Biological activities of pomegranate peels

14.1. Antibacterial activity

The antibacterial activity of strictly water extracts (SWEs), 75% ethanol extracts (E75s), and dehydrated ground peels of *Punica granatum* L. were evaluated against skin infections, including methicillin-resistant *S. aureus* (MRSA) and other resistant isolates. With the increasing resistance to conventional antibacterial agents, the demand for natural antibacterial compounds is growing, as these adaptable bacteria pose a significant public health concern (Kupniket *al.*, 2021).

While recent research has primarily focused on other bacteria, this study highlights the potential of pomegranate peels, a significant byproduct of the pomegranate industry, as a sustainable resource. These peels are rich in secondary metabolites, and findings reveal

that their antibacterial activity is comparable to that of the seeds (**Salimet *et al.*, 2023**; **Podettiet *et al.*, 2023**).

The study also investigated the effects of the extracts on animal-source biofilms; however, the experiments were conducted on planktonic cultures, which do not fully reflect real-world food safety scenarios. Further research on the safety, biocompatibility, and monitoring of essential oils and pomegranate peel extracts is necessary, as some natural compounds may be toxic or have adverse health effects (**Krivokapić *et al.*, 2022**; **Chavan *et al.*, 2023**).

14.2. Antioxidant activity

Pomegranate fruit peels, which represent about 40% of the fruit's weight, are often discarded despite their considerable health benefits against oxidative stress. Composed of 50-60% moisture, 5-10% organic acids, and various phenolic compounds, these peels have gained attention for their potential in medicine, nutraceuticals, disinfectants, and other commercial uses, particularly in developing countries (**Alazbet *et al.*, 2021**). Recent research has thoroughly examined the composition and safety of Pomegranate Peel Extract (PPE), which is rich in flavan-3-ol, phenolic acids, and flavonoid glycosides (**Daset *et al.*, 2021**). PPE shows promise as an antioxidant and can serve as a food-grade colorant for fresh pasta. Object Mapper may help optimize the industrial production of PPE due to its favorable physiochemical properties (**Montefusco *et al.*, 2021**).

Combating oxidative stress is crucial for human health and exploring food supplements with antioxidant properties. Pomegranate peel contains polyphenols, including tannins, flavonoids, and phenolic acids, which act as antioxidants and may delay the onset of chronic diseases. Standard methods to assess antioxidant activity include Folin–Ciocalteu reagent, DPPH, and ABTS. Entrepreneurs and academics are encouraged to utilize pigmented PPE's advantageous attributes (**Mo *et al.*, 2022**). Optimizing actor and color can lead to high-yield, food-grade antioxidant PPE extraction for specific tasks. Pressurized liquid extraction and purification offer a green, efficient method for producing pigmented PPE, providing excellent antioxidant and colorant properties. Considering factors affecting PoPx's total phenolic content through orthogonal experiments enables the extraction of high-quality, low-toxic phytochemicals to harness PoPx's beneficial effects safely (**Azmat *et al.*, 2024**).

Chapter 02

Pathogenicmicroorganisms

1. Definition of pathogenic microorganisms

A harmful microbe is commonly defined as a living entity capable of inducing damage to its target host during the host–microorganism interaction, directly by the microbe (toxins or virulence factors) or indirectly through the body's immune system (Casadevall, Pirofski, 1999).

Disease-causing microbes are classified into opportunistic, facultative, and obligate pathogens. Strict pathogens, such as *Mycobacterium tuberculosis*, the agent of tuberculosis (TB), also induce multidrug-resistant infections. They infect only specific hosts but can also target both healthy and vulnerable host species. Facultative pathogens similarly infect a specific host range but can also survive outside the host. Opportunistic pathogens can grow on various organic materials and typically show low pathogenicity towards a broad spectrum of living hosts. If the host is damaged or immunocompromised, opportunistic pathogens become more aggressive. It is believed that obligate and facultative pathogens have evolved from microbes that were originally opportunistic (Van Baarlen *et al.*, 2007).

1.1. Antimicrobial resistance and the need for natural alternatives

Microbial drug resistance develops when microbes, such as bacteria, viruses, fungi, and parasites, combat microbicidal agents, making traditional medical interventions powerless and raising the threat of infection and mortality (Ahmed *et al.*, 2024). There are various types of anti-infectives, including antibiotics, antifungals, and food preservatives, that inhibit the growth and proliferation of microorganisms or eliminate them. Antibiotics are a category of microbicides primarily used to combat pathogenic bacterial infections, and antibiotic resistance is more widespread than any other antimicrobial resistance (Salam *et al.*, 2023).

Bacteria and other germs have a notable ability to rapidly adjust, mutate, and transfer flexible genomic elements through horizontal gene transfer processes, allowing them to develop different resistance mechanisms. Treating human and animal infections can be challenging due to antimicrobial resistance (AMR) pathogens. Typical resistance mechanisms involve biochemical alterations, decomposition of antibiotics, restricting antibiotic access into cells to prevent their buildup, modification of metabolic pathways, changing binding sites such as ribosomes to lower treatment potency, and enhancing the performance of export systems that eject the antibiotics from cells before

they can reach high levels. Protective extracellular matrices are also frequently synthesized by bacteria, with diverse nutrient concentrations and minimal antibiotic entry (**Ahmed et al., 2024**).

Many antibiotics, particularly methicillin, are tolerated by Methicillin-resistant *Staphylococcus aureus*. Additionally, plasmids frequently carry resistance genes in Carbapenem-resistant Enterobacteriaceae, such as *Klebsiella pneumoniae* and *Escherichia coli*, promoting their spread among bacterial populations. Fluconazole-resistant *Candida* species cause opportunistic mucosal and genital area infections. Similarly, viruses, including HIV and influenza, also commonly develop Treatment-defying mutations (**Ahmed et al., 2024**).

Antibacterials are crucial in infighting pathogenic infections. However, the overuse and misuse, such as inappropriate uses and treatment lengths over the decades, have resulted in selection pressure with the emergence of resistant bacteria. Besides human medical treatment, the rise and propagation of drug resistance from the non-scientific utilization of antibiotics in feed given to livestock in several low and middle-income countries has been a key contributor. It demands intensified surveillance of the consequences of the overuse and misuse of antibiotics in livestock feeds to lower the rate of antibiotic resistance (AMR) (**Salam et al., 2023**).

In addition to improper antibiotic use, the absence of an official system to collect and report data, the lack of standard rules, and the failure to require stewardship make it challenging to share helpful information and control antibiotic abuse in hospitals and clinics. (**Abbas, 2024**).

Given the concerning growth of AMR and its severe consequences, there is an urgent need to discover natural alternatives that can effectively treat infections while minimizing the risk of resistance development.

A. Plant-derived compounds

In recent years, in some high-income countries, such as the United States, medicinal drugs obtained from botanicals constitute nearly a quarter of the total. While in densely populated nations with accelerating development and decadent cultural heritage, as seen in China (**Abdallah, Sadeek., 2019**).

- **Phytochemicals**

Botanical bioactive substances sourced from plants have emerged as highly effective Germ-inhibiting agents due to their diverse functional pathways and minimal chance of drug resistance. Moreover, contrary to typical antibiotics, which usually inhibit specific bacterial activities, phytochemicals act via different mechanisms, including altering the cell walls, suppressing protein production, and altering microbial metabolism, which lowers the risk of resistance emergence. The wide-ranging effectiveness of specific bioactive compounds enables them to combat a variety of infectious agents, including bacteria, fungi, and viruses, making them adaptable alternatives to traditional antibiotics. Beyond the antimicrobial properties of these compounds, such as tannins, terpenoids, and alkaloids, they also exhibit inflammation-reducing and oxidation-inhibiting traits, thereby strengthening immunity against illness (Adam *et al.*, 2024).

- **Fruit and vegetable peels**

Researchers have extracted a wide range of bioactive substances from different outer layers, such as those of oranges, lemons, and bananas, demonstrating properties that fight germs and have oxidation-preventing and anti-inflammatory effects. These substances are active against several bacterial strains. Like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella enteritidis*, *Micrococcus aureus*, and *Pseudomonas fluorescens* (Saleem *et al* 2020).

Exploiting fruit and vegetable peels for pharmaceutical purposes will reduce agro-waste and, on the other hand, provide a solution to AMR in a cost-effective and eco-friendly way (Naghavi, *et al.*, 2024).

1.2. Importance in public health

Antimicrobial resistance (AMR) has emerged as a significant concern for public health, food system security, and development. Across the three-decade period (1990 to 2021), fatality rates dropped by over half among children below age five. However, it has escalated by more than 80% for the older population (those over 70 years old).4.71 million Deaths were projected to be attributed to drug resistance in 2021, including 1.14 million mortality directly caused by bacterial AMR (Naghavi *et al.*, 2024).

Microbes that develop resistance are progressively turning human and animal conditions hard to manage. Once-curable infections are now evolving into stubbornly resistant ones. The ineffectiveness of initial antimicrobial therapies has led to increased dependence on backup therapies, which are often costly, have more severe side effects, and require prolonged treatment durations. Resistant infections equally raised clinical consultations, laboratory analyses, and infection control strategies. The gap between developing new effective drugs and the rapid worsening of drug resistance among microbes complicates the threat even more (Ahmed *et al.*, 2024).

2. Pathogenetic bacteria

2.1. *Staphylococcus aureus*

Staphylococcus aureus is a ubiquitous and pathogenic bacterium. Around half to more than half of hosts are occasionally or chronically inhabited with *Sare occasionally or chronically inhabited with S. aureus* (Kobayashi *et al.*, 2015); it is Gram-positive bacteria that can proliferate in both aerobic and anaerobic conditions (facultative) and thermal conditions between 18 C and 40 C. catalase-producing (all pathogenic *Staphylococcus* species), coagulase-secreting, Susceptible to novobiocin and ferments mannitol (Taylor *et al.*, 2023).

2.1.1. Classification

Sir Alexander Ogston first isolated *Staphylococci*, who linked them to wound infections. Later, in 1884, the German Physician Friedrich Rosenbach identified *S. aureus* as a distinct species and named it for its distinctive golden colonies (Table 4) (Horswill, Keim., 2023).

Table 3. Classification of *Staphylococcus aureus* (Horswill, Keim., 2023).

Domain	Bacteria
Kingdom	Bacteria
Phylum	<i>Firmicutes</i>
Class	<i>Bacilli</i>
Order	<i>Bacillales</i>
Family	<i>Staphylococcaceae</i>
Genus	<i>Staphylococcus</i>
Species	<i>Staphylococcus aureus</i>

2.1.2. Morphology

Staphylococcus aureus is a Gram-positive microbe (violet-staining), cocci-shaped, and frequently organized into clusters “grape-like.” On growth medium, these bacteria multiply in saline concentrations up to 10%. Their colonies are commonly golden or yellow (aureus means golden or yellow) (Figure 7) (Taylor *et al.*, 2023).

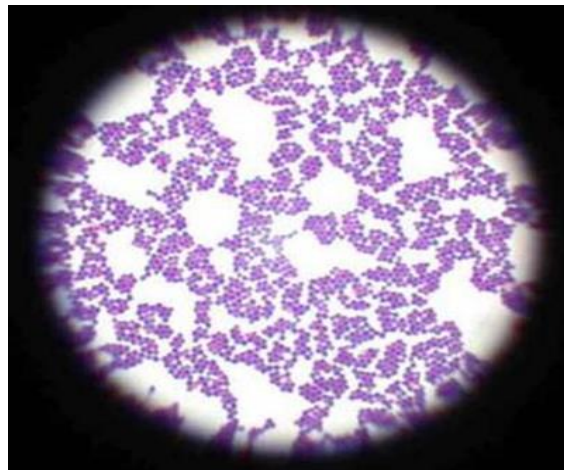


Figure 5. *Staphylococcus aureus* Under the microscope (Ragab *et al.*, 2021)

2.1.3. Habitat

S. aureus mainly inhabits the front part of the nostrils and also resides in the skin, oropharynx, Inner vaginal surface, and digestive tract. Being colonized by *Aureus* increases the risk of infection; it develops when the disease-causing agent invades through the protective layers of the skin and mucosa (Keim *et al.*, 2023).

2.1.4. Pathogenicity

Staphylococcus aureus is a leading bacterial pathogen in humans. It is responsible for many infections, such as bacteremia, infection of the heart valves, dermal and soft tissue diseases (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), bone infection, septic arthritis, Infections involving medical implants, respiratory infections (e.g., pneumonia and empyema), Stomach and intestinal infection, meningitis, toxic shock syndrome, and infection of the urinary system (Taylor *et al.*, 2023).

2.2. *Klebsiella pneumoniae*

K. pneumonia is classified under a family of facultative anaerobic Gram-negative rods, often opportunistic, lacks motility, and does not produce spores. It can metabolize lactose, produce catalase, lacks cytochrome oxidase activity, and can develop biofilms (Abbas *et al.*,2024).

2.2.1. Classification

Klebsiella pneumoniae was first described by Carl Friedlander in 1882 as an encapsulated bacillus after recovering the pathogen from the lungs of pneumonia victims. Initially called Friedlander's bacillus. In 1886, the organism was officially named *Klebsiella* (Table 5) (Ashurst, Dawson, 2023).

Table 4. Taxonomic Classification of *Klebsiella pneumoniae* (Dong *et al.*, 2022).

Domain	Bacteria
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	<i>Klebsiella</i>
Species	<i>Klebsiella pneumoniae</i>

2.2.2. Morphology

Klebsiella pneumoniae is a bacillus-shaped, immobile, and non-sporulating microbe, measuring between 0.3–2.0 µm wide and 0.6–6.0 µm long, with a gelatinous and shiny appearance (Abbas *et al.*, 2024)

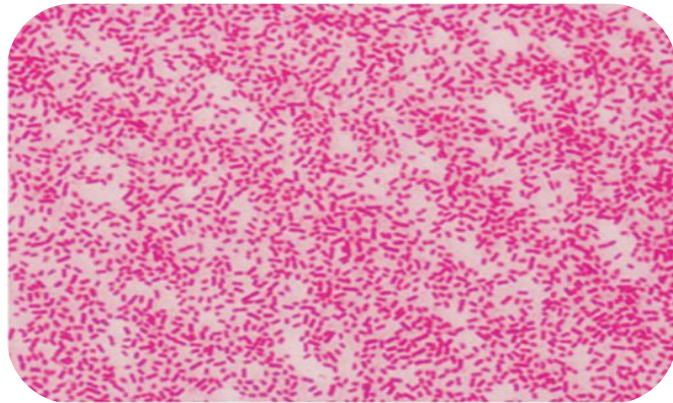


Figure 6. *Klebsiella pneumoniae* isolates (Kulkarni *et al.*, 2019).

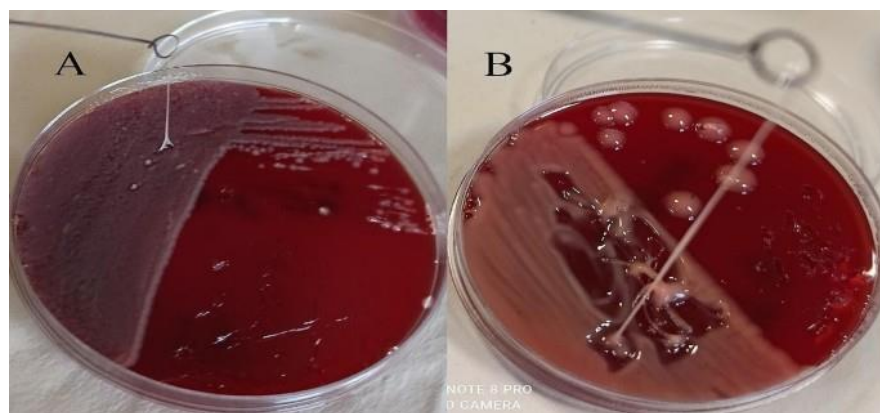


Figure 7. Mucoid appearance of *Klebsiella pneumoniae* (Sohrabi *et al.*, 2024).

2.2.3. Habitat

Humans are the main reservoir for *K. pneumoniae*. About 5% to 38% of people host the microbe in their feces and 1% to 6% in their nasal passages. Infections mainly originate from the patient's gut and the contaminated hands of medical staff; increased colonization rates have been linked to Chinese ancestry and chronic alcohol users (Ashurst ,Dawson., 2023). Additionally, humanists also inhabit animals, as well as other environments, such as wastewater, potable water, soils, lakes and rivers, factory discharges, and vegetation (Bagley, 1985).

2.2.4. Pathogenicity

K. pneumoniae severely targets immune-compromised hosts as a result of prior infections. However, this hyper-virulent pathogen also targets healthy people, causing Non-hospital-associated infections like a pyogenic liver abscess, meningitis, necrotizing fasciitis, eye infections, and life-threatening. Nosocomial infection development is often initiated by

gut colonization. Additionally, such an invasion may further spread to the urinary system, pulmonary system, and bloodstream. *K. pneumoniae* also produces biofilm as another infectious aspect, which can grow on clinical devices (such as catheters and endotracheal tubes), leading to a significant source of infection in individuals who use catheters. Furthermore, the clinical infections caused by this pathogen are usually chronic due to two key reasons: the formation of biofilms that resist immunity within the organism and the synthesis of enzymes that may lead to toxin-secreting, facultative bacteria (Abbas *et al.*, 2024).

2.3. Bacillus cereus

Bacillus cereus is a toxin-secreting facultative anaerobic gram-positive bacterium. It is ubiquitous and can spoil food. *Bacillus cereus* can rapidly proliferate at ambient temperatures. It is a catalase-producing, anaerobic, facultative bacterium, yet it may occasionally show variation in Gram staining. It is spore-forming in response to temperature extremes. Consequently, it is found as a contaminant of various foods, i.e., beef, turkey, rice, beans, and vegetables (McDowell *et al.*, 2023).

2.3.1. Classification

Frankland and Frankland were the first to detect *Bacillus cereus* in the cowshed air back in 1887. Since 1950, numerous cases of food poisoning related to this pathogen have been reported in Europe, affecting various food items, including meat dishes, seafood, soups, fish, milk, and ice cream. Moreover, the first related illness was reported in the USA in 1971 (Table 6) (Islam, 2019).

Table 5. Taxonomic classification of *Bacillus cereus* (Islam, 2019).

Domain	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	Bacillus
Species	<i>Bacillus cereus</i>

2.3.2. Morphology

When cultivated on blood and chocolate media, *Bacillus cereus* shows a regular rod-like (bacillus) structure, measuring between $3 \times 0.4 \mu\text{m}$ to $9 \times 2 \mu\text{m}$. Colonies on sheep blood media appear non-transparent with irregular edges. When inoculated onto egg yolk medium, it generates an opaque halo, indicating the presence of lecithinase. In biological fluids, it appears as straight or mildly curved rods, either alone or in short chains on Gram staining. Within tissue samples, they may be observed as elongated filamentous shapes. Spores may or may not be detectable (Figure 8 and 9) (McDowell *et al.*, 2023).



Figure 8. Gram stain of *B. cereus* (Rostom, Selim ., 2017).



Figure 9. Gray, opaque colonies of *B.cereus* on Blood agar (Adugna *et al*, 2024).

2.3.3. Habitat

B. cereus-related species are naturally occurring in soil and inhabit various ecosystems. Due to the production of stress-resistant endospores, they can survive in a dormant state, making it challenging to define their primary habitats. Besides soil, it has been recovered from raw and preserved foods, arthropods, and plants (Ehling-Schulz *et al.*, 2019).

2.3.4. Pathogenicity

The pathogenic potential of *B.* depends on its ability to infect and survive in the organism and penetrate tissues. This ability differs among strains, complicating both the evaluation and management of the associated risks. It is known to lead to other illnesses, such as airway infections, hospital-acquired infections, eye inflammation, and neurological infections, and it is a significant issue in the food sector due to its resistance to food decontamination procedures (Mohammadi *et al.*, 2022).

2.4. *Escherichia coli* (E. coli)

E. coli species are nearly entirely harmless bacteria. It is a commensal microbe (Martinson *et al.*, 2020). It is negatively stained, lacks spores, has a rod-like shape, can survive with or without oxygen, and can ferment lactose. Belonging to the *Escherichia* genus, which is often found in the environment, food, and intestinal tract of warm-blooded animal species, it is the most studied bacterial model. Its peak growth occurs at 37°C (98°F), but some experimental strains can reproduce at temperatures as high as 49°C (120.2°F). It requires only 20 minutes to multiply in ideal conditions (Basavaraju, Gunashree., 2022).

2.4.1. Classification

Escherichia coli was initially discovered and characterized in 1885 by Theodor Escherich, a Bavarian pediatrician, who was investigating the role of microorganisms in newborn digestion and illness. He identified the bacterium and initially named it *Bacterium coli commune* based on microscopic examination and Gram staining of neonatal stool. In 1919, it was given the new name *Escherichia coli* in honor of his work. The first isolate, which lacks virulence genes, remains a primary lab model (Martinson *et al.*, 2020).

Table 6. Taxonomic Classification of *Escherichia coli* (Basavaraju, Gunashree ., 2022)

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	<i>Escherichia</i>
Species	<i>Escherichia coli</i> (<i>E.coli</i>)

2.4.2. Morphology

E. coli is a Gram-negative bacillus, non-sporulating, non-acid fast, and found as single units or in pairs. Measuring between 1–3 μm in length and 0.4–0.7 μm in width and 0.6–0.7 μm^3 in volume. It is motile via peritrichous flagella, and only a few lack mobility (Figure 10) (**Basavaraju, Gunashree., 2022**).



Figure 10.Gram-stained *E.coli* (**Hossainet et al., 2021**).

2.4.3. Habitat

It is a commensal microorganism in the gut and a frequent contaminant in healthcare environments. *E.coli* is the most prevalent gram-negative species in the intestines, and it is generally nonpathogenic. It also exists in the environment, including soil, water, raw vegetables, and poorly cooked meat (**Mueller et al., 2023**).

2.4.5. Pathogenicity

While most colonic *E. coli* are nonpathogenic, those with virulence factors can lead to various infections. The intestinal pathogenic *E.coli* is frequently implicated in extraintestinal infections, such as cholecystitis, bacteremia, cholangitis, UTI, traveler's diarrhea, septicemia, as well as neonatal meningitis. Except for infant meningitis and gastroenteritis, infections are endogenous and develop when host defenses are compromised by trauma or illness. To become pathogenic, *E. coli* must produce virulence factors, including fimbrial and fimbrial adhesins, capsules, toxins (such as exotoxins, hemolysins, and enterotoxins), and iron uptake systems (**Basavaraju, Gunashree., 2022**). Also, Drug resistance in *E. Coli*. This poses a serious and critical challenge on a global scale for both humans and animals (**Poirelet et al., 2018**).

2.5. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-stain-negative, aerobic, sporogenous rod-shaped pathogenic bacterium that can cause multiple infections in both healthy and immunocompromised Individuals. It is challenging to control because it often targets individuals with weakened immune systems, and also due to its ability to resist treatment, harsh environmental conditions, and its broad range of survival strategies. (Pandey, Wilson., 2023).

2.5.1. Classification

Sédillot, in 1850, was the first to detect that the change in the color of medical bandages was due to a communicable agent. In 1860, Fodos isolated the pigment responsible for the blue stain, and in 1862, Lucke was the first to link this pigment to a bacterial organism (Table 7) (Lister *et al.*, 2009).

Table 7. Taxonomic Classification of *Pseudomonas aeruginosa* (Diggle, Whiteley., 2020)

Kingdom	Monera
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	<i>Pseudomonas</i>
Species	<i>Pseudomonas aeruginosa.</i>

2.5.2. Morphology

Mobile, Gram-negative bacillus microbe measures between 1–5 µm long and 0.5–1.0 µm wide (Figure 11) (Hussein, 2022).



Figure 11. Gram-stained *Pseudomonas aeruginosa* (Mubbunuet *al.*, 2023).

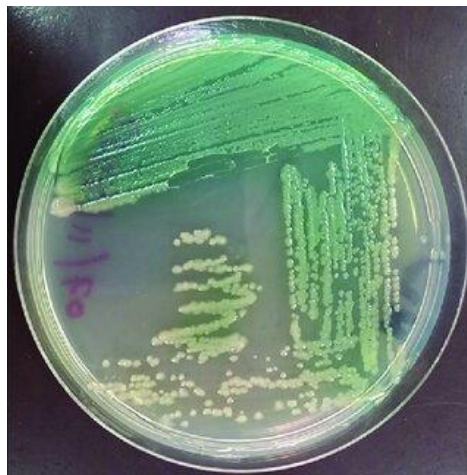


Figure 12. The growth of *Pseudomonas aeruginosa* on nutritional agar (Mubbunuet *al.*, 2023).

2.5.3. Habitat

Pseudomonas aeruginosa typically occupies soil, water, and plants. It is detected in the epidermis of some healthy individuals and has been recovered from the pharynx (5%) and feces (3%) of outpatients. Digestive tract colonization rates rise in inpatients to 20 percent after three days following their entry to the hospital. Inside hospitals, *P. aeruginosa* colonizes various reservoirs, such as antiseptics, food, basins, and floor wipers. Additionally, transmission occurs between patients through hand contact with medical staff, infected surfaces, and unsafe food (Iglewski, 1996).

2.5.4. Pathogenicity

Diseases resulting from *P. aeruginosa* may be severe if insufficient treatment is applied, especially if antibiotic-resistant strains are implicated. Antimicrobial resistance has posed a

challenge to both public and veterinary health over the past three decades. Moreover, it is a persistent infectious agent in healthcare facilities, leading to over half of the nosocomial infections. Despite the introduction of novel antibiotics, death rates associated with *P. aeruginosa* remain elevated (**Tuon *et al.*, 2022**). Even though new antimicrobial drugs have been developed, mortality rates due to *P. aeruginosa* continue to be high (**Tuon *et al.*, 2022**).

Chapter 03

Material and methods

1. Preparation of Plant Material

Fresh fruits of orange (*Citrus sinensis*) and pomegranate (*Punica granatum*) were obtained from a local market in **Khenchela, Algeria** in December 2024. Fruits were cleaned and washed with tap water, and then rinsed in sterile distilled water.

This research was conducted at the Khenchela University Educational Laboratory.

1.1. Solvents

Two solvents were used in this experiment: 80% methanol, 80% ethanol.

1.2. Preparation of peels

The peels were manually separated using sterile scalpel and knife. Then cut into small pieces and spread on clean trays and dried naturally at room temperature, in a shaded and well-ventilated area, away from direct sunlight. This drying method was used to preserve sensitive bioactive compounds that might degrade under heat or UV exposure. The drying process continued for several days (up to 10 days) until the peels became completely dry and brittle. Once completely dried, the peels were ground into fine powder using electrical grinder, and then stored in sterile, airtight containers at room temperature, away from light and humidity until used (**Pandey *et al.*, 2011**).

1.3. Preparation of peel extracts

To extract using hydroalcoholic solvents, 60 g of finely powdered fruit peels were soaked in a mixture of 600 ml of hydroalcoholic solvents (20% distilled water and 80% ml of methanol or ethanol). The mixtures were stirred for 15 minutes at room temperature and then left to rest for 24 hours at room temperature (**Cowan, 1999**). The samples were agitated for 30 to 60 minutes and covered with aluminum foil, then allowed to macerate for 24 to 72h hours at room temperature, protected from the sunlight (**Mau *et al.*, 2001; Abhary, AL-Hazmi., 2015**). Thereafter, Whatman number 1 filter paper was used to remove the impurities and supernatant was preserved and kept in the refrigerator at 4°C.

1.4. Solvent Removal

The solvents (ethanol and methanol) were removed from the extracts using a rotary evaporator under reduced pressure and controlled temperature (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.

1.5. Yield determination

The following equation determines the extraction yield: Extraction yield (%) = $(W1/W2) \times 100$; W1 is the mass of crude extract (g), and W2 is the sample (g) mass (Ngamkhae *et al.*, 2022).

2. Phytochemical screening

The identification of any compounds was conducted utilizing the techniques outlined by Vijayalakshmi *et al.* (2012), Lawal *et al.* (2019); Solanki *et al.* (2019) and Wilde *et al.* (2013).

Polyphenols

To detect polyphenols, add a drop of 2% alcoholic ferric chloride solution to 2 mL of either aqueous or alcoholic plant extract in a test tube. The development of a blue-black or green coloration, varying in intensity, indicates a positive result (Vijayalakshmi *et al.*, 2012).

Flavonoids

The presence of flavonoids is assessed using the Shinoda reaction (Soulama *et al.*, 2014). To 1 mL of extract, add a few drops of concentrated hydrochloric acid and approximately 0.5 g of metallic magnesium. Allow the reaction to proceed for 3 minutes. The appearance of a red, orange, pink, or purplish-red color confirms the presence of flavonoids (Solanki *et al.*, 2019).

Tannins

In a test tube, mix 1 mL of extract with 2 mL of distilled water, followed by 1 mL of 2% aqueous ferric chloride (FeCl₃). The appearance of a greenish color indicates the presence of catechic tannins, while a blue-black color indicates gallic tannins (Vijayalakshmi *et al.*, 2012).

Terpenoids

To detect terpenoids, combine 0.5 mL of the aqueous extract with 2 mL of chloroform in a test tube, then carefully add a few drops of concentrated sulfuric acid. A positive reaction is indicated by the formation of a reddish-brown ring at the interface of the two liquids (Solanki *et al.*, 2019)

Saponins

Add 3 mL of distilled water to 1 mL of extract. Vortex the mixture vigorously for 30 seconds and let it stand for 15 seconds. Persistent frothing during this time indicates the presence of saponins (Vijayalakshmi *et al.*, 2012)

Quinones

Mix 1 mL of the extract with 1 mL of concentrated sulfuric acid. The development of a red coloration signifies the presence of quinines (Solanki *et al.*, 2019; Wilde *et al.*, 2013).

Anthraquinones

To 0.5 mL of extract, add a few drops of hydrochloric acid. The formation of a red precipitate confirms the presence of anthraquinones (Wilde *et al.*, 2013).

Anthocyanins

Mix 2 mL of extract with 2 mL of sulfuric acid (H₂SO₄) and 2 mL of ammonium hydroxide (NH₄OH). An increase in coloration under acidic conditions followed by a blue shift in a basic medium indicates the presence of anthocyanins (Vijayalakshmi *et al.*, 2012; Wilde *et al.*, 2013).

3. Flavonoids content determination

The aluminum chloride (AlCl₃) method is frequently utilized to assess the flavonoid content in extracts derived from the studied fruit peels. The process entails adding 500 µL of each extract or standard solution (after suitable dilution) into a test tube, followed by the incorporation of 500 µL of a 2% AlCl₃ solution that has been prepared in methanol. Following a 10-minute incubation period at room temperature in the absence of light, the absorbance is recorded at 430 nm (Baghiani *et al.*, 2012). The total flavonoid content is reported as milligrams of quercitrine equivalent per gram 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* (*E. coli* 01, 02, 03, and 04) from clinical source, isolated from tract urinary infection were also tested.

4.2. Preparation of extracts dilutions

The extracts were dissolved in diméthylsulfoxyde (10%) (DMSO) to prepare three concentrations for each extract: 100, 50, and 25 mg/mL. Therefore, 100 mg, 50 mg, and 25 mg of the extracts were weighted. Then, each weighed extract was solubilized in 1 ml of DMSO in a sterile tube and mixed well until the total solubilization was achieved.

4.3. Solid media disc diffusion method

A fresh 24-hour cultures of selected bacterial species grown on nutrient agar were used to prepare suspensions in 9 ml of sterile physiological water, adjusted to to a turbidity level of 0.5 McFarland standard tube (about 10^8 cell/ml). These suspensions served as inoculums to test the effect of crude extracts by the agar diffusion method on Mueller Hinton agar plates. Petri dishes poured with the Muller-Hinton agar are seeded a sterile swab soaked in the bacterial suspension prepared (**Konemanet al., 1997; Karou et al., 2005**).

Furthermore, sterile filter disc (diameter 6 mm, Whatman paper no. 3) impregnated with 20 µl of the various prepared extracts concentration were placed on the surface of these plates, Petri dishes were preincubated at +4°C for 2 h to allow uniform diffusion of extracts into the agar. After preincubation, the plates with bacteria were incubated at 37°C for 24 h. Additionally, a negative control was included, which consists of a disc containing 20 µl DMSO. The antibacterial activity was evaluated using the well diffusion method. In the second and third repetitions, the wells were carefully filled with the plant extract using a Pasteur pipette to ensure precision and consistency (**Yazdani et al., 2012**).

5. Determination of the minimum inhibitory concentration (MIC)

5.1. 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 100 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 = 100 mg/ml; C2=50 mg/ml; C3=25 mg/ml; C4=12.5 mg/ml; C5=6.25mg/ml; C6=3.125; C7=3.125; C8= 1.56 mg/ml ; C9= 0.78mg/ml (**Kouamé *et al.*, 2008; Ouattara *et al.*, 2013**).

5.2. 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 10⁶ CFU (**Yapo Yomeh Cynthia Viviane *et al.*, 2020**).

5.3. 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.

Chapitr 04

Results and discussion

1. Extraction

1.1 Determination of Extraction Yield

The extraction yields varied depending on the plant material and solvent used (Table 8) Among the extracts, methanol extraction of pomegranate peel produced the highest total extracts weight with 26.2g. Indicating that methanol is a highly efficient solvent for extraction compounds from pomegranate (*Punica granatum*) peel under the conditions used. Similarly ethanol extraction of pomegranate peel yielded a substantial amount with 19.39g, slightly lower than methanol but still higher than other extracts.

For Orange (*Citrus sinensis*) peel, the ethanol extract produced a considerably greater yield than the methanol extract with 14.5g for ethanol extract and 11.7g for methanol extract. Suggesting that ethanol may be better solvent than methanol for extracting compounds from Orange (*Citrus sinensis*) in this study.

Table 8. Characteristics and yield of fruit peel extracts

Plant material	Solvent	Total extract weight (g)	Yield (%)	Aspect	Color
Orange peel	Ethanol	14.5 g	24.16%	Viscous	Dark orange to brownish
Orange peel	Methanol	11.7g	19.5%	Viscous	Orange to light brown
Pomegranate peel	Ethanol	19.39	32.31%	Viscous	Dark reddish brown
Pomegranate peel	Methanol	26.2	43.66%	Viscous	Dark brown to blackish brown

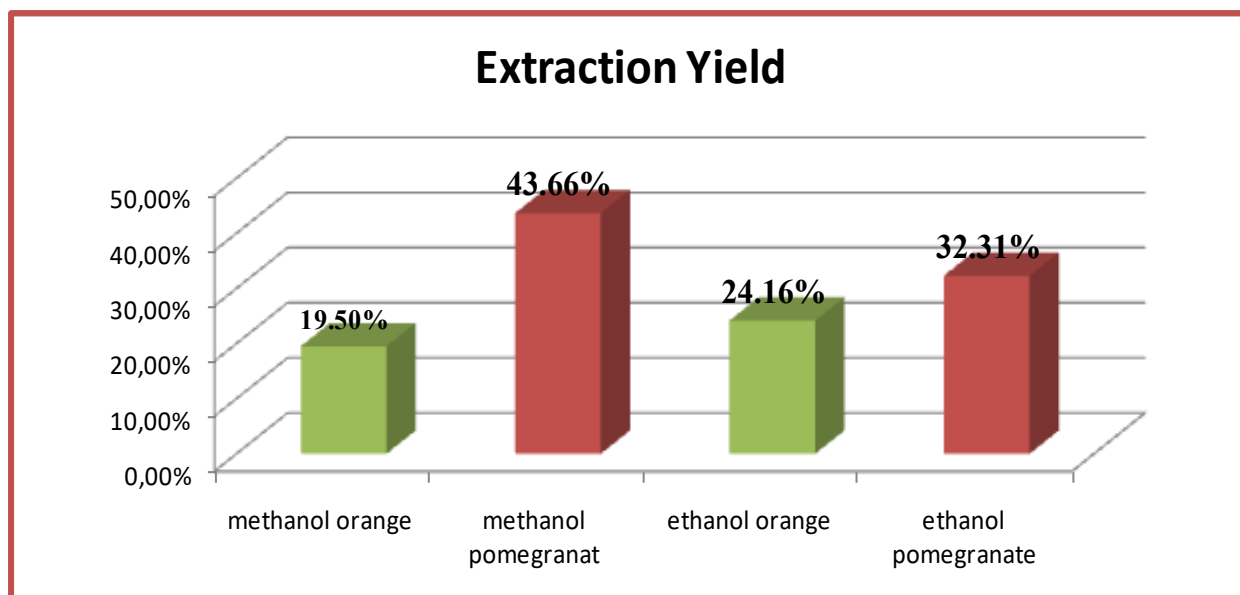


Figure 13: Comparative Yield Analysis of Extracts (Orange and Pomegranate).

According to the Bar graph (figure 13), pomegranate revealed a higher extraction yield than orange with both solvents (Methanol and Ethanol), while ethanol gave a slightly higher yield 24.16% than methanol 19.50% in the case of orange.

Discussion

Abkhoo and Jahani (2017) indicated that the yields of plant extracts vary depending on the extraction solvent and the plant extract used. The yields of extracts obtained from the same organ and the same species can be influenced by several factors, such as the extraction method and the conditions applied, such as the drying time of the plant material, the quantity of the plant to be extracted, the time, stirring speed, temperature, and polarity of the solvent (**Koné et al., 2017**). It is highly recommended to use solvents with varying polarities to extract a wide range of phytochemicals with high precision. The variations in values can be attributed to the genetic variety of these plants, which impacts the production of bioactive metabolites (**Ellafi et al., 2023**).

The extraction yields obtained from the fruit peel powders varied depending on the solvent used. Methanolic extraction of pomegranate peels resulted in the highest yield (43.66%), followed by ethanolic extraction (32.31%). This is consistent with **Ahmed et al. (2023)**, who reported that methanol, due to its higher polarity, is more efficient at extracting phenolic compounds and other bioactives from pomegranate peels compared to ethanol.

Elzoghbiy *et al.* (2022) confirmed that pomegranate peels contain significant amounts of polyphenols and flavonoids, which contribute to the high extraction yield observed with methanol. The viscous texture and dark brown to blackish coloration of the methanolic pomegranate extracts further suggest a high concentration of these compounds.

In contrast, orange peel extracts showed lower yields with ethanol (24.16%) and methanol (19.5%). This difference likely reflects the comparatively lower content of extractable phenolic compounds in orange peels, as supported by previous phytochemical studies (Ahmed *et al.*, 2023)

Also, the ethanolic extracts of orange peels showed a higher yield (24.16%) than methanolic extracts (19.5%), which differs from some previous reports where methanol extracted higher yields (Gupta *et al.*, 2021; Shukre *et al.*, 2020). This discrepancy could be due to differences in peel composition, solvent polarity, and extraction parameters such as time, temperature, and peel preparation. Ethanol, being less polar than methanol, may have better solubilized certain compounds present in the orange peels used here, resulting in a higher extraction yield (Ellafi *et al.*, 2023).

2. Phytochemical screening results

The phytochemical screening of *Punica granatum* (pomegranate) and *Citrus sinensis* (orange) peel extracts revealed a diverse array of bioactive compounds, with notable variations influenced by both plant species and extraction solvents. According to Table n°5, Polyphenols, flavonoids, and tannins, are strongly present in all extracts, which can be explained by the fruits' high concentration of these three metabolites.

Table 9. Phytochemical screening Results

Compound	Solvent	Plant	Results
Polyphenols	Ethanol	Orange	+
		Pomegranate	+
	Methanol	Orange	+
		Pomegranate	+
Flavonoids	Ethanol	Orange	+
		Pomegranate	+
	Methanol	Orange	+
		Pomegranate	+
Tannins	Ethanol	Orange	+

		Pomegranate	+
	Methanol	Orange	+
Terpenoids	Ethanol	Orange	-
		Pomegranate	+
	Methanol	Orange	-
		Pomegranate	+
Saponosides	Ethanol	Orange	-
		Pomegranate	+
	Methanol	Orange	-
		Pomegranate	-
Quinones	Ethanol	Orange	+
		Pomegranate	+
	Methanol	Orange	+
		Pomegranate	+
Anthraquinones	Ethanol	Orange	-
		Pomegranate	+
	Methanol	Orange	+
		Pomegranate	+
Anthocyanins	Ethanol	Orange	+
		Pomegranate	-
	Methanol	Orange	+
		Pomegranate	+

The qualitative phytochemical screening showed the presence of several phytochemical classes (phenols, flavonoids, anthocyanins, coumarins, quinones, tannins, saponins, and triterpenoid,) in pomegranate and orange peel extracts. Polyphenols, Flavonoids and Tannins were detected in all extracts. Studies conducted on pomegranate peels have reported that among the various phytochemical classes present in pomegranate peels, phenols, flavonoids, and tannins were considered the major phytochemical classes found in pomegranate peels (Sweidan *et al.*, 2023).

Terpenoids were exclusively identified in pomegranate extracts for both solvents. Saponins were present only in the ethanolic pomegranate extract, while quinones appeared consistently across all samples, Anthraquinones showed variable distribution, present in all samples except ethanolic orange extract, Lastly, anthocyanins were detected in all extracts except ethanolic pomegranate, suggesting variations in the anthocyanin profile influenced by both solvent and plant source. The peels of these fruit have been reported to be rich in

various bioactive components, in particular the phytochemicals, which in turn are responsible for their numerous reported health benefits including antioxidant and antimicrobial activities (Altarawneh *et al.*, 2022).

Despite the increased research interest in pomegranate and orange peels, few studies have been made on pomegranate peels and orange of Algerian origin. Moreover, factors such as variations in these fruits cultivars, geographical region, and extraction methods affect the phytochemical composition and concentrations of pomegranate peels, and hence their health outcomes (Sweidan *et al.*, 2023).

Among the various pomegranate peel extracts used, the ethanol extract was the only extract that showed positive detection for all phytochemical classes. Previous studies done on the pomegranate peels showed similar results in which ethanol (absolute or diluted) was one of the best solvent for the extraction of their phytochemicals (Altarawneh *et al.*, 2022). These results demonstrate that active compounds present in plant materials differ in their polarity, and their extraction depends greatly on the choice of solvent used and the method of extraction applied

3. Flavonoid content results

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 10).

Table 10. Flavonoids content

Extract	Ethanolic orange	Methanolic orange	Ethanolic pomegranate	Methanolic pomegranate
<i>Flavonoids</i> mgCE/ g	20± 0.014	22±0.048	53 ± 0.86	32±0.38

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 (Naczka, 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). These findings align with the study by Elzoghbiy et al. (2022), which reported substantial quantities of total flavonoids in pomegranate peel extracts. Hence Omoba et al. (2015) detected similar values for orange ethanolic and methanolic extracts.

4. Antibacterial activity results

The antimicrobial activities of the plant extracts against microbial strains are presented in Table 12. The inhibition zone diameters ranged from 06 to 28 mm at three concentrations: 25, 50 and 100 mg/ml (Table 11). Overall, the four extracts of the studied fruit peels have potent antibacterial effect against the tested bacteria.

Table 11. Antibacterial activity results in mm

Strains	Extract Concentration (mg/ml)	Hydroalcoholic extracts inhibition zone (mm)			
		Methanol Orange peel	Ethanol Orange peel	Methanol pomegranate Peel	Ethanol pomegranate Peel
<i>E. coli R</i>	100	00	12±00	27±00	19.66± 0.57
	50	00	12±00	27±00	19.33± 1.15
	25	00	9±00	20±00	19± 1.73
<i>E. coli 01.</i>	100	7.66±2.88	14±00	2.5±2.12	23.5±2.12
	50	8±2.82	12±00	23.5±3.53	22.33±3.78
	25	8±2.82	9 ±00	20±00	16.66±2.08
<i>E. coli 02.</i>	100	6±00	11.5±00	22±00	23±00
	50	6±00	10.5±00	26±00	22±00

	25	6±00	14 ±00	20±00	19.25±1.06
<i>E. coli 03.</i>	100	13.75±5.03	14±00	25.5±2.12	24±2.82
	50	11±4.24	10.5±0.70	23.5±4.94	27 ±2.82
	25	6±00	14±00	25.5 ±2.12	20.5±2.12
<i>E. coli 04.</i>	100	18.25±1.06	10±00	23.5±2.12	27±1.41
	50	11.66±2.08	11 ±00	25.5±0.70	22±00
	25	12±2.64	11.5±0.70	21.5±3.53	20±1.41
<i>Bacillus cereus</i>	100	00	00	25±1.41	19±7,07
	50	00	14±00	23.5±0.70	20± 5,65
	25	00	9±00	20.5±0.70	16,83±1,04
<i>Klebsiella pneumoniae</i>	100	21±00	14.5±0.70	24±1.41	22± 2,82
	50	6±00	12±1.41	23.5±0.70	21,5± 0,70
	25	6±00	10.5±0.70	20.5±0.70	19,5±2,12
<i>Staphylococcus aureus</i>	100	10±3.46	14±00	26±00	25± 1
	50	7.33±1.15	13±00	28±3.46	23± 2.64
	25	8.66±2.30	11±00	23±00	22 3.46
<i>Pseudomonas aeruginosa</i>	100	15±00	8.5±3.53	22±4.24	21±7.07
	50	10.33±5.68	11±00	23±4.24	21.66±3.51
	25	12±00	11.5±0.70	20.5±0.70	19.5 ±0.70

The antibacterial activity of methanolic and ethanolic extracts of orange and pomegranate showed significant variation against different bacterial strains. Methanolic pomegranate extracts showed potent antibacterial properties against *E. coli R*, *E. coli 03*, and *Klebsiella pneumoniae*. Ethanolic pomegranate extracts also showed substantial efficacy against *E. coli 03*, *E. coli 04*, and *Staphylococcus aureus*. Orange extracts generally showed lower activity, although methanolic orange extract displayed moderate effectiveness against *E. coli 04* and *Klebsiella pneumoniae*. Overall, pomegranate extracts significantly outperformed orange extracts, indicating their strong potential as natural antibacterial agents.

The findings highlight that pomegranate extracts possess significant antibacterial properties, suggesting their potential as natural antibacterial agents compared to orange extracts. Methanolic and ethanolic orange extracts exhibited prominent activity against *E.*

coli 03 and *E. coli* 04, with inhibition zones up to 18.25 mm. However, limited antibacterial effects were observed against *Klebsiella pneumoniae* for both orange extracts, suggesting selective antibacterial efficacy depending on the bacterial strain, extraction solvent, and plant source.

The methanolic and ethanolic extracts of pomegranate peel exhibited significant inhibitory effects, with inhibition zones reaching up to 28 mm against *S. aureus* and 27 mm against *Escherichia coli* R with methanolic extract and *Pseudomonas aeruginosa* methanolic extract. These findings were consistent with those reported by **Ahmed et al. (2023)**, who demonstrated that both methanolic and ethanolic extracts of *P. granatum* peels exerted vigorous antibacterial activity against bacterial strains, including *E. coli* and *K. pneumoniae*, with methanol showing superior efficacy. Overall, pomegranate extracts significantly outperformed orange extracts, indicating their strong potential as natural antibacterial agents across the tested bacterial species. Similar results were reported by **Boukezzoula et al. (2024)** regarding the antibacterial activity results for *Punica granatum* against *E. coli*, *Bacillus cereus*, *klebsiella pneumoniae*, *staphylococcus aureus*, *Pseudomonas aeruginosa*.

In addition, our findings on the efficacy of *pomegranate* hydroalcoholic extracts, particularly the ethanolic extract, against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* are based on other studies. **Al-Bouneb et al. (2021)** revealed that the highest antibacterial activity was recorded against *Staphylococcus aureus*, as well as *Staphylococcus aureus* ATCC29213 with the ethanolic extracts (29.3 ± 0.3 mm). However, moderate inhibition zones were observed against *Escherichia coli* ATCC 25922 within the ethanolic extracts (14.6 ± 0.3 - 17 ± 0.7 mm), as well as *Pseudomonas aeruginosa*, which was less potent than those in our results.

Moreover, orange peel extracts generally showed lower activity, although the methanolic orange extract displayed moderate effectiveness against *E. coli* 04 (18.25 mm) and *Klebsiella pneumoniae* (21 mm). Similarly, **Mehmood et al. (2015)** highlighted the antibacterial properties of *C. sinensis* peel extracts, noting moderate inhibition zones, particularly against *K. pneumoniae* and *E. coli*, in alignment with the comparatively lower activity observed in our study for orange extracts. Previous results with Citrus indicated that peel extracts obtained with methanol and ethanol exhibit moderate antibacterial activity against pathogenic bacteria. For instance, Phattayakorn and Wanchaitanawong (2009) reported the antimicrobial properties of an ethanol extract from the fruit peel of Thailand

against *Bacillus*; however, the obtained results, between 11.00 and 14.00 mm, were similar to those observed in our study with the methanolic extract against the species *Bacillus cereus*. Likewise, the result reported by Dayanand *et al.* (2011) of hydroethanolic and hydroethanolic extracts of orange peel at different concentrations exhibited antibacterial against *Staphylococcus aureus* (6-14mm), *Bacillus sp* (6- 9mm), *E. coli* (7-12 mm), *Pseudomonas aeruginosa* (6-9 mm) which were in concordance with our results.

The superior performance of pomegranate peel extracts is likely attributed to their high content of polyphenols, tannins, and flavonoids such as punicalagin and ellagic acid, which are known to disrupt bacterial cell walls and inhibit enzymatic systems. Collectively, these findings underscore the relevance of pomegranate peel as a promising natural antibacterial agent and support its integration into alternative antimicrobial strategies (Boukezzoula *et al.*, 2024).

The mechanism of action behind the antibacterial properties of Citrus and pomegranate peel extracts may involve the disruption of bacterial cell walls, interference with enzyme activity within the bacterial cells, or the impairment of bacterial communication systems, known as quorum sensing (Bouyahya *et al.*, 2022). The exact mechanisms are likely as diverse as the phytochemicals present in the peels, and further research into these areas could unlock new antibacterial strategies and compounds (Jubair *et al.*, 2021).

These variations of the inhibition zone diameters of the extracts of the three plants are likely attributed to the phytochemical composition of the plants utilized and the method of extraction, whether it be water or solvents (Rafailidis, Kofteridis, 2022). Likewise, an absence of antimicrobial effects is not due to the presence of bioactive substances in the plant or a lack of antimicrobial activity against microorganisms. Several factors, including solvent quantity, extraction time, and process, can also influence the activity of the crude extracts (Hanoun *et al.*, 2023).

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

5.1. Results and interpretation

The antibacterial activity of fruit peels (orange and pomegranate) extracts was evaluated in 96-well microplate using broth microdilution method. Bacterial growth was assessed visually based on turbidity. A clear well indicated inhibition (no growth), While turbidity indicated bacterial proliferation.

The following table 4 shows the MIC values (in mg/ml) of each fruit peel extracts against tested bacteria.

Table 12. Minimum inhibitory concentrations of the studied extracts

Extract Strains	Ethanollic pomegranate peels	Methanollic pomegranate peels	Ethanollic orange peels	Methanollic orange peels
<i>Pseudomonas aeruginosa</i>	0.78mg/ml	0.78mg/ml	1.56mg/ml	0.78mg/ml
<i>Bacillus cereus</i>	0.78mg/ml	0.78mg/ml	3.125mg/ml	0.78mg/ml
<i>Klebsiella pneumoniae</i>	0.78mg/ml	0.78mg/ml	0.78mg/ml	0.78mg/ml
<i>E. coli R</i>	0.78mg/ml	0.78mg/ml	/	3.125mg/ml
<i>Staphylococcus aureus</i>	0.78mg/ml	0.78mg/ml	0.78mg/ml	0.78mg/ml
<i>E. coli 1</i>	0.78mg/ml	0.78mg/ml	0.78mg/ml	0.78mg/ml
<i>E. coli 2</i>	0.78mg/ml	0.78mg/ml	1.56mg/ml	0.78mg/ml
<i>E. coli 3</i>	0.78mg/ml	0.78mg/ml	1.56mg/ml	0.78mg/ml
<i>E. coli 4</i>	0.78mg/ml	0.78mg/ml	1.56mg/ml	0.78mg/ml

The MIC values obtained with pomegranate extract show that both the the ethanollic and ethanollic extracts of pomegranate peels exhibits exceptionally strong antibacterial activity against all tested bacterial strains. Since lower MIC values indicate higher antimicrobial potency, the results demonstrate the extract’s significant effectiveness. Notably, all strains were fully inhibited at the lowest tested concentration 0.78mg/ml.

Moreover, the MIC values obtained show that the ethanollic extract of orange peels extract exhibited notable s antibacterial activity against the tested bacterial strains. Likewise, the MIC values obtained with the methanollic extract of Orange peels exhibits exceptionally strong antibacterial activity against almost all tested bacterial strains. Hence, eight out of nine bacterial strains, both gram-negative and gram positive showed complete inhibition of

growth at lowest tested concentration 0.78mg/ml, except for *E.coli* ATCC 25922 (Reference strain) that showed MIC at 3.125mg/ml.

The present study demonstrates a significant antibacterial activity of both pomegranate and orange peel extracts, with notable difference depending on the solvent and the fruit type.

Among all tested extracts, pomegranate peel extracts regardless of the solvent used, are the most effective, achieving complete bacterial inhibition at the lowest concentration 0.78mg/ml. these findings are in strong agreement with those of **Ahmed et al. (2023)** who reported that methanolic and ethanolic extracts of (*Punica granatum*) peels exhibited broad spectrum antibacterial activity against clinical isolates like *E. coli* and *klebsiella pneumoniae* with methanol showing slightly higher efficacy.

This strongest antibacterial activity of (*Punica granatum*) compared to (*Citrus Sinensis*) may of be due to the high content of bioactive compounds in pomegranate peel, such as polyphenols tannins, and flavonoids, which are known to damage bacterial membranes and inhibit growth, as reported by **Elzoghbiy et al. (2022)**, who identified high levels of these compounds in (*Punica granatum*) peel extracts. In contrast, studies on orange have shown comparatively lower levels of these compounds (**Musa et al., 2023**).

Moreover, Orange peel extract exhibited moderate antibacterial activity, with superior performance of Methanol-orange peels extract compared to the ethanolic extract, especially in inhibiting a wider range of clinical strains. This is consistent with the findings of (**Mehmood et al., 2015**).

These findings indicates that pomegranate peels are significantly more potent sources of antibacterial compounds than orange peels, and the methanol may be a more effective solvent for extracting antibacterial agents from orange peels.

Conclusion

Conclusion

Conclusion

The continuous rise of antimicrobial resistance presents a critical challenge to public health, emphasizing the urgent need for novel and effective natural alternatives. This study highlights the significant potential of *Punica granatum* and *Citrus sinensis* fruit peels as valuable sources of antibacterial compounds against various bacterial strains including *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, and against four strains of clinical *E. coli*.

The extraction processes demonstrated that solvent choice profoundly influences the yield and potency of bioactive constituents, with pomegranate peels consistently showing superior efficacy compared to orange peels. 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.

Despite these encouraging results, this investigation represents an initial step toward fully unlocking the medicinal value of these natural resources. Further comprehensive studies are required to evaluate their safety profiles, elucidate the mechanisms of action, and expand testing across diverse microbial pathogens. Additionally, exploring their synergistic effects with existing antibiotics and other biological activities could pave the way for innovative, multifunctional phototherapeutics.

Conclusion

Ultimately, this research reinforces the vital role of botanical source in the fight against antimicrobial resistance and encourages the integration of traditional knowledge with modern science to discover sustainable solutions for global health challenges.

***Bibliographical
references***

Bibliographical references

References

Abbas, A., Naqvi, S. A. R., Rasool, M. H., Noureen, A., Mubarik, M. S., & Tareen, R. B. (2021). Phytochemical analysis, antioxidant and antimicrobial screening of *Seriphidium oliverianum* plant extracts. *Dose-Response: A Publication of International Hormesis Society*, 19(1), 15593258211004739. <https://doi.org/10.1177/15593258211004739>

Abbas, R., Chakkour, M., Zein El Dine, H., Obaseki, E. F., Obeid, S. T., Jezzini, A., Ghssein, G., & Ezzeddine, Z. (2024). General overview of *Klebsiella pneumoniae*: Epidemiology and the role of siderophores in its pathogenicity. *Biology*, 13(2), 78. <https://doi.org/10.3390/biology13020078>

Abdel-Naem, H. H., Elshebrawy, H. A., Imre, K., Morar, A., Herman, V., Paşcalău, R., & Sallam, K. I. (2022). Antioxidant and antibacterial effect of fruit peel powders in chicken patties. *Foods*, 11(3), 301. [mdpi.com](https://www.mdpi.com)

Adam, A. B., Abubakar, M. Y., Aikhoje, E. F., & Nair, N. (2024). Phytochemicals for infectious diseases: A systematic review. *Conference Paper*.

Adujna, D., Diriba, S., Diriba, M., Girma, A., & Mekonen, H. (2024). Growing role of *Bacillus cereus* as an emerging potential food pathogen of humans: A review. *Austin Journal of Veterinary Science & Animal Husbandry*, 11(5), 1154. <https://austinpublishinggroup.com/veterinary-science-research/fulltext/avsah-v11-id1154.pdf>

Ahati, P., Xu, T., Chen, L., & Fang, H. (2022). Cytotoxicity, and antioxidant properties of gold nanoparticles mediated by *Citrus sinensis* seed aqueous extract: Introducing a novel chemotherapeutic drug. *Inorganic Chemistry Communications*. [\[HTML\]](#)

Ahmed, J., Abdu, A., Mitiku, H., & Ataro, Z. (2023). In vitro Antibacterial Activities of Selected Medicinal Plants Used by Traditional Healers for Treating Urinary Tract Infection in Haramaya District, Eastern Ethiopia. *Infection and drug resistance*, 16, 1327–1338. <https://doi.org/10.2147/IDR.S398204>

Ahmed, J., Abdu, A., Mitiku, H., & Ataro, Z. (2023). In vitro Antibacterial Activities of Selected Medicinal Plants Used by Traditional Healers for Treating Urinary Tract Infection in

Bibliographical references

Haramaya District, Eastern Ethiopia. *Infection and drug resistance*, 16, 1327–1338. <https://doi.org/10.2147/IDR.S398204>.

Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. (2024). Antimicrobial resistance: Impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health*, 2, 100081. <https://doi.org/10.1016/j.glmedi.2024.100081>

Akthar, M. S., Degaga, B., & Azam, T. (2014). Antimicrobial activity of essential oils extracted from medicinal plants against pathogenic microorganisms: A review. *Issues in Biological Sciences and Pharmaceutical Research*, 2(1), 1–7.

Alazb, B. R., El-Sahy, K. M., Sulieman, A., & Youssif, M. R. (2021). Physicochemical and organoleptic characteristics of cakes supplemented with tomato pomace, mango seeds kernel and pomegranate peels powders. *Plant Archives*. plantarchives.org

Alharthi, S., Noah Badr, A., Gromadzka, K., Stuper-Szablewska, K., Gabr Abdel-Razek, A., & Selim, K. (2021). Bioactive Molecules of Mandarin Seed Oils Diminish Mycotoxin and the Existence of Fungi. ncbi.nlm.nih.gov

Ali, M. S., Lee, E. B., Hsu, W. H., Suk, K., Sayem, S. A. J., Ullah, H. M. A., Lee, S. J., & Park, S. C. (2023). Probiotics and postbiotics as an alternative to antibiotics: An emphasis on pigs. *Pathogens (Basel, Switzerland)*, 12(7), 874. <https://doi.org/10.3390/pathogens12070874>

Altarawneh RM, Al-Jaafreh AM, Qaralleh H, Al-Qaralleh OS. (2022). Chemical profiling of Punica granatum peels from Jordan using LC–MS/MS and study on their biological activities. *International Journal of Food Science & Technology*, 57(8):5256–67.

American Type Culture Collection (ATCC). (n.d.). Discover the ATCC Genome Portal. ATCC. Retrieved June 1, 2025, from <https://www.atcc.org/applications/reference-quality-data/discover-the-atcc-genome-portal>

Bibliographical references

Ana KRIVOKAPIĆ, S., Otović, M., Perović, S., & Vratnica, B. D. (2022). Total phenols, flavonoids, anthocyanins and antioxidant activity of wild pomegranate (*Punica granatum* L.) biowaste from Montenegro. [academia.edu](https://www.academia.edu)

Andrade, M. A., Barbosa, C. H., Shah, M. A., Ahmad, N., Vilarinho, F., Khwaldia, K., ... & Ramos, F. (2022). Citrus by-products: valuable source of bioactive compounds for food applications. *Antioxidants*, 12(1), 38. [mdpi.com](https://www.mdpi.com)

Angiosperm Phylogeny Group (2016). "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV". *Botanical Journal of the Linnean Society*.

Antimicrobial Resistance Collaborators. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. *The Lancet*, 404(10459), 1199–1226. [https://doi.org/10.1016/S0140-6736\(24\)01867-1](https://doi.org/10.1016/S0140-6736(24)01867-1)

Arbona, V., J Iglesias, D., & Gómez-Cadenas, A. (2015). Non-targeted metabolite profiling of citrus juices as a tool for variety discrimination and metabolite flow analysis. [ncbi.nlm.nih.gov](https://www.ncbi.nlm.nih.gov)

Arbona, V., J Iglesias, D., & Gómez-Cadenas, A. (2015). Non-targeted metabolite profiling of citrus juices as a tool for variety discrimination and metabolite flow analysis. [ncbi.nlm.nih.gov](https://www.ncbi.nlm.nih.gov)

Asgary, S., Azadeh, Z., Dodman, S., Soleymani, A., Mirjalili, M. H., Farzaei, M. H., Sedaghat, M. (2021). Pomegranate bioactive compounds and health. In *The pomegranate: botany, production and uses* (pp. 504-547). Wallingford UK: CAB International. [\[HTML\]](#)

Ashurst, J. V., & Dawson, A. (2023, July 20). *Klebsiella pneumonia*. In *StatPearls*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK519004/>

Attah, A. F., Fagbemi, A. A., Olubiyi, O., Dada-Adegbola, H., Oluwadotun, A., Elujoba, A., & Babalola, C. P. (2021). Therapeutic potentials of antiviral plants used in

Bibliographical references

traditional african medicine with COVID-19 in focus: a Nigerian perspective. *Front Pharmacol* 12: 596855. *Frontiers in Pharmacology* | www.frontiersin.org, 12. [scienceopen.com](https://www.frontiersin.org)

Azmat, F., Safdar, M., Ahmad, H., Rizwan Jamil Khan, M., Abid, J., Sadiq Naseer, M., Aggarwal, S., Imran, A., Khalid, U., Mahvish Zahra, S., Islam, F., Arif Cheema, S., Shehzadi, U., Ali, R., Befar Kinki, A., Amer Ali, Y., & Ansar Rasul Suleria, H. (2024). Phytochemical profile, nutritional composition of pomegranate peel and peel extract as a potential source of nutraceutical: A comprehensive review. [ncbi.nlm.nih.gov](https://pubmed.ncbi.nlm.nih.gov/)

Baghiani, A., Ameni, D., Boumerfeg, S., Adjadj, M., Djarmouni, M., Charef, N., Khennouf, S., & Arrar, L. (2012). Studies of antioxidants and xanthine oxidase inhibitory potentials of root and aerial parts of medicinal plant *Capparis spinosa* L. *American Journal of Medicine and Medical Sciences*, 2(1), 25–32.

Bagley, S. T. (1985). Habitat association of *Klebsiella* species. *Infection Control*, 6(2), 52–58. <https://doi.org/10.1017/S0195941700062603>

Bailly, C. (2021). The subgroup of 2'-hydroxy-flavonoids: Molecular diversity, mechanism of action, and anticancer properties. *Bioorganic & Medicinal Chemistry*. [\[HTML\]](#)

Bar-Ya'akov, I., Amir, R., Tian, L., & Holland, D. (2019). Primary metabolites, anthocyanins, and hydrolyzable tannins in the pomegranate fruit. *Frontiers in plant science*, 10, 620.

Basavaraju, M., & Gunashree, B. S. (2022). *Escherichia coli*: An overview of main characteristics. IntechOpen. <https://doi.org/10.5772/intechopen.105508>

Bolou G.E.K., Bagré I., Ouattara K. & Djaman A.J. (2011).- Evaluation of the Antibacterial Activity of 14 Medicinal Plants in Côte d'Ivoire. *Tropical Journal of Pharmaceutical Research*, 10 (3): 335-340.

Bolou, G. E. K., Bagré, I., Ouattara, K., & Djaman, A. J. (2011). Evaluation of the antibacterial activity of 14 medicinal plants in Côte d'Ivoire. *Tropical Journal of Pharmaceutical Research*, 10(3), 335–340.

Bibliographical references

Bongoni, R. N., & Rajakumari, M. (2017).Antimicrobial activity on peels of different fruits and vegetables. *Zenodo*.<https://doi.org/10.5281/zenodo.1133694>

Boukezzoula A., Djalila B., Palko. N ., Maria G., Chawki B., (2024). Quantification, phytochemical evaluation, and identification of pomegranate yellow peel constituents using LC-MS/MS, the effect of the solvent extract on the antioxidant, in vitro anti-inflammatory, and antibacterial activities. 8(2):146-161.

Bouyahya A, Chamkhi I, Balahbib A, Rebezov M, Shariati MA, Wilairatana P, Mubarak MS, Benali T, «El Omari N. (2022). Mechanisms, antiquorum-sensing actions, and clinical trials of medicinal plant bioactive compounds against bacteria: a comprehensive review. *Molecules*. 22; 27(5):1484. 21.

Bureš, M. S., Maslov Bandić, L., & Vlahoviček-Kahlina, K. (2023). Determination of bioactive components in mandarin fruits: A review. *Critical reviews in analytical chemistry*, 53(7), 1489-1514. [researchgate.net](https://www.researchgate.net)

Cai, X., Chen, J., Chen, J., Li, A., Li, Q., Bai, L., ... & Liu, W. (2023). Identification and quality evaluation of *Citrus sinensis* cv. Chachiensis varieties based on SNP markers. *Arabian Journal of Chemistry*, 16(8), 104948. [sciencedirect.com](https://www.sciencedirect.com)

Caputo, L., Quintieri, L., Maddalena Cavalluzzi, M., Lentini, G., & Habtemariam, S. (2018). Antimicrobial and antibiofilm activities of citrus water-extracts obtained by microwave-assisted and conventional methods. [\[PDF\]](#)

Casadevall, A., & Pirofski, L. A. (1999). Host-pathogen interactions: Redefining the basic concepts of virulence and pathogenicity. *Infection and Immunity*, 67(8), 3703–3713. <https://doi.org/10.1128/IAI.67.8.3703-3713.1999>

Chandra, R., Babu, K. D., Jadhav, V. T., Jaime, A., & Silva, T. D. (2010). Origin, history and domestication of pomegranate. *Fruit, Vegetable and Cereal Science and Biotechnology*, 2, 1-6.

Bibliographical references

Chavan, M. S., Suryawanshi, S., & Kumbhar, A. (2023). Bio-waste originated, heterogeneous catalysts based on pomegranate peel for Knoevenagel condensation: a green approach. *Reaction Kinetics, Mechanisms and Catalysis*, 136(4), 2167-2180. vivekanandcollege.ac.in

Chen, Q., Wang, D., Tan, C., Hu, Y., Sundararajan, B., & Zhou, Z. (2020). Profiling of Flavonoid and Antioxidant Activity of Fruit Tissues from 27 Chinese Local Citrus Cultivars. ncbi.nlm.nih.gov

Chen, S., Wang, X., Cheng, Y., Gao, H., & Chen, X. (2023). A review of classification, biosynthesis, biological activities and potential applications of flavonoids. *Molecules*. mdpi.com

Chen, Y., Pan, H., Hao, S., Pan, D., Wang, G., & Yu, W. (2021). Evaluation of phenolic composition and antioxidant properties of different varieties of Chinese citrus. *Food Chemistry*. [[HTML](#)]

Ciupei, D., Colișar, A., Leopold, L., Stănilă, A., & Diaconeasa, Z. M. (2024). Polyphenols: From Classification to Therapeutic Potential and Bioavailability. *Foods*, 13(24), 4131. mdpi.com

Costanzo, G., Vitale, E., Iesce, M. R., Naviglio, D., Amoresano, A., Fontanarosa, C., ... & Arena, C. (2022). Antioxidant properties of pulp, peel and seeds of phlegrean mandarin (*Citrus sinensis* Blanco) at different stages of fruit ripening. *Antioxidants*, 11(2), 187. mdpi.com

Das, A. K., Nanda, P. K., Chowdhury, N. R., Dandapat, P., Gagaoua, M., Chauhan, P., ... & Lorenzo, J. M. (2021). Application of pomegranate by-products in muscle foods: oxidative indices, colour stability, shelf life and health benefits. *Molecules*, 26(2), 467.

Dayanand D, K., Balamurugan, R.C. Agrawal, R. V., and Rahi. J. (2011). Evaluation of Antibacterial and Antioxidant Activity of Methanolic and Hydromethanolic Extract of Sweet Orange Peels. *Recent Research in Science and Technology*, 3(11): 22-25

Bibliographical references

Diggle, S. P., & Whiteley, M. (2020). Microbe profile: *Pseudomonas aeruginosa*: Opportunistic pathogen and lab rat. *Microbiology*, 166(1), 30–33. <https://doi.org/10.1099/mic.0.000860>

Dilip, M. W. S. (2024). Effect of micronutrients on growth, yield and fruit quality of acid lime (*Citrus aurantifolia* Swingle) cv. Sai sharbati. Egranth.ac.in

Djihad, T., Alghorbany, A., Izzuddin Bin Muhamad, A., & Mahmudul Alam, M. (2021). Government Policies, Financial Scopes and Technological Usages for Agricultural Development and Post-Harvest Loss Reduction in Algeria. osf.io

Dong, N., Yang, X., Chan, E. W., Zhang, R., & Chen, S. (2022). Klebsiella species: Taxonomy, hypervirulence and multidrug resistance. *EBioMedicine*, 79, 103998. <https://doi.org/10.1016/j.ebiom.2022.103998>

Dorji, N., Khandu, Y., Dendup, T., Chozang, J., & Wangmo, U. (2024). Determination of Growth Performance of Mandarin (*Citrus sinensis* Blanco) in Relation to Surrounding Chir pine (*Pinus roxburghii* Sarg.) at Dagana District Through Dendrochronological Analysis. *International Journal of Environment*, 13(1), 1-20.

Durmus, N., Gulsunoglu-Konuskan, Z., & Kilic-Akyilmaz, M. (2024). Recovery, Bioactivity, and Utilization of Bioactive Phenolic Compounds in Citrus Peel. *Food Science & Nutrition*, 12(12), 9974-9997. wiley.com

Edo, G. I., Nwachukwu, S. C., Makia, R. S., Jikah, A. N., Yousif, E., Gaaz, T. S., & Umar, H. (2024). Unveiling the Chinese or red date (*Ziziphus jujuba*); its phytochemical, botanical, industrial and pharmacological properties: a review. *Phytochemistry Reviews*, 1-34. [\[HTML\]](#)

Elzoghbiy, A. S., Mansour, M. M., Elhaw, M. H., & Mekky, A. E. (2022). Phytochemical analysis of pomegranate peel extract (PPE) with the evaluation of its efficacy as anti-rancidity of oils, anti-bacterial and anti-candida agent. *Al-Azhar Medical Journal*, 51(3), 1851–1866. <https://doi.org/10.21608/amj.2022.246585>

Bibliographical references

Facciola, S. (1990). *Cornucopia: A source book of edible plants* (p. 166). Vista, CA: Kampong Publications.

Fahmy, N. M., Elhady, S. S., Bannan, D. F., Malatani, R. T., & Gad, H. A. (2022). *Citrus sinensis* Leaves Essential Oil as an Antiaging Agent: A Comparative Study between Different Cultivars and Correlation with Their Chemical Compositions. *Plants*, 11(23), 3335. [mdpi.com](https://www.mdpi.com)

Farsi, M., Kalantar, M., Zeinalabedini, M., & Vazifeshenas, M. R. (2023). First assessment of Iranian pomegranate germplasm using targeted metabolites and morphological traits to develop the core collection and modeling of the current and future spatial distribution under climate change conditions. *Plos one*, 18(2), e0265977. [plos.org](https://www.plos.org)

Ferrara, G., Porfido, C., Terzano, R., Sarkhosh, A., & Mazzeo, A. (2023). A study on the characteristics of buds and flowers in pomegranate: differences among cultivars. *Horticulturae*, 9(1), 117. [mdpi.com](https://www.mdpi.com)

Ge, S., Duo, L., Wang, J., Yang, J., Li, Z., & Tu, Y. (2021). A unique understanding of traditional medicine of pomegranate, *Punica granatum* L. and its current research status. *Journal of ethnopharmacology*. [\[HTML\]](#)

Ghasemi-Soloklui, A. A., Kordrostami, M., & Gharaghani, A. (2023). Environmental and geographical conditions influence color, physical properties, and physiochemical composition of pomegranate fruits. *Scientific Reports*, 13(1), 15447. [nature.com](https://www.nature.com)

Goh, R. M. V., Pua, A., Luro, F., Ee, K. H., Huang, Y., Marchi, E., ... & Yu, B. (2022). Distinguishing citrus varieties based on genetic and compositional analyses. *PLoS One*, 17(4), e0267007. [plos.org](https://www.plos.org)

Goldenberg, L., Yaniv, Y., Porat, R., & Carmi, N. (2017). *Mandarin fruit quality: a review. Journal of the Science of Food and Agriculture*, 98(1), 18–26. doi:10.1002/jsfa.8495

Guellier, N. (2021). Mandarinier (*Citrus sinensis*), un hybride remarqué par le père Clément. *Binette & Jardin*. <https://jardinage.lemonde.fr/dossier-4102-clementine.html>

Bibliographical references

Guentzel, M. N. (1996). *Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus*. In S. Baron (Ed.), *Medical microbiology* (4th ed., Chap. 26). University of Texas Medical Branch at Galveston. <https://www.ncbi.nlm.nih.gov/books/NBK8035/>

Gupta, S., Nath, A., Gupta, M. K., & Sundaram, S. (2021). Phytochemical analysis and antibacterial activity of different citrus fruit peels. *International Journal of Pharmaceutical Sciences and Research*, 12(11), 1234–1240.

Hassani Moghaddam, E., & Sepahvand, A. (2020). Medicinal properties of pomegranate. *Herbal Medicines Journal*, 4(3), 127-139.

Heena, Kaushal, S., Kalia, A., & Kaur, V. (2022). Proximate, mineral, chemical composition, antioxidant and antimicrobial potential of dropped fruits of *Citrus sinensis* Blanco. *Journal of Food Measurement and Characterization*, 16(6), 4303-4317. [\[HTML\]](#)

Hibstu, Z., Belew, H., Akelew, Y., & Mengist, H. M. (2022). Phage therapy: A different approach to fight bacterial infections. *Biologics: Targets and Therapy*, 16, 173–186. <https://doi.org/10.2147/BTT.S381237>

Holland, D., & Bar-Ya'akov, I. (2018). Pomegranate (*Punica granatum* L.) breeding. *Advances in Plant Breeding Strategies: Fruits: Volume 3*, 601-647.

Hooks, T., Niu, G., Masabni, J., Sun, Y., & Ganjegunte, G. (2021). Performance and phytochemical content of 22 pomegranate (*Punica granatum*) varieties. *HortScience*. ashs.org

Hossain, M. S., Rahman, W., Ali, M. S., Hossain, K. M. M., (2021). Identification and antibiogram assay of *Escherichia coli* isolated from chicken eggs. *Journal of Bio-Science*, 29, Article 54828. <https://doi.org/10.3329/jbs.v29i0.54828>

Hussein, E. F. (2022). *Pseudomonas aeruginosa* represents a main cause of hospital-acquired infections (HAI) and multidrug resistance (MDR). *IntechOpen*. <https://doi.org/10.5772/intechopen.108759>

Bibliographical references

Iglewski, B. H. (1996). Pseudomonas. In S. Baron (Ed.), *Medical microbiology* (4th ed., Chap. 27). University of Texas Medical Branch at Galveston. <https://www.ncbi.nlm.nih.gov/books/NBK8326/>

Islam, M. N. (2019). Fried Rice Syndrome, a disease of fast world: Scientific analysis. *American Journal of Biomedical Science & Research*, 5, 512–514. <https://doi.org/10.34297/AJBSR.2019.05.000979>

Jacquemond, C., Curk, F., & Heuzet, M. (2013). *Les clémentiniers et autres petits agrumes*. Quae.

John, E. & Makris, C. (2022). Presentation of species. [\[HTML\]cabidigitallibrary.org/doi/full/10.5555/20220344165](https://cabidigitallibrary.org/doi/full/10.5555/20220344165)

Joshi, M., Schmilovitch, Z., & Ginzberg, I. (2021). Pomegranate fruit growth and skin characteristics in hot and dry climate. *Frontiers in Plant Science*. frontiersin.org

Jubair N, Rajagopal M, Chinnappan S, Abdullah NB, Fatima A. (2021). Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria (MDR). *Evidence-Based Complementary and Alternative Medicine*. 17;2021.

Keim, K., & Horswill, A. R. (2023). Staphylococcus aureus. *Trends in Microbiology*, 31(3), 222–235. <https://doi.org/10.1016/j.tim.2022.09.008>

Khadivi, A., Hosseini, A. S., & Naderi, A. (2024). Morphological and pomological variation of pomegranate (*Punica granatum L.*) genotypes. *Genetic Resources and Crop Evolution*, 71(4), 1521-1528.

Khalid, K. A., Darwesh, O. M., & Ahmed, A. M. (2021). Peel essential oils of citrus types and their antimicrobial activities in response to various growth locations. *Journal of Essential Oil Bearing Plants*, 24(3), 480-499. [\[HTML\]](#)

Kouamé, R. O., Yolou, S., Boti, J. B., Guessennd, K. N., Kanko, C., Ahibo, C., & Casanova, J. (2008). Étude chimique et activité antidiarrhérique des huiles essentielles de deux

Bibliographical references

plantes aromatiques de la pharmacopée ivoirienne. *European Journal of Scientific Research*, 24(1), 94–103.

Kulkarni, S., Deshmukh, R., Agivale, S., & Awandkar, M. (2019). [An antibiogram of pathogens from nasal affections in equines]. [Details unavailable].

Kumari, A., Dora, J., Kumar, A., & Kumar, A. (2012). Pomegranate (*Punica granatum*)—overview. *Int. J. Pharm. Chem. Sci*, 1(4), 1218-1222.

Kupnik, K., Primožič, M., Vasić, K., Knez, Željko, & Leitgeb, M. (2021). A Comprehensive Study of the Antibacterial Activity of Bioactive Juice and Extracts from Pomegranate (*Punica granatum* L.) Peels and Seeds. ncbi.nlm.nih.gov

Lado, J., Cuellar, F., Rodrigo, M. J., & Zacarías, L. (2016). Nutritional composition of mandarins. In *Nutritional composition of fruit cultivars* (pp. 419-443). Academic Press.

Langgut, D. (2017). *The Citrus Route Revealed: From Southeast Asia into the Mediterranean*. *HortScience*, 52(6), 814–822. doi:10.21273/hortsci11023-16

Lawal AM, Lawan MM, Apampa SA. Phytochemical analysis and thin layer chromatography profiling of crude extracts from *Guiera Senegalensis* (Leaves). *J Biotechnol Biomed Sci*. 2019;3(3):7–12.

Lee, K. H., Chun, Y., Lee, J. H., Lee, J. U., Lee, T., & Yoo, H. Y. (2023). Sustainable utilization strategy of organic waste via fabrication of bioelastomer with antibacterial and antioxidant activities using mandarin peel extracts. *Agriculture*. mdpi.com

Li, Y., Liu, S., Zhao, C., Zhang, Z., Nie, D., Tang, W., & Li, Y. (2022). The chemical composition and antibacterial and antioxidant activities of five citrus essential oils. *Molecules*. mdpi.com

Lin, X., Cao, S., Sun, J., Lu, D., Zhong, B., & Chun, J. (2021). The chemical compositions, and antibacterial and antioxidant activities of four types of citrus essential oils. *Molecules*. mdpi.com

Bibliographical references

Lister, P. D., Wolter, D. J., & Hanson, N. D. (2009).Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews*, 22(4), 582–610. <https://doi.org/10.1128/CMR.00040-09>

Liu, H., Wen, J., Xu, Y., Wu, J., Yu, Y., Yang, J., Liu, H., & Fu, M. (2022).Evaluation of dynamic changes and formation regularity in volatile flavor compounds in *Citrus sinensis* 'chachi' peel at different collection periods using gas *Lwt*. [sciencedirect.com](https://www.sciencedirect.com)

Liu, Y., Wen, H., Kong, J., Hu, Z., Hu, Y., Zeng, J., ... & Xu, J. (2024). Flavor characterization of Citri reticulatae Pericarpium (*Citrus sinensis* 'Chachiensis') with different aging years via sensory and metabolomic approaches. *Food Chemistry*, 443, 138616. [\[HTML\]](#)

Mabberley, D. J. (2004). Citrus (Rutaceae): a review of recent advances in etymology, systematics and medical applications. *Blumea-Biodiversity, Evolution and Biogeography of Plants*, 49(2-3), 481-498.

Malik, A., Najda, A., Bains, A., Nurzyńska-Wierdak, R., & Chawla, P. (2021).Characterization of Citrus nobilis peel methanolic extract for antioxidant, antimicrobial, and anti-inflammatory activity. *Molecules*, 26(14), 4310. [mdpi.com](https://www.mdpi.com)

Maqbool, Z., Khalid, W., Atiq, H. T., Koraqi, H., Javaid, Z., Alhag, S. K., ... & Al-Farga, A. (2023). Citrus waste as source of bioactive compounds: Extraction and utilization in health and food industry. *Molecules*, 28(4), 1636. [mdpi.com](https://www.mdpi.com)

McDonnell, A., Countryman, A., Laurence, T., Gulliver, S., Drake, T., Edwards, S., Kenny, C., Lamberti, O., Morton, A., Shafira, A., Smith, R., & Guzman, J. (2024). Forecasting the fallout from AMR: Economic impacts of antimicrobial resistance in humans. Center for Global Development. <https://www.cgdev.org/publication/forecasting-fallout-amr-economic-impacts-antimicrobial-resistance-humans>

McDowell, R. H., Sands, E. M., & Friedman, H. (2023). *Bacillus cereus*. In *StatPearls*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK459121/>

Bibliographical references

Mehmood, B., Dar, K. K., Ali, S., Awan, U. A., Nayyer, A. Q., Ghous, T., & Andleeb, S. (2015). Short communication: in vitro assessment of antioxidant, antibacterial and phytochemical analysis of peel of *Citrus sinensis*. *Pakistan journal of pharmaceutical sciences*, 28(1), 231–239.

Melgarejo-Sánchez, P., Núñez-Gómez, D., J. Martínez-Nicolás, J., Hernández, F., Legua, P., & Melgarejo, P. (2021). Pomegranate variety and pomegranate plant part, relevance from bioactive point of view: a review. [ncbi.nlm.nih.gov](https://pubmed.ncbi.nlm.nih.gov/)

Mo, Y., Ma, J., Gao, W., Zhang, L., Li, J., Li, J., & Zang, J. (2022). Pomegranate Peel as a Source of Bioactive Compounds: A Mini Review on Their Physiological Functions. [ncbi.nlm.nih.gov](https://pubmed.ncbi.nlm.nih.gov/)

Mohammadi, B., Gorkina, N., & Smith, S. A. (2022). Pathogenicity, toxin production, control and detection of *Bacillus cereus*. IntechOpen. <https://doi.org/10.5772/intechopen.104228>

Montefusco, A., Durante, M., Migoni, D., De Caroli, M., Ilahy, R., Pék, Z., ... & Lenucci, M. S. (2021). Analysis of the phytochemical composition of pomegranate fruit juices, peels and kernels: A comparative study on four cultivars grown in southern Italy. *Plants*, 10(11), 2521. [mdpi.com](https://www.mdpi.com/)

Mouas, T. N., Kabouche, Z., Benssuici, C., & Chaoui, L. (2021). *Punica granatum* L. Fruit Parts from Algerian Cultivar Bioactive Compounds and In Vitro Biological Activities: A Comparative Study. Proceedings.

Mubbunu, L., Simukoko, H., Hang'ombe, M. B., & Mwaanga, E. (2023). Effects of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on the immune system of *Pseudomonas aeruginosa*-challenged hamsters. *Asian Pacific Journal of Health Sciences*, 10(2), 31–36. <https://doi.org/10.21276/apjhs.2023.10.2.08>

Mueller, M., & Tainter, C. R. (2023). *Escherichia coli* infection. In *StatPearls*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK564298/>

Bibliographical references

- Musa, D. D., Sangodele, F., & Hafiz, S. S. (2023). Phytochemical analysis and antibacterial activity of orange (*Citrus sinensis*) peel. *FUDMA Journal of Sciences*, 3(1), 375–380. <https://fjs.fudutsinma.edu.ng/index.php/fjs/article/view/1467>
- Musa, D. D., Sangodele, F., & Hafiz, S. S. (2023).**Phytochemical analysis and antibacterial activity of orange (*Citrus sinensis*) peel. *FUDMA Journal of Sciences*, 3(1), 375–380. <https://fjs.fudutsinma.edu.ng/index.php/fjs/article/view/1467>
- Myles, I. A., & Datta, S. K. (2012).***Staphylococcus aureus*: An introduction. *Seminars in Immunopathology*, 34(2), 181–184. <https://doi.org/10.1007/s00281-011-0301-9>
- Naghavi, M., Vollset, S. E., Ikuta, K. S., Swetschinski, L. R., Gray, A. P., Wool, E. E, (2024).** Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. *The Lancet*, 404(10459), 1199–1226. [https://doi.org/10.1016/S0140-6736\(24\)01867-1](https://doi.org/10.1016/S0140-6736(24)01867-1)
- Narayanankutty, A., U. Visakh, N., Sasidharan, A., Pathrose, B., Joshua Olatunji, O., Al-Ansari, A., Alfarhan, A., & Ramesh, V. (2022).** Chemical Composition, Antioxidant, Anti-Bacterial, and Anti-Cancer Activities of Essential Oils Extracted from *Citrus limetta* Risso Peel Waste Remains after Commercial Use. [ncbi.nlm.nih.gov](https://pubmed.ncbi.nlm.nih.gov)
- Naser, A., AM Abd–Alrazik, A. M., & Khalifa, S. M. (2021).**Effect of some nutrients on growth, Yield and fruit quality of “Wonderful” cultivar pomegranate. *Al-Azhar Journal of Agricultural Research*, 46(1), 1-15. [ekb.eg](https://www.ekb.eg)
- National Academies of Sciences, Engineering, and Medicine. (2021).***Combating antimicrobial resistance and protecting the miracle of modern medicine* (G. H. Palmer & G. J. Buckley, Eds.; Chapter 3). National Academies Press. <https://www.ncbi.nlm.nih.gov/books/NBK577288/>
- Ngamkhae, N., Monthakantirat, O., Chulikhit, Y., Boonyarat, C., Maneenet, J., Khamphukdee, C., Kwankhao, P., Pitiporn, S., & Daodee, S. (2022).** Optimization of extraction method for Kleeb Bua Daeng formula and comparison between ultrasound-assisted and microwave-assisted extraction. *Journal of Applied Research on Medicinal and Aromatic Plants*, 28, Article 100369.

Bibliographical references

Noor, U., Soni, S., & Gupta, E. (2022). Punica granatum peel waste-an underutilized substrate of therapeutic potential: an overview. *Recent Advances in Food Nutrition & Agriculture*, 13(2), 103-119. [\[HTML\]](#)

Ochora, D. O., Moodley, T., & Hayeshi, R. (2023). Phytochemistry, Pharmacological Activities, and Drug Interactions of Pomegranate, *Punica granatum L.* (Punicaceae).

Oluwatomide Oyeyinka, B. & Jide Afolayan, A. (2020). Comparative and Correlational Evaluation of the Phytochemical Constituents and Antioxidant Activity of *Musa sinensis L.* and *Musa paradisiaca L.* Fruit Compartments (Musaceae). [ncbi.nlm.nih.gov](https://pubmed.ncbi.nlm.nih.gov/)

Oluwole, O., Fernando, W. B., Lumanlan, J., Ademuyiwa, O., & Jayasena, V. (2022). Role of phenolic acid, tannins, stilbenes, lignans and flavonoids in human health—a review. *International Journal of Food Science & Technology*, 57(10), 6326-6335. [researchgate.net](https://www.researchgate.net/)

Omoba, O. S., Obafaye, R. O., Salawu, S. O., Boligon, A. A., & Athayde, M. L. (2015). HPLC-DAD Phenolic Characterization and Antioxidant Activities of Ripe and Unripe Sweet Orange Peels. *Antioxidants (Basel, Switzerland)*, 4(3), 498–512.

Ouattara, S., Kporou, K. E., Kra, A. K. M., Zirihi, G. N., N'guessan, J. D., Coulibaly, A., & Djaman, A. J. (2013). Antifungal activities of *Terminalia ivorensis* A. Chev. bark extracts against *Candida albicans* and *Aspergillus fumigatus*. *Journal of Intercultural Ethnopharmacology*, 2, 49–52.

Pandey A., Kaushik A., Tiwari S. (2011). Evaluation of antimicrobial activity and phytochemical analysis of Citrus limon. *J. Pharmaceut. Biomed. Sci*, 13(17):1–7.

Pandey, A. K., & Pandey, Y. (2023). Fruit Production in NEH Region with Major Emphasis on Quality Transplant Production. *Quality Transplant Production of Fruit Crops*, 1. [researchgate.net](https://www.researchgate.net/)

Phattayakorn K, Wanchaitanawong P. (2009). Antimicrobial activity of Thai herb extracts against coconut milk spoilage microorganisms. *Nat Sci*, 43: 752-759

Bibliographical references

Podetti, C., Riveros-Gomez, M., Román, M. C., Zalazar-García, D., Fabani, M. P., Mazza, G., & Rodríguez, R. (2023). Polyphenol-Enriched Pectin from Pomegranate Peel: Multi-Objective Optimization of the Eco-Friendly Extraction Process. *Molecules*, 28(22), 7656. [mdpi.com](https://doi.org/10.3390/molecules28227656)

Poirel, L., Madec, J., Lupo, A., Schink, A., Kieffer, N., Nordmann, P., & Schwarz, S. (2018).Antimicrobial resistance in *Escherichia coli*. *Microbiology Spectrum*, 6(4), ARBA-0026-2017. <https://doi.org/10.1128/microbiolspec.arba-0026-2017>

Ragab, T., Rady, A., & Mahmoud, M. (2021). The antimicrobial effect of Bacillus secondary metabolites on the human pathogen [Thesis]. <https://doi.org/10.13140/RG.2.2.32627.04642>

Rahimi, H. R., Arastoo, M., & Ostad, S. N. (2012).A Comprehensive Review of *Punica granatum* (Pomegranate) Properties in Toxicological, Pharmacological, Cellular and Molecular Biology Researches. *Iranian journal of pharmaceutical research : IJPR*, 11(2), 385–400.

Ranjha, M. M. A. N., Shafique, B., Wang, L., Irfan, S., Safdar, M. N., Murtaza, M. A., ... & Nadeem, H. R. (2021). A comprehensive review on phytochemistry, bioactivity and medicinal value of bioactive compounds of pomegranate (*Punica granatum*). *Advances in Traditional Medicine*, 1-21. [researchgate.net](https://www.researchgate.net/publication/354111111)

Razi, S., Soleimani, A., Zeinalabedini, M., Vazifeshenas, M. R., Martínez-Gómez, P., Mohsenzade Kermani, A., Raiszadeh, A. R., Tayari, M., & Martínez-García, P. J. (2021). Development of a Multipurpose Core Collection of New Promising Iranian Pomegranate (*Punica granatum* L.) Genotypes Based on Morphological and Pomological Traits. *Horticulturae*, 7(10), 350. <https://doi.org/10.3390/horticulturae7100350>

Rima, M., Rima, M., Fajloun, Z., Sabatier, J. M., Bechinger, B., & Naas, T. (2021).Antimicrobial peptides: A potent alternative to antibiotics. *Antibiotics (Basel, Switzerland)*, 10(9), 1095. <https://doi.org/10.3390/antibiotics10091095>

Bibliographical references

Sadeek, A. M. M., & Abdallah, E. M. (2019). Phytochemical compounds as antibacterial agents: A mini review. *Global Journal of Pharmacy & Pharmaceutical Sciences*, 7(4), 131–136. <https://doi.org/10.19080/GJPPS.2019.07.555720>

Sager, M. & Wiche, O. (2024). Rare Earth Elements (REE): Origins, dispersion, and environmental implications—a comprehensive review. *Environments*. [mdpi.com](https://www.mdpi.com)

Saini, R. K., Ranjit, A., Sharma, K., Prasad, P., Shang, X., Gowda, K. G. M., & Keum, Y. S. (2022). Bioactive compounds of citrus fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes. *Antioxidants*, 11(2), 239. [mdpi.com](https://www.mdpi.com)

Saini, R. K., Ranjit, A., Sharma, K., Prasad, P., Shang, X., Gowda, K. G. M., & Keum, Y. S. (2022). Bioactive compounds of citrus fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes. *Antioxidants*, 11(2), 239. [mdpi.com](https://www.mdpi.com)

Sajid, A., Manzoor, Q., Sajid, A., Nazir, A., Mumtaz, M. A., Fatima, N., ... & Younas, U. (2023). Downregulation of NF- κ B activation pathways using essential oil derived from Citrus pseudolimon: Anticancer and anti-inflammatory potential. *Biocatalysis and Agricultural Biotechnology*, 47, 102599. [\[HTML\]](#)

Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. A. (2023). Antimicrobial resistance: A growing serious threat for global public health. *Healthcare (Basel, Switzerland)*, 11(13), 1946. <https://doi.org/10.3390/healthcare11131946>

Saleem, M., & Saeed, M. T. (2020). Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agents. *Journal of King Saud University—Science*, 32(1), 805–810. <https://doi.org/10.1016/j.jksus.2019.02.013>

Salim, A., Deiana, P., Fancello, F., Molinu, M. G., Santona, M., & Zara, S. (2023). Antimicrobial and antibiofilm activities of pomegranate peel phenolic compounds: Varietal

Bibliographical references

screening through a multivariate approach. *Journal of Bioresources and Bioproducts*, 8(2), 146-161.

Saparbekova, A. A., Kantureyeva, G. O., Kudasova, D. E., Konarbayeva, Z. K., & Latif, A. S. (2023). Potential of phenolic compounds from pomegranate (*Punica granatum* L.) by-product with significant antioxidant and therapeutic effects: A narrative review. *Saudi Journal of Biological Sciences*, 30(2), 103553.

Sarangi, P. K., Srivastava, R. K., Sahoo, U. K., & Vivekanand, V. (2024). Resource recovery from Pomelo byproducts for zero waste biorefinery and bioeconomic perspectives. *Process Safety and Environmental Protection*. [\[HTML\]](#)

Selim, K., & Rostom, M. (2017). Bioflocculation of (iron oxide–silica) system using *Bacillus cereus* bacteria isolated from Egyptian iron ore surface. *Egyptian Journal of Petroleum*, 27(2), 209–215. <https://doi.org/10.1016/j.ejpe.2017.07.002>

Sen, D., & Mukhopadhyay, P. (2024). Antimicrobial resistance (AMR) management using CRISPR-Cas based genome editing. *Gene and Genome Editing*, 7, 100031. <https://doi.org/10.1016/j.ggedit.2024.100031>

Shah, A. J., Mir, R. H., Mir, P. A., Sabreen, S., Jan, R., Wani, T. U., ... & Masoodi, M. H. (2021). Clove: The Spice of Polyvalent Merit. *Science of Spices and Culinary Herbs-Latest Laboratory, Pre-clinical, and Clinical Studies: Volume 5*, 1-26. [\[HTML\]](#)

Sheidai, M., Khandan, M., & Nasre, E. S. (2007). B-chromosomes in Iranian pomegranate (*Punicagranatum*) cultivars. *Pakistan Journal of Botany*, 39, 85–91.

Shin, S. D., Kim, C. S., & Lee, J. H. (2021). Compositional characteristics and antibacterial activity of essential oils in citrus hybrid peels. *Food Science and Technology*. scielo.br

Shorbagi, M., Fayek, N. M., Shao, P., & Farag, M. A. (2022). *Citrus sinensis* Blanco (the common mandarin) fruit: An updated review of its bioactive, extraction types, food quality, therapeutic merits, and bio-waste valorization *Food Bioscience*. [\[HTML\]](#)

Bibliographical references

Shorbagi, M., Fayek, N. M., Shao, P., & Farag, M. A. (2022). *Citrus sinensis* Blanco (the common mandarin) fruit: An updated review of its bioactive, extraction types, food quality, therapeutic merits, and bio-waste valorization Food Bioscience. [\[HTML\]](#)

Shorbagi, M., Fayek, N. M., Shao, P., & Farag, M. A. (2022). *Citrus sinensis* Blanco (the common mandarin) fruit: An updated review of its bioactive, extraction types, food quality, therapeutic merits, and bio-waste valorization Food Bioscience. [\[HTML\]](#)

Showkat, M., Alam, M. G., Nahar, S., & Hossain, M. J. (2016). Partial purification and characterization of alkaline protease enzyme from *Pseudomonas aeruginosa* for tannery in Bangladesh. *Asian-Australasian Journal of Bioscience and Biotechnology*, 2016(12), 221–229.

Shukre, V. M., Kore, G. V., & Tope, T. S. (2020). Extraction of flavonoid from sweet orange (*Citrus sinensis*) peels by using different organic solvents. *Journal of Pharmacognosy and Phytochemistry*, 9(6), 627–629.

Silva, D. R., Sardi, J. de C. O., Pitangui, N. de S., Roque, S. M., da Silva, A. C. B., & Rosalen, P. L. (2020). Probiotics as an alternative antimicrobial therapy: Current reality and future directions. *Biomedicine & Pharmacotherapy*, 129, 110409. <https://doi.org/10.1016/j.biopha.2020.110409>

Singh, I. P., Gore, D. D., Karkhele, S., & Vairappan, C. S. (2023). The Chemistry and Pharmacology of Mandarin Orange (*Citrus sinensis*). In *Recent Advances in Pharmaceutical Innovation and Research* (pp. 305-320). Singapore: Springer Nature Singapore. [\[HTML\]](#)

Singh, R., Gupta, M., Singhal, P., Goyal, S., & Upadhyay, S. K. (2021). In vitro antimicrobial activities of vegetables (potato, cucumber, sweet potato and ginger) peel wastes for ecofriendly microbial management. *International Journal of Botany Studies*, 6(4), 134–137. <https://www.botanyjournals.com/archives/2021/vol6/issue4/6-4-22>

Snoussi, H., Duval, M. F., Garcia-Lor, A., Belfalah, Z., Froelicher, Y., Risterucci, A. M., Perrier, X., Jacquemoud-Collet, J. P., Navarro, L., Harrabi, M., & Ollitrault, P. (2012). Assessment of the genetic diversity of the Tunisian citrus rootstock germplasm. ncbi.nlm.nih.gov

Bibliographical references

Sohrabi, M., Pirbonyeh, N., AlizadehNaini, M., &Others. (2024). A challenging case of carbapenem-resistant *Klebsiella pneumoniae*-related pyogenic liver abscess with capsular polysaccharide hyperproduction: A case report. *BMC Infectious Diseases*, 24, 433. <https://doi.org/10.1186/s12879-024-09314-z>

Solanki SL, Modi CM, Patel HB, Patel UD. Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh, Gujarat, India. *J Pharmacogn Phytochem*. 2019;8(4):3122–3126.

Stover, E. (2012). Center of origin for pomegranate (*Punica granatum* L.) [Figure]. Retrieved from [ResearchGate](#).

Sun, Y., Wang, J., Gu, S., Liu, Z., Zhang, Y., & Zhang, X. (2010). Simultaneous Determination of Flavonoids in Different Parts of *Citrus sinensis* ‘Chachi’ Fruit by High Performance Liquid Chromatography—Photodiode Array Detection. ncbi.nlm.nih.gov

Sweidan, N., Abu Rayyan, W., Mahmoud, I., & Ali, L. (2023). Phytochemical analysis, antioxidant, and antimicrobial activities of Jordanian Pomegranate peels. *PLoS one*, 18(11), e0295129.

Tarantino, A., Difonzo, G., Disciglio, G., Frabboni, L., Paradiso, V. M., Gambacorta, G., &Caponio, F. (2022). Fresh pomegranate juices from cultivars and local ecotypes grown in southeastern Italy: Comparison of physicochemical properties, antioxidant activity and bioactive compounds. *Journal of the Science of Food and Agriculture*, 102(3), 1185-1192. wiley.com

Tarantino, A., Difonzo, G., Disciglio, G., Frabboni, L., Paradiso, V. M., Gambacorta, G., &Caponio, F. (2022). Fresh pomegranate juices from cultivars and local ecotypes grown in southeastern Italy: Comparison of physicochemical properties, antioxidant activity and bioactive compounds. *Journal of the Science of Food and Agriculture*, 102(3), 1185-1192. wiley.com

Taylor, T. A., &Unakal, C. G. (2023). Staphylococcus aureus infection. In *StatPearls*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK441868/>

Bibliographical references

Tinebra, I., Scuderi, D., Sortino, G., Mazzaglia, A., & Farina, V. (2021). Pomegranate cultivation in mediterranean climate: plant adaptation and fruit quality of 'mollar de elche' and 'wonderful' cultivars. *Agronomy*. [mdpi.com](https://doi.org/10.3390/ag1105078)

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G., Jr. (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, 28(3), 603–661. <https://doi.org/10.1128/CMR.00134-14>

Tong, Y., Abbas, Z., Zhang, J., Wang, J., Zhou, Y., Si, D., Wei, X., & Zhang, R. (2025). Antimicrobial activity and mechanism of novel postbiotics against foodborne pathogens. *LWT – Food Science and Technology*, 217, 117464. <https://doi.org/10.1016/j.lwt.2025.117464>

Tshireletso, P., Ateba, C. N., & Fayemi, O. E. (2021). Spectroscopic and antibacterial properties of CuONPs from orange, lemon and tangerine peel extracts: Potential for combating bacterial resistance. *Molecules*. [mdpi.com](https://doi.org/10.3390/molecules22051788)

Tuon, F. F., Dantas, L. R., Suss, P. H., & Tasca Ribeiro, V. S. (2022). Pathogenesis of the *Pseudomonas aeruginosa* biofilm: A review. *Pathogens (Basel, Switzerland)*, 11(3), 300. <https://doi.org/10.3390/pathogens11030300>

Türker, S., Göze, H., Çetinkaya, H., & Haspolat, Y. K. (2024). *Impacts of global climate change on pomegranate cultivation*. In *Climate change and fruit farming*. ResearchGate.

Turkyilmaz, M. (2013). Anthocyanin and organic acid profiles of pomegranate (*Punica granatum* L.) juices from registered varieties in Turkey. *Int. J. Food Sci. Technol.* 48, 2086–2095. doi: 10.1111/ijfs.12190

Van Baarlen, P., Van Belkum, A., Summerbell, R. C., Crous, P. W., & Thomma, B. P. H. J. (2007). Molecular mechanisms of pathogenicity: How do pathogenic microorganisms develop cross-kingdom host jumps? *FEMS Microbiology Reviews*, 31(3), 239–277. <https://doi.org/10.1111/j.1574-6976.2007.00065.x>

Bibliographical references

Venat, O., Peticilă, A. G., Nicolae, I. C., & Hoza, D. (2021). Secondary metabolites and health importance of *Punica granatum*.

Vincent, L. & Meena, S. (2025). Genetic Diversity of Pomegranate (*Punica granatum* L.) and Sustainable Utilization. Genetic Diversity of Fruits and Nuts. [\[HTML\]](#)

Wang, F., Chen, L., Chen, H., Yan, Z., & Liu, Y. (2022). Discovery of the key active compounds in Citri Reticulatae Pericarpium (*Citrus sinensis* “Chachi”) and their therapeutic potential for the treatment of COVID-19 based *Frontiers in Pharmacology*. frontiersin.org

Wang, P., Wang, H., Zou, J., Chen, L., Chen, H., Hu, Y., ... & Liu, Y. (2023). Electronic Nose and Head Space GC–IMS Provide Insights into the Dynamic Changes and Regularity of Volatile Compounds in Zangju (*Citrus sinensis* cv. Manau Gan) Peel at Different Maturation Stages. *Molecules*, 28(14), 5326. mdpi.com

Wang, W., Li, Z. J., Zhang, Y. L., & Xu, X. Q. (2021). Current Situation, Global Potential Distribution and Evolution of Six Almond Species in China. ncbi.nlm.nih.gov

Wang, Y., Qian, J., Cao, J., Wang, D., Liu, C., Yang, R., Li, X., & Sun, C. (2017). Antioxidant Capacity, Anticancer Ability and Flavonoids Composition of 35 Citrus (*Citrus sinensis* Blanco) Varieties. ncbi.nlm.nih.gov

Wang, Z., Zhong, T., Mei, X., Chen, X., Chen, G., Rao, S., ... & Yang, Z. (2023). Comparison of different drying technologies for brocade orange (*Citrus sinensis*) peels: Changes in color, phytochemical profile, volatile, and biological availability and activity of bioactive compounds. *Food Chemistry*, 425, 136539. [\[HTML\]](#)

Wilde TJJ, Ben IO, Woode E, Abotsi WKM (2013). Preliminary phytochemical screening and in vitro antioxidant properties of *Trichilia monadelpha* (Thonn.) JJ De Wilde (Meliaceae). *J Med Biomed Sci*, 2:6–15.

Wilson, M. G., & Pandey, S. (2023). *Pseudomonas aeruginosa*. In *StatPearls*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK557831/>

Bibliographical references

World Health Organization. (2017). The world is running out of antibiotics, WHO report confirms. <https://www.who.int/news/item/20-09-2017-the-world-is-running-out-of-antibiotics-who-report-confirms>

Wu, G. A., Terol, J., Ibanez, V., López-García, A., Pérez-Román, E., Borredá, C., ... Talon, M. (2018). *Genomics of the origin and evolution of Citrus. Nature, 554(7692), 311–316.* doi:10.1038/nature25447

Wu, W., Zhu, Q., Wang, W., Grierson, D., & Yin, X. (2022). Molecular basis of the formation and removal of fruit astringency. *Food Chemistry.* [[HTML](#)]

Yang, J., Cai, W., Rizwan Khan, M., Ahmad, N., Zhang, Z., Meng, L., & Zhang, W. (2023). Application of tannic acid and Fe³⁺ crosslinking-enhanced pectin films for passion fruit preservation. *Foods, 12(18), 3336.* [mdpi.com](https://www.mdpi.com)

Yapo Yomeh, C. V., GuédéKipré, B., Tra Bi Otis, I., ZirihiGuédé, N., & Guessennd Kouadio, N. (2020). Antibacterial activity of leaves' aqueous crude extract (ETA) of *Mallotus oppositifolius* (Geisel.) Müll-Arg. (Euphorbiaceae) on methicillin-resistant *Staphylococcus aureus* (MRSA) and phytochemical screening. *Revue Bio-Africa (23), 38–49.*

YéoSounta, O., Guessennd Kouadio, N., Ouattara, K., Konan, F., Djaman, A. J., Dosso, M., & Coulibaly, A. (2014). Triphytochemistry and in vitro antibacterial activity of root extracts of *Cochlospermumplanchonii* Hook f. ex Planch. (Cochlospermaceae) on multiresistant strains. *Scholars Academic Journal of Biosciences, 2(10), 663–670.*

Yilmaz, C., Rezaei, M., & Sarkhosh, A. (2021). Environmental requirements and site selection. In *The pomegranate: Botany, production and uses* (pp. 225-246). Wallingford UK: CAB International. [researchgate.net](https://www.researchgate.net)

Zouaoui, S. S., Rawda, B., & Mohammed, B. (2019). Evaluation of bioactive properties and antioxidant capacity of native and imported pomegranate fruit bark (*Punica granatum*, L) cultivars found in Algeria.

Appendices

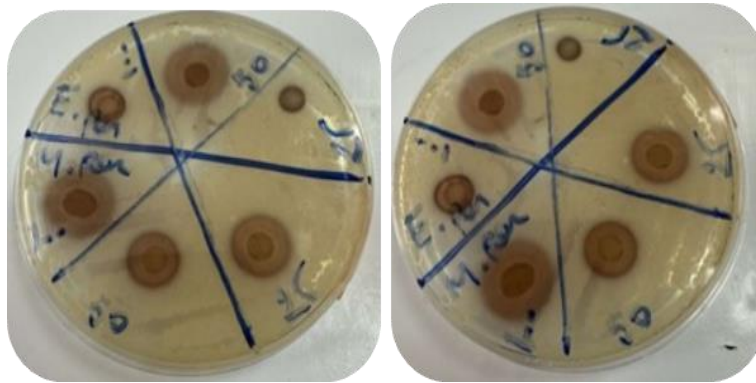
Appendices

Appendix 1

Result of the antimicrobial activity



A. *E. coli* 3 with orange extracts

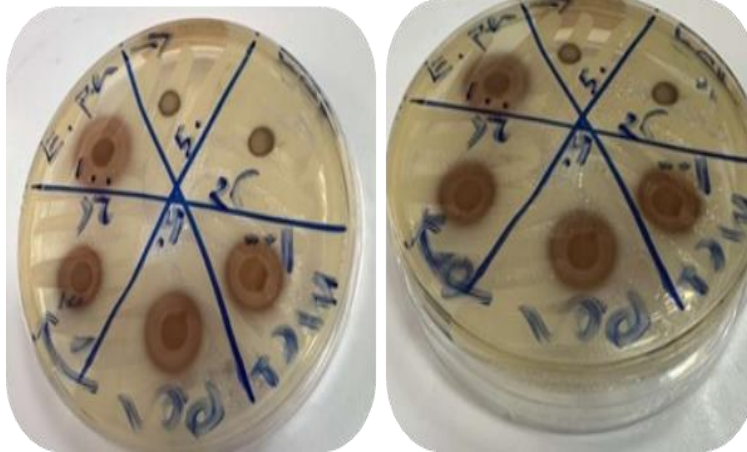


B. *Pseudomonas aerogenosa* with pomegranate extracts.

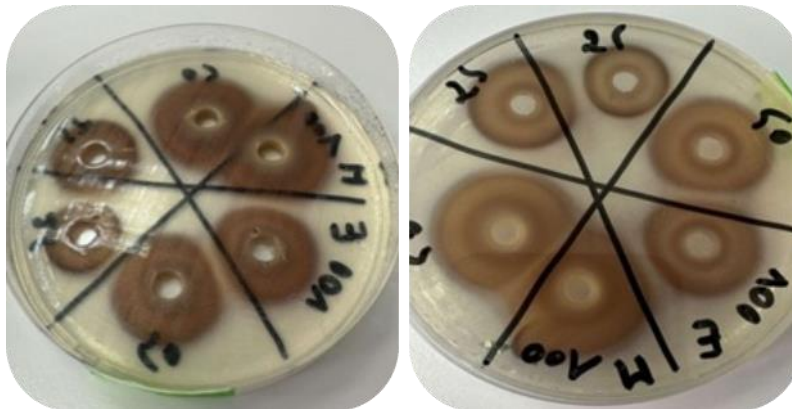


C. *Klebsiella pneumoniae* pomegranate extracts

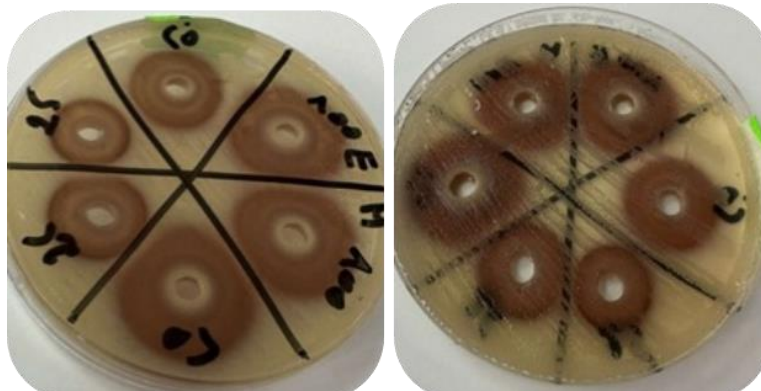
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D. *E. coli* 02 pomegranate extracts

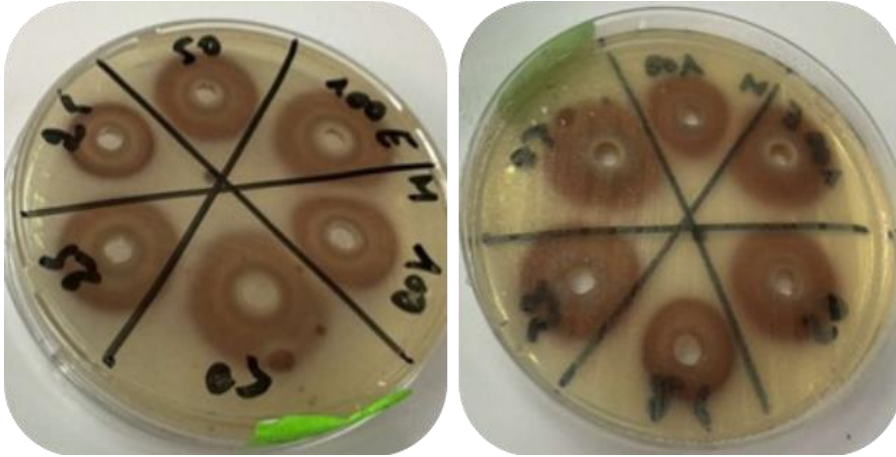


E. *E. coli* 1 with pomegranate extracts

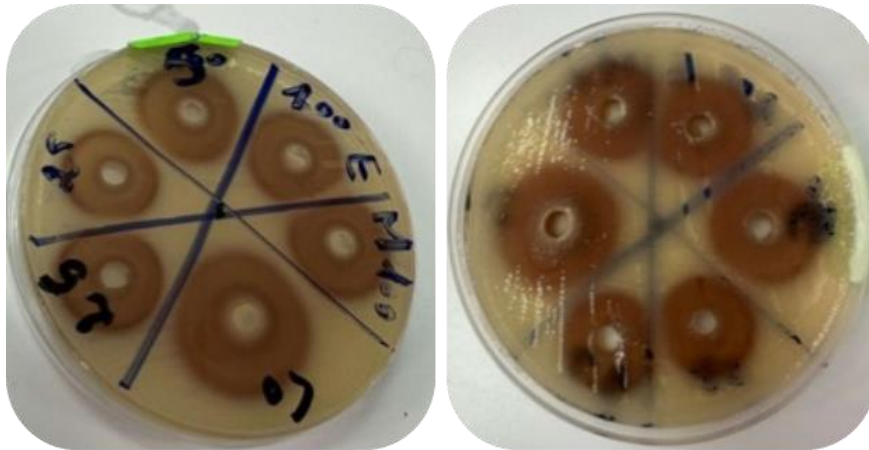


F. *E. coli* 02 with pomegranate extracts

Appendices



G. *E coli* 04 with pomegranate extracts



H. *E coli* 2 with pomegranate extract

Photograph 1. Antimicrobial activities of the studied extracts against the tested bacteria.