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Antimicrobial activity of hydro-alcoholic extracts of two plants used in oral care.

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List of abbreviations

ATB : Antibiotic.

ATCC : American Type Culture Collection.

Aw : Water Activity .

DMSO : Dimethyl Sulfoxide .

ExPEC : Extracted Pathogens .

ICU : Intensive Care Units .

PDA : Potato Dextrose Agar.

S.aromaticum : *Syzygium aromaticum*

***S.persica*, S.P** : *Salvadora persica*.

Antimicrobial activity of hydro-alcoholic extracts of two plants used in oral care.

Abstract

The current study aimed to enhance the value of two naturally occurring medicinal plants: *Syzygium aromaticum* (Kourounfoul) and *Salvadora persica* (Miswak), commonly used for oral hygiene in Arabic countries. In this study three solvents were used: distilled water, ethanol, and methamol, to investigate the antibacterial and antifungal activities of these plants at different concentrations: 200, 100, 50, and 25 mg/ml. The different extracts were tested against five pathogenic bacterial strains: *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, and two fungi: *Aspergillus niger* and *Candida albicans*, using the solid medium diffusion method. The methanolic extracts of *S.persica* and *Syzygium aromaticum* worked well, but the ethanolic and methanolic extracts of *S.persica* were more effective against bacteria than those of *Syzygium aromaticum*. However, for fungal activity, *Syzygium aromaticum's* ethanolic and methanolic extracts were superior to *Salvadora persica's*. The boiled water extracts of *Salvadora persica*was more effecient against the tested bacteria than *Syzygium aromaticum*. It also had the strongest effect against Aspergillus niger, with an inhibition diameter of 20 mm. On the other hand, the cold water extract of *Syzygium aromaticum* has a better effect against bacteria than *Salvadora persica.* The cold water from Salvadora persica had no effect on *Aspergillus niger. The Syzygium aromaticum* ethanolic extract, when combined with *Aspergillus niger,* demonstrated the highest efficiency among all the two studied plants, with a mean diameter of the inhibition zone of 24 mm.

Key words: antimicrobial activity, *Syzygium aromaticum*, medicinal plants, *Salvadora persica*

Activité antimicrobienne des extraits hydro-alcooliques de deux plantes utilisées dans les soins bucco-dentaires.

Résumé

La présente étude est une contribution à la valorisation de deux plantes médicinales spontanées : *Syzygiumaromaticum* (Clou de girofle) et *Salvadorapersica* (Miswak) ; qui sont largement utilisées dans les pays arabes pour l'hygiène buccale. Quatre extraits préparés avec de l'eau distillée, de l'éthanol et du méthanol organique ont été utilisés pour étudier les activités antibactériennes et antifongiques de ces plantes à quatre concentrations différentes pour chaque extrait : 200, 100, 50 et 25 mg/ml. Les différents extraits ont été utilisés pour étudier l'activité antimicrobienne contre cinq souches bactériennes pathogènes : gram positif : *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923 et gram négatif : *Escherichia coli* ATCC 25922, *Klebsiellapneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853 et contre un champignon : *Aspergillus niger* et *Candida albicans*, en utilisant la méthode de diffusion en milieu solide. Les résultats ont montré que les extraits éthanoliques et méthanoliques de *S. persica* et de *Syzygium aromaticum* ont montré des résultats satisfaisants, mais les extraits éthanoliques et méthanoliques de *S. persica* ont eu un meilleur effet que *Syzygiumaromaticum* contre l'activité bactérienne. En revanche, pour l'activité fongique, les extraits éthanoliques et méthanoliques de *Syzygium aromaticum* étaient meilleurs que ceux de *Salvadorapersica*. En ce qui concerne les extraits aqueux, les résultats montrent que l'extrait de *Salvadorapersica* à l'eau chaude a un meilleur effet contre les bactéries que *Syzygiumaromaticum*, et il a également montré l'effet le plus élevé contre *Aspergillus niger* avec un diamètre d'inhibition de 20 mm. En revanche, l'extrait de *Syzygiumaromaticum* à l'eau froide a un meilleur effet contre les bactéries que Salvadorapersica. L'eau froide de *Salvadorapersica* n'a montré aucun effet sur *Aspergillus niger*. La plus grande efficacité de tous les extraits des deux plantes étudiées a été observée avec l'extrait éthanolique de *Syzygiumaromaticum* contre *Aspergillus niger* (moyenne des diamètres des zones d'inhibition = 24 mm).

Mots clés: activité antimicrobienne, *Syzygium aromaticum* , plantes médicinales, *Salvadora persica.*

النشاط المضاد للميكروبات لمستخلصات كحولية مائية من نباتين مستخدمين في العناية بالفم.

الملخص

تعد هذه الدراسة مساهمة في تثمين اثنين من النباتات الطبية العطرية : القرنفل (*Syzygiumaromaticum*) والمِسواك (*Salvadorapersica*)؛ اللذان يستخدمان على نطاق واسع في البلدان العربية من أجل نظافة الفم. تم استخدام أربعة مستخلصات محضرة بالماء المقطر والإيثانول والميثانول العضوي لدراسة النشاط المضاد للبكتيريا والفطريات لهذه النباتات بتركيزات مختلفة لكل مستخلص؛ 200، 100، 50 و25 ملغ/مل. استخدمت المستخلصات المختلفة لدراسة النشاط المضاد للميكروبات ضد خمس سلالات بكتيرية ممرضة: البكتيريا الموجبة لصبغة جرام *Bacillus cereus* ATCC 11778، *Staphylococcus aureus* ATCC 25923والبكتيريا السالبة لصبغة جرام *Escherichia coli* ATCC 25922، *Klebsiellapneumoniae* ATCC 4352، *Pseudomonas aeruginosa* ATCC 27853و ضد الفطريات *Aspergillus niger*و*Candida albicans*، باستخدام طريقة الانتشار في الوسط الصلب. أظهرت النتائج أن كل من المستخلصات الإيثانوليةوالميثانولية للمِسواك والقرنفل أظهرت نتائج مرضية، لكن المستخلصات الإيثانوليةوالميثانولية للمِسواك كان لها تأثير أفضل من القرنفل ضد النشاط البكتيري. أما بالنسبة للنشاط الفطري، فقد كانت المستخلصات الإيثانوليةوالميثانولية للقرنفل أفضل من المِسواك. أما بالنسبة لمستخلصات الماء، فإن النتائج تظهر أن مستخلص الماء الساخن للمِسواك له تأثير أفضل ضد البكتيريا من القرنفل، كما أظهر أعلى تأثير ض د *Aspergillus niger*بقطر تثبيط 20 مم. من ناحية أخرى، فإن مستخلص الماء البارد للقرنفل له تأثير أفضل ضد البكتيريا من المِسواك. لم يظهر مستخلص الماء البارد للمِسواك أي تأثير على *Aspergillus niger*.أعلى كفاءة لجميع مستخلصات النباتين المدروسين كانت لمستخلص القرنفل الإيثانوليمع*Aspergillus niger*(متوسط أقطار منطقة التثبيط = 24 مم.)

الكلمات المفتاحية: نشاط مضاد للميكروبات ،*Syzygium aromaticum*، نباتات طبية ، *Salvadora persica*

INTRODUCTION

Introduction

Plants have medicinal properties as a result of containing secondary metabolites, which possess various therapeutic benefits. They are used in pharmaceutical production and play a role in protecting plants from microbial invasions (Boudjouref, 2011). A wide variety of plants have been documented in the pharmacopeia for their effectiveness in treating oral bacterial infections (Ranjan *et al.,* 2012).

Oral hygiene is one of the most important daily routine practices because it keeps the mouth and teeth clean and prevents many health problems. The design of modern dental care tools incorporates both mechanical and chemical methods to eliminate plaque and food residues from the teeth's surface and interstitial spaces. Throughout history, people have been using different tools and chemicals to maintain their oral health, such as chewing sticks, tooth brushes, gum, mouth wash, toothpaste, and floss, which are all believed to have evolved from botanical origins(Dutta and Shaikh, 2012; Riggs *et al*., 2012)..

Salvadora persica, commonly known as Miswak in Islamic culture, is a member of the Salvadoraceae family and is used as chewing sticks or toothbrush trees (Al-Sieni *et al*., 2014). The Sunnah of Prophet Muhammad (peace and blessings of Allaah be upon him) recommends Miswak for oral hygiene (Abhary et al., 2016). Salvadora persica demonstrates significant antibacterial properties, and studies have reported its antifungal activity (Mohammed, 2013). People widely utilize Miswak in various forms, such as sticks, extracts, and toothpastes, due to its potent properties, availability, and low cost, or as part of their traditional practices. The presence of benzyl isothiocyanate in the plant is believed to play a pivotal role in preventing acid formation and bacterial growth. Furthermore, it exhibits antiviral and antifungal properties (Jassoma *et al*., 2019).

S. aromaticum (Cloves) are a well-known spice derived from plants belonging to the Myrtaceae family. People have used cloves for their anesthetic, analgesic, anti-inflammatory, and antibacterial properties across generations. Secondary metabolites like alkaloids, phenols, flavonoids, and coumarins in the plant are responsible for this diverse array of therapeutic effects. These compounds are believed to underpin its potential antibacterial activity (Bruneton, 2009).

This research aimed to assess the antimicrobial properties of extracts from two plants *Syzygium aromaticum* and the stems of *Salvadora persica*against various bacterial and dungul species.

We organize this work into two primary sections and a concluding summary. The initial section begins with a literature review consisting of two chapters. The first chapter provides a description of the plants utilized in this study, detailing their biological activities. The second chapter focuses on presenting the microorganisms found in oral flora, including information on the strains used. The second section delves into the experimental methodologies and findings. It elaborates on the materials and analytical approaches utilized in this study, present the results obtained, and engages in discussions surrounding those results.

Part One: Bibliography Study

I. *Salvadora persica*

1. History of *Salvadora persica*

Around 7,000 years ago, the Babylonians were the first to use chewing sticks, followed by the Greek and Roman Empires. Many parts of the world, mainly in Africa and the Middle East, still use chews today (Almas, 2002; Niazi *et al*., 2016).

Ancient Islamic texts, including hadiths (the words and deeds of Prophet Muhammad), mention using peach trees for oral care. Ayurvedic texts in traditional Indian medicine also emphasize the dental benefits of *Salvadora persica* (Figure 1, 2) **(**Almas, 2001; Darout *et al*., 2002; Sofrata *et al*., 2011).

Figure 1.*Salvadora persica* tree (Sudhir *et al*., 2018)

Figure 2. *Salvadora persica* toothbrush (Niazi *et al*., 2016)

2. Origin of *Salvadorapersica*

The word "Salvadora" was first coined in 1749 by Dr. Laurent Garcin (1683-1752), a botanist, traveler, and plant collector. He mentioned Barcelona's famous Spanish pharmacist, Juan SalvadoraBosca (1598-1681). "Persica" refers to the Persian Empire, the original region of this plant species **(**Hilal and Rajagopal, 2014**).**

3. Nomenclature of *Salvadorapersica*

Salvadorapersica, commonly known as Miswak, is a tooth or chewing stick that is recommended by the prophet "Muhammed" and belongs to the family Salvadoraceae. Name in Japanese: Koyoji, in Hebrew is qesam; in Aramaic are visas, in Latin is mastic (Bos, 1993).

4. The taxonomic classification of *Salvadorapersica*

Salvadorapersica belongs to the family Salvadoraceae. The complete taxonomic classification of *Salvadorapersica*is listed below **(**Mohammad *et al*., 2015).

Kingdom: Plantae

Division: Magnoliphyta

Class: Magnoliopsida

Order: Brassicales

Family: Salvadoraceae

Genus: *Salvadora*

Species: *Salvadora persica* (Congo, 2012)

Binomial name: *Salvadora persica* (Khari Jaal)

S*alvadora oleoides* (MeethiJaal)

5. Geographic dispersion

*Salvadorapersica*is a native species to dry areas of the Middle East and Africa **(**Elvin, 1980; Eid*et al* 1990) and the Indian subcontinent. It thrives in dry climates and it is distributed in countries such as Saudi Arabia, Yemen, Egypt, Sudan, and India **(**Tackholm, 1947).

Salvadora persica occurs primarily on moist rocks and canyon banks **(**Ozenda, 1983**).** It is a Sudano-Decanian species. It is distributed throughout the central Sahara: Khojar and Tibesti; in the Arabian Peninsula, Iran, and India. Moreover, it is spread throughout Mauritania's river valleys, where the landscape is dotted with green spots during drought **(**Abdellahi, 2001**).** It is widely distributed in India's arid areas and usually grows on saline soils **(**Khatak*et al*., 2010**).**

6. Botanical characteristics

Salvadorapersica is a toothbrush tree with a short trunk, white bark, smooth green leaves, and a life span of 25 years (Figure 3, 4) **(**Hilal and Nizar, 2012**).**

It is considered a small tree or shrub because it can reach a height of 6-7 m and a diameter of 20-23 feet. The main trunk is more than 30 cm in diameter, upright or drooping, and the branches droop strongly and spread out. Young branches are green. The bark on the main trunk is slightly rough and grayish-brown, but the bark elsewhere is lighter **(**Panday, 2004**).**

Salvadora persica produces clusters of small red edible fruits, fleshy, globosely, single-layered, seedless, smooth surface, 5-10 mm in diameter, spherical (Figure 5). Fruits are pink to scarlet when mature **(**Sher *et al*., 2010**).**

Figure 3: leaves of *S.persica***Figure 4:** Flower of *S.persica* (Arora and Gupta, 2011; Lababidi,2019).

Figure 5: Fruits of *S.persica* (Lababidi, 2019).

7. Chemical composition

The broad phytochemical investigation uncovered the nearness of carbohydrates, flavonoids, terpenes, sterols, alkaloids, and glycosides, and natural sulfur compounds and elemental sulfur are moreover displayed, as well as small sums of fluoride, calcium, phosphorus, silica, and ascorbic corrosive **(**Aumeeruddy*et al*., 2017**).**

Fixed Oil and vitamins: Seeds of *S. persica*containabout 40% oil, composed of myristicacid (55%), lauricacid (20%), cetylacid (20%) and cis-9-octadecenoic acid (5%) **(**Ahmed *et al*., 2008**).**

Flavonoids: *Salvadorapersica* contains flavonoids, Salvador, cyanogenic glycosides, lignans, saponins, alkaloids, tannins, linoleic corrosive, stearic corrosive, Salvador, and other compounds **(**Abdelrahman *et al*., 2003**).**

Alkaloids and nitrogenous compounds: The roots are rich in salvadorea and benzyl isothiocyanate, which have antiviral activity against the dangerous oral virus, herpes simplex **(**Kamil*et al*., 1999**).** Also, high alkaloids such as salvadorisin and trimethylamine are found in the roots **(**lafi and Ababneh, 1995**).**

Glycosides and phenolic compounds: The roots of both Egyptian and Saudi plantsproducetwoglucosinolates; glucotropaelin and sinigrin**(**Ezmirly and El-Nasr, 1981., Abdel-Wahab*et al*., 1990**)**Salvadoside, salvadoraside, syringin, liriodendron, and lignan glycosides werealsoisolatedfromthisstrain**(**Ohtani*et al*., 1992**).** Furthermore, cyanogenic glycosides are also present (Khalil, 2006**).**

Minerals: The root of *Salvadorapersica* encompasses a tall substance of minerals, with 27.06% **(**Almas *et al*., 2000**).**

Phytochemicals: Phytochemical examinations have uncovered the nearness of benzyl-isothiocyanate, saponins, tannins, silica, a small amount of tar, trimethylamine, and other compounds within the root (Gupta *et al*., 2015).

8. Traditional uses of *S.persica*

The traditional medicinal use of *S.persica* as an antibacterial toothbrush for oral hygiene and treatment of gingivitis is a centuries-old practice and part of the Greco-Arabic

medical system **(**Sher *et al*., 2011**).**Miswakchewingsticksare used by the majority of Muslims **(**Goyal *et al*., 2011).

The severity of dental lack of oral hygiene can be explained by socioeconomic conditions, infrequent medical care (expensive and painful), and sociocultural background, especially in children. For these reasons, the World Health Organization recommends using snacking as an effective means of oral hygiene in areas where their use is frequent **(**Sofrata *et al*., 2007**).**

In recent years, many researchers around the world have studied miswak as a plant for oral and dental hygiene and demonstrated that aqueous extracts of various chewing sticks possess antibacterial, anticaries, and antiperipheral properties **(**Darout*et al*., 2003; Sarfaraz Khan *et al*., 2009; Sofrata *et al*., 2011).

9. Medicinal uses of *S.persica*

*S.persica*is is used to treat various diseases. They are used to make toothpaste. The leaves are boiled in sour milk, and pepper is added to treat rhinitis and the common cold **(**Renie, 1933**)**.

It is widely used to treat coughs, bronchitis, asthma, flatulence, and indigestion. The roots are effective as repellents and can combat fever, headaches, and rheumatism. The decoction of branches and leaves is effective in treating dysuria. The bark powder of the root is used to treat jaundice and improve fertility in women **(**Arbonnier, 2002).

Moreover, Salvadora persica is also effective against anemia and post-malarial inflammation. It is effective against respiratory and hepatic diseases **(**Abdellahi, 2001).

The factory also has the following medicinal uses: eliminates lousy smell, Improves taste, Improves memory, Improves intelligence, eliminates mucus, prevents caries, Treats headaches, eliminates dental pain, eliminates yellow color of teeth, and promotes digestion **(**Abdellahi, 2001).

10. Use of *S. persica* as a toothbrush for oral hygiene

Miswak has been scientifically proven to be very useful in preventing cavities even without using other tooth cleaning methods **(**Farooqi and Srivatava, 2008). However, chewing Miswakextract gum may promote periodontal health by reducing plaque, bleeding, and gingival index **(** Babak Amoian *et al*., 2010**)**.

In addition, it is known that patients with diabetes and renal transplantation (often treated with immunosuppressants) are susceptible to oral Candida infections **(**Lamey *et al.,* 1988; Peddi *et al*., 1998) because these conditions reduce the patient's immune response. In tests, miswak showed excellent antimicrobial activity against certain substances. The tannins and resins in miswak have an astringent effect on the mucous membranes, forming a layer on the enamel that protects the teeth **(**Almas and Al-Lafi, 1995; Elvin-Lewis *et al.,* 1980**)**showed that adult tooth loss rates are meager in these countries where miswak is widespread.

11. Biological Activities

Several experimental studies were conducted has proved that*Salvadorapersica*, has a biologically activity and contains active compounds. It has proven that this plant have significant antibacterial activity against aerobic and anaerobic bacteria collected from gingivally inflamed teeth Necrotic pulp **(**Hilal and Rajagopal, 2014).

11.1. Antimicrobial activity

Consistent with previous studies, peach blossoms have been confirmed to exhibit significant antibacterial activity against aerobic and anaerobic bacteria collected from teeth with gingival inflammation and pulp necrosis **(** Al-Sabawi *et al*., 2007; Sher *et al*.,2011**).**

Peach blossom has also been found to have anti-plasmodial activity and is used as part of a medication to treat malaria (Ali *et al*., 2002).

Recently, Hoggar miswak extract exhibited potent antimicrobial activity both in vitro and in vivo, inhibiting the growth of Gram-negative bacteria in dental plaque more significantly than Gram-positive bacteria **(**Chelli-Chentouf*et al.,* 2012*).*

11.2. Antifungal activity

Fungal infections have increased dramatically over the past two decades as the at-risk population continues to grow—resistance to pathogenic fungi, especially *Candida albicans* and non-Candida species. There has also been an increase in the number of antifungal *Candida albicans* species isolated from patients. Throughout history, essential oils and plant extracts have generated significant interest as a source of natural products. Their potential as alternative treatments for various infectious diseases has been extensively studied **(**Naeini*et al*., 2014**).**

Other studies include a Comparison of the antifungal properties of solid Miswak versus grounded Miswak granules against different bacterial strains Candida. The conclusion is that solid miswak has more robust antifungal properties while powder miswak has more vital antifungal properties. It does not exhibit any antifungal properties. Similarly, in vitro studies by Naeini*et al*. Research shows that alcohol extracts of *S. persica* exhibits antifungal properties against all Candida strains except *C. parapsilosisCandida krusei*. Additionally, the hexane content in Miswak root is beneficial against *Candida albicans* and Enterococcus faecalis **(**Fayez *et al*., 2016**).**

11.3. Antiviral effects

Al-Bagieh *et al* conducted a study explored the impact of BITC, a compound extracted from *Salvadorapersica* root, on herpes simplex virus-1 (HSV-1). Their findings from the plaque reduction assay revealed that BITC exhibited virucidal activity against HSV-1, notably at a concentration of 133 µg/ml. Consequently, the authors advocated using miswak (*Salvadorapersica*) as a preventive measure for managing oral infections **(**Haque and Alsareii, 2015).

11.4. Antioxidant activity

Antioxidants protect the body from oxidative stress caused by free radicals. Current literature suggests that miswak has antioxidant properties. A study by Mohamed and Khan concluded that antioxidant enzymes (catalase, peroxidase, polyphenol oxidase) in Miswak are responsible for the antioxidant properties of peach blossoms. The synergistic action of antioxidant compounds and enzymes makes Miswak a good remedy for maintaining oral hygiene **(**Mohamed and Khan, 2013).

Another study conducted by Ibrahim *et al,* (2015) showed that antioxidant properties and the content of flavonoids and phenolics are more prominent in the southern region plants of Saudi Arabia than in the central area plants.

Moreover, Antioxidant and phytochemical studies were conducted on peach blossoms, and the results showed that the chloroform extract of Miswak showed the most potent antioxidant activity in vitro, followed by the ethanol extract **(**Gupta *et al*., 2015**).** Based on all these findings and evidence, it can be safely concluded that peach blossoms are a potential source of antioxidant compounds and can be used in pharmaceutical preparations against oxidative stress-related diseases **(**Anand and Sati , 2013; Ibrahim*et al*., 2015 **).**

11.5. Anti-Inflammatory and Antiulcer Properties

Inflammation is a complex biological response of neurovascular tissues to harmful stimuli such as pathogens, damaged cells, and irritants. It attempts to protect by removing and neutralizing harmful stimuli to initiate healing. Interestingly, *S. persica*has anti-inflammatory properties, as reported by Hoor *et al.* (2014**).** Anti-inflammatory effects were measured by measuring paw edema volume (in milliliters) using a plethysmograph immediately before injection and then hourly for up to five hours. Then, calculate the average. Researchers demonstrated the anti-inflammatory effect of peach kernels on carrageenan-induced edematous paw volume reduction (Hoor *et al*., 2014**).**

In addition, studies have examined the antiulcer effects of peach blossoms. Sanogo *et al*. (1999); reviewed the impact of peach blossoms given to rats before they developed gastric ulcers and compared them with a placebo. The results showed that peach blossom decoction significantly protected ulcers caused by ethanol and cold stress. A recent study by Lebda *et al.*(2018). The effects of peach water extract on pro-inflammatory cytokines, nitric oxide synthase, apoptotic pathways, and oxidative/antioxidative pathways involved in ethanol-induced gastric ulcers in rats were studied. They concluded that peach blossom could relieve severe gastric ulcers caused by ethanol and confirmed its effectiveness as an antiulcer agent.

They concluded that peach blossom could relieve severe gastric ulcers caused by ethanol and confirmed its effectiveness as an antiulcer agent. Its mechanism of action is thought to be strengthening the antioxidant defense system, minimizing pro-inflammatory cytokines, upregulating apoptotic pathways, increasing mucus content, and remodeling nitric oxide synthase (N.O.S.) isoforms(Lebda *et al* ., 2018**).**

II. *Syzygiumaromaticum*

1. History

For decades, clove has been utilized for its culinary and medicinal virtues. It is widely employed in dental medicine for its local anesthetic properties, effectively eliminating oral germs **(**Kozam, 1977; Ohkubo *et al*., 1997**).** Around the 16th century, the Portuguese broke the Arab monopoly on spice trade at sea **(**François, 1936**).** In the early 17th century, the Dutch, to increase prices, removed clove trees from all islands except Amboina **(**Charles,

2013**).** Until the 18th century, production control was even more stringent -to artificially maintain prices **(**Razafimamonjison *et al*., 2014**).**

2. Origin

Cloves grow in Tanzania, Madagascar, western India, and Brazil. Unopened flower buds are harvested at maturity twice a year and dried in the sun (Figure 6) (Anejaand Joshi, 2010; Pandey and Singh, 2011)**.**

Today, the tree is grown at lower altitudes in many tropical countries and kept in a shrub state to facilitate harvesting—the plant requires the following conditions: partial shade, light, and sun. The trees thrive in the deep, calm, nutrient-rich, and well-drained soils typically found on the moist eastern slopes of the islands exposed to the trade winds. It is less susceptible to attack and disease, possibly due to its aromatic and antiseptic properties (Ghedira *et al*., 2010).

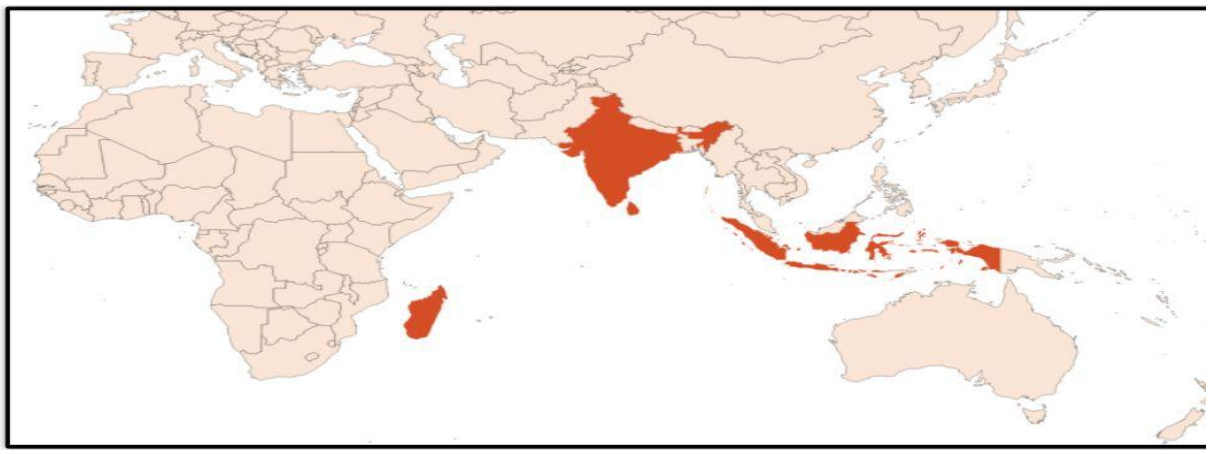


Figure 6. Geographic map of the world's major clove producers (Ghedira *et al*., 2010).

3. Etymology

The word clove was first used in English in the 15th century and is derived from the Middle English word "clow of silverthe Anglo-French word "Clowes de gilofre" and the Old French word "clou de girofle." The name of the genus is derived from the Greek term syzygiesch means joined about the paired leaves and branchlets of a Jamaican plant species (*Calyptranthes suzygium*) that was initially given this name. The specific epithet aromaticum signifies that the plant has an aromatic quality **(**Uchibayashi, 2001**).**

4. The taxonomic classification of *Syzygiumaromaticum*

The complete taxonomic classification of *Syzygium aromaticum* is listed below in Table 1 (Sophie, 2015)**.**

Table1. Scientific classification of plant *Syzgium aromaticum* (Sophie, 2015).

Kingdom	Plantae
Division	Trachophyta
Class	Magnoliosida
Order	Mytrales
Family	Myrtaceae
Genus	*Syzygium*
Species	*Syzygiumaromaticum*

5. Ecology

The clove tree, belonging to the Myrtaceae family, thrives in tropical climates, requiring ample sunlight, humidity, warmth, and low altitudes below 300 meters. It benefits from maritime climates, well-draining soils on lower slopes of hills, and volcanic or sedimentary soil near the sea. It is essential to avoid stagnant water as it harms the roots. The ideal conditions include high rainfall, increased sunlight during inflorescence, and avoiding highly clayey or sandy soils **(**Barbelet , 2015)

6. Description

It is a tall tree native to the small islands of the Moluccas, slender, with an average height of 10 to 12 meters, reaching up to 20 meters. It has a pyramidal shape and a lightly grayish, wrinkled trunk. Its leaves, 8 to 10 cm long, are leathery, evergreen, opposite, petiolate, oval, with lanceolate blades, reddish-green on the upper surface, and dark Green on the lower surface, slightly punctuated**(**Goetz and Ghedira, 2012**).**

These leaves are aromatic, emitting a strong clove scent when crushed **(**Goetz and Ghedira, 2012).

The inflorescence consists of small, compact, and branched cymes (4-5 cm) grouped in panicles of three to five small fragrant flowers. The calyx is tubular and off-white, turning red (four fleshy and persistent red sepals), and the corolla is white-pink (four white dialypetalous). The hermaphroditic flower has numerous stamens (forming a pompom) and a pistil with an inferior ovary in two compartments. The "antholfe" fruit is an ellipsoidal purple-brown drupe containing a single seed approximately 1.5 cm long (Figure 7) **(**Ghedira*et al*., 2010).

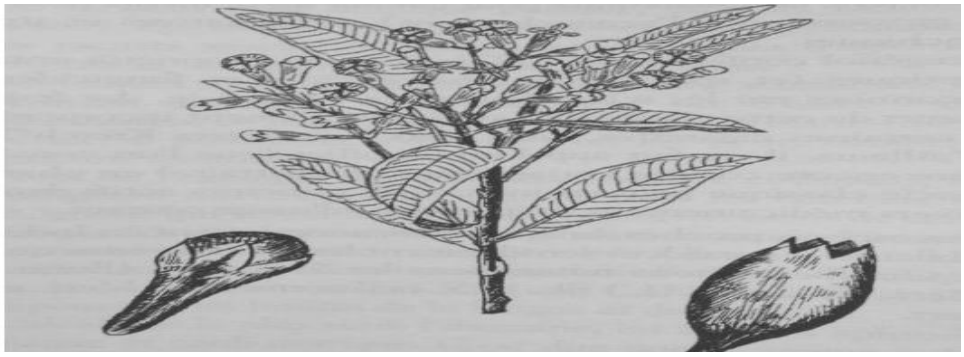


Figure 7. *Syzygium aromaticum*. The flowering shoot and floral bud are below. On the right is fruit topped with the calyx remnants (Boullard, 2001).

7. Chemical composition

Syzygium aromaticum represents one of the primary plant sources of phenolic compounds, such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenylpropenes, as well as terpenoids(Bao*et al*., 2012 ;Cortés-Rojas *et al*., 2014).

Eugenol (1) is the compound primarily responsible for the aroma of cloves, constituting 72 to 90% of the essential Oil of cloves **(**Kamatou*et al*., 2012**).** Other common constituents of the essential Oil include eugenol acetate (2), β-caryophyllene (3), methyl salicylate (4), pinene (5), vanillin (6), and α-humulene (7) (Figure 8) (Kuete, 2017**).**

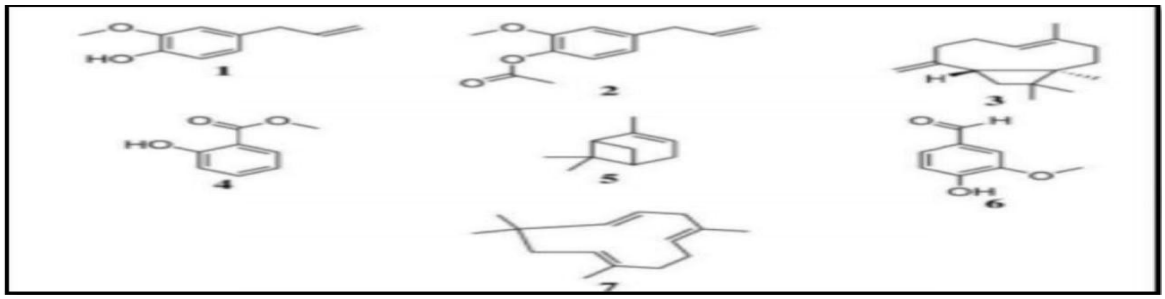


Figure 8. Main constituents of *S. aromaticum* oil (Jirovetz*et al*., 2006).

8. Traditional uses in medicine

The cloves are used in traditional folk medicine as diuretics, antalgic, cardiotonic, spices, condiments, and carminative and simulative properties **(** Kamatou *et al*., 2012**)**.
S. aromaticum (Myrtaceae) is widely used in traditional Moroccan medicine to treat dental problems, rheumatism, and lung diseases; antiseptic is used as an anti-inflammatory **(ElHaouari*et al*., 2018).**

In general, decoctions of seeds, fruits, leaves, and flowers can also be used to treat diabetes, heart disease, high blood pressure, and toothache **(**Skalli*et al*., 2019; Mrabti*et al*., 2019; Mrabti *et al*., 2021). Clove powder mixed with the leavesofLawsoniainermis is used for hair care **(**Elhassan *et al*., 2020**).**

9. Potential of Clove (*Syzygiumaromaticum*) for treatment of periodontal disease

The onset and Progression of periodontal disease are as follows: Consequences of the host response to microorganisms found in tooth biofilm **(**Haffajee, 1994; Aptazidou *et al*., 2004**).**

Host-modulated therapeutic strategies aim to Promote inflammatory bone loss and suffering from periodontitis (Preshaw, 2008**).** Hence, the pharmacological activities of clove have been reported. Thus, research on its therapeutic potential needs to be improved. Table 2 describes the therapeutic use of clove for dental application.

Table 2: Pharmacological effects of clove for dental application (Preshaw, 2008).

Activity studied	Effect	Reference
Antibacterial	• Effective against gram-positive and gram-negative bacteria.	13, 42, 52
Anti-inflammatory	• Cytokine inhibition • Suppression of the NF-κBpathway	16, 10
Antioxidant	• Suppression LPO • Reactive oxygen scavenging activity • DPPH scavenging activity • Hydroxyl radical scavenging activity	54, 30
Antifungal	• Effective against *Candida albicans*	15, 50, 29,36
Antiviral	• Effective against HSV 1 and HSV 2	11, 51
Analgesics	• Antinociceptive effect	17, 25
Anesthetic	• Local anaesthesia	32, 37

10. Biological Activities

Han and Parker (2017) revealed the antiviral, antibacterial, antifungal, anticancer, antioxidant, and anti-inflammatory activities of *Syzygiumaromaticum.*

10.1. Antibacterial activity

Cloves contain 70 to 90 percent eugenol and over 15 percent essential oil. It has antiseptic, antibacterial, and antifungal properties, includes 9 to 15% eugenol acetate, and is known for its antimicrobial effects **(**Rakotoatimanana*et al*.,1999**).** Due to phenol groups, high-concentration of eugenol , it exhibits bactericidal effects **(**Dobler*et al*., 2020**).**

Eugenol induces bacterial lysis in various strains, particularly affecting Gram-negative bacteria. Phenolic hydroxyl groups interact with cell membranes and cause leakage of cytoplasmic compounds. The reaction also causes changes in the structure of fatty acids and

phospholipids and disrupts the synthesis of genetic material. Like phenolic compounds, terpenes act on cell membranes, diffusing through them, causing swelling and inhibiting respiratory enzymes (Bouacida, 2021**).**

In *E.coli*, eugenol in the bacterial cytoplasm increases the concentration of saturated fatty acids and decreases unsaturated fatty acids, resulting in changes in bacterial morphology. Eugenol inhibits the effects of various bacterial proteins or compounds, including virulence factors such as pyogenes, violaceins, and elastase (Bouacida, 2021**).**

10.2. Antiviral activity

Viruses react very sensitively to essential ingredients, Oil. Eugenol's antiviral effects have been tested on Herpes simplex 1 (HSV-1) and HSV-2 viruses **(**Benencia and Courrges , 2000; Tragoolpua and Jatisatienr, 2007).

Hence, several studies found that clove extract was highly active in inhibiting the replication of the Hepatitis C virus. A synergistic interaction was observed between the combination of acyclovir and eugenol **(**Hussein *et al.*, 2000**)**.

10.3. Antifungal activity

Clove's phenolic components, carvacrol, and eugenol, exhibit fungicidal properties in laboratory settings and living organisms **(**Chami *et al*., 2005; Chaieb *et al*., 2007**).** Research indicates a synergistic interaction when employing eugenol and methyl-eugenol, alone or in combination with fluconazole or amphotericin B **(**Khan *et al*., 2012**).**

Clove exhibits antifungal activity against *Candida albicans, Trichophytonmentagrophytes, Onychomycosis, Saccharomyces cerevisiae, E. Caryophyllata,* and *Aspergillus niger* **(**Pawar and Thaker , 2006)**.**

10.4. Anti-inflammatory and analgesic effects

Eugenol's anti-inflammatory and analgesic properties are attributed to its effects on receptors and antigens responsible for inflammation. Regarding anti-inflammation, eugenol relieves pain by directly destroying pathogenic bacteria, affecting cytokines and immune cells, changing the inflammatory response, and indirectly relieving pain. Additionally, eugenol directly acts on cells and nerve receptors to relieve pain **(**Bouacida, 2021**).**

10.5. Anticancer activity

Clove bud essential oil has been studied as a potential carcinogen **(**Zheng *et al*., 1992**).** One study found that it had antiproliferative and antimetastatic effects on triple-negative and HER2-positive breast cancer cells. In both cases, the compound increased the expression of genes involved in apoptosis, such as caspase 3, caspase 7, and caspase 9. On the other hand, another study showed that combined treatment with eugenol and 5-fluorouracil had cytotoxic activity against HeLa. Cell lines that induce apoptosis **(**Diniz do Nascimento *et al*., 2020**).**

10.6. Antioxidant activity

Reactive oxygen species produced by our bodies that cause tissue damage and cell death can inhibit the normal functioning of cellular lipids, D.N.A. and R.N.A.This can lead to many chronic diseases, such as heart disease, cancer, and even periodontitis. The antioxidant activity of clove could be due to the higher concentration of phenolic compounds such as eugenol acetate and thymol (Yadav and Bhatnagar ,2007**).**

1. Oral microflora

The oral microbiome is the collective genome of the microorganisms living in the mouth. It is the second largest microbial community in humansafter the gut. Comparedwithother body parts, they show a surprisingdiversity of predicted protein functions. The human microbiome consists of acoreand variable microbiome (Zaura *et al*., 2014).

The core microbiome is standard to all individuals, while the variable microbiomeis unique to the individualdepending on lifestyle and physiologicaldifferences. Bacteria can colonize two types of mouth surfaces: the teeth' hard tissue and the soft tissue or oral mucosa (Zaura*et al*., 2014).Teeth, tongue, cheeks, gingival sulcus, tonsils, hard palate, and soft palateprovide a richenvironment for microorganisms to thrive (Dewhirstand *et al*., 2010). Oral surfaces are coveredwith various bacteria, known as bacterial biofilms (Zhao *et al*., 2017).

2. Varieties of oral microflora

The oral cavity is a bustling habitat for many microorganisms, encompassing bacteria, fungi, and viruses. Firmicutes, Bacillus, Proteobacteria, and Actinomycetes stand as prominent bacterial cohorts. Notably, Candida, the foremostfungal constituent, exerts no pathogenic influence when the oral microbiota maintains equilibrium but becomes detrimental to oral tissues whenthis balance isdisrupted. Streptococcus and Candida collaborate within biofilms, manifesting pathogenic repercussions. Additionally, the oral microbiota harbors various viruses, predominantly phages, whose presenceremains constant throughout life, barring instances such as infections by the mumps virus or HIV, whichmayintroduceother viral entitiesinto the oral cavity (Lu Gao *et al*., 2018; Camille, 2020).

This microbial communitythrives in diverse niches within the oral cavity, including saliva, soft tissues likemucosa and tongue surfaces, and hard tissues such as teeth, where dental biofilms reside in fissures or supra- or subgingivalregions, as well as on artificial structures like dentures and oral implants (Nicole and Lutz, 2016).

During childbirth, the infant's oral cavity typically begins in a pristine state, save for a few microorganisms possibly transmitted rom the mother'sbirth canal. Withinhours, microorganismsfrom the mother's or nurse's mouth, facilitated primarily by saliva and potential environmental sources, populate the infant's oral environment (vertical

transmission). While water, food, and other nutritive fluidscan serve as additional sources, saliva remains the primaryvector for microbialtransfer (Lakshman and Victor, 2017).

Moreover, the non-shedding surfaces of enamel and cementum, alongside the gingival crevice, offeradditional habitats for bacterialcolonizationduring and post-tootheruption, marking a pivotalevolutionaryjuncture in microbial population dynamics:Gram-positive bacterialike *Streptococcus mutans*, *Streptococcus sanguinis*, *Actinomyces spp*., Lactobacillus, and Rothia preferentially colonize enamel surfaces.

Conversely, gram-negativeorganisms, includingnon-pigmenting*Prevotella spp*.,*Porphyromonas spp*., Neisseria, and Capnocytophaga, favoringanaerobic conditions, inhabitcrevicular tissues (Lakshman and Victor, 2017).

3. Characterization of the oral microbial flora and notion of the species studied

The community of microorganisms in our body iscalled the microbiome. The term "microbiome" wascoined by Nobel Prize winner Joshua Lederberg to describeecologicalcommunities of commensal and pathogenicmicroorganisms. Thesemicroorganismsshareour body space.(Kilian andal., 2016) .The number of microorganismspresent in our bodies isalmostequal to or evenhigherthan the number of ourcells. (Scotti *et al*., 2017).

3.1. Yeast

3.1.1. Candida albicans

Candida albicans is a yeast that belongs to the commensal flora of healthy individuals. However, when the fragile balance between the parasite and the host is disrupted, it becomes opportunistic and colonizes many mammals' mucocutaneous surfaces and oral and gastrointestinal cavities. This yeast poses a severe health problem in humans, especially in immunocompromised patients and those undergoing immunosuppressive therapies (Céline, 2007).

As a commensal fungus, *C. albicans* peacefully inhabit the oral mucosa, skin, vagina, and gastrointestinal tract withoutcausingsymptoms in healthy individuals. It constitutes over 80% of vaginal and oral yeaststrainsfound in asymptomatichumans. However, I tpossesses characteristics that can transition from commensal to virulent, enablingit to be a part of the

body'snaturalmicrobiome and, under conditions of immunocompromise, potentiallyinvade tissues and organs(Sobel, 2007; Taff*et al*., 2012; Cottier and Hall, 2020).

3.1.2. Morphology and characteristic

*Candida albican s*exhibits a range of yeast-like morphologies, including white, opaque, gray, and intestinal forms, as well as two types of hyphae (linear and sinusoidal), pseudohyphae, and chlamydospores (see Figure 1 and Figure 2). Pseudohyphae, distinct for remaining attached post-cytokinesis, candevelopintomyceliathrough successive cell divisions akin to hyphalcells. Besides this morphological diversity, *C. albicans*grows in single-cell cultures, biofilms, and microcolonies(Cottierand Hall, 2020).

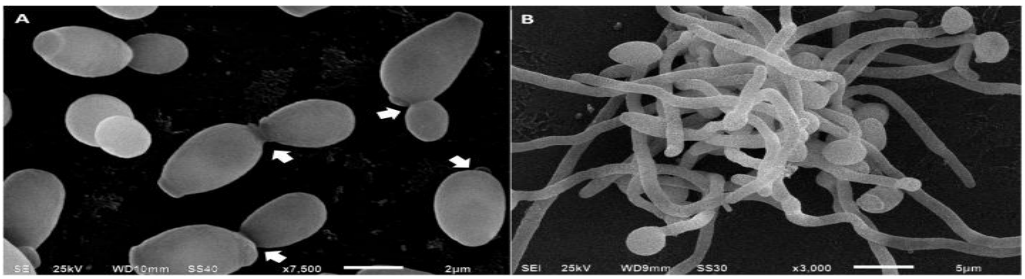


Figure 9.*Candida albicans*cellsare analyzed by scanning electron microscopy (Cottier and Hall, 2020).

In (A), yeasts are budding; the arrows indicate the site of cell division between the motherand daughtercells.

In (B), the mycelia ofC. *albicans* is involved in tissue invasion during the infectiousprocess.

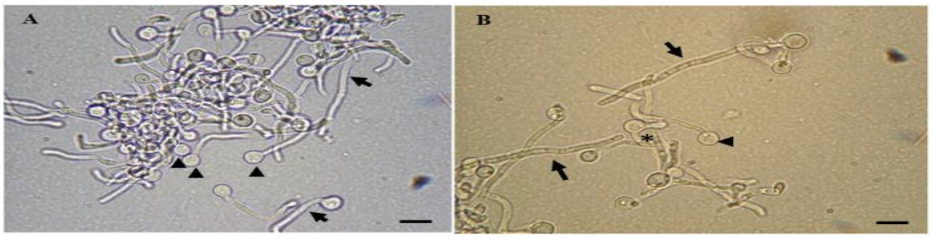


Figure 10. *Light microscopy analyzed C. albicans*cells in cell transition (Cottierand Hall, 2020).

In (A), a combination of chlamydospores (arrowheads) and hyphae (arrows) are found.

In (B), pseudohyphae (asterisk), chlamydospores (arrowhead), and multicellularhyphae (arrows) are seen. Scale bars represent five μm.

3.1.4. Taxonomy and Pathogenicity

The categorization of fungi has significantlyevolved, considering bothasexual and sexual reproduction. The Classification is presentedbased on its modes of reproduction, encompassingbothsexual and asexualmethods (Tab 2) (Maruyama *et al.,* 2005).

Table 3: Classification of *Candida albicans*presentedbased on its modes of reproduction, encompassingbothsexual and asexual methods (Maruyama *et al.,* 2005).

According to sexual reproduction	According to asexual reproduction
Division: Fungi perfect	Division: Fungi imperfecti
Phylum: Ascomycetes	Phylum: Deuteromycotina
Class: Saccharomycetes	Class: Blastomycetes
Order: Saccharomycetal	Order: Cryptococcal
Family: Candidaceae	Family: Cryptococcaceae
Gender: Candida	Gender: Candida
Species: *C.albicans*	Species: *C.albicans*

Candida speciesrankamong the prevalentfungalpathogensaffectinghumans, accounting for a spectrum of infections rangingfromsuperficialmucosal and cutaneous to systemic(Papon*et al*., 2013). Candida speciescontribute to about 8% of bloodstream infections acquired in healthcare settings (Pfaller and Diekema, 2007).

3.2. Bacteria

3.2.1. *Echerichia coli*

One of the typical intestinal flora of the human body is*Escherichia coli* (*E.coli*), whichusuallyexists in the human digestive tract (Enciso-Martinez *et al*., 2022). *E. coli* is an opportunistic pathogenthatis generally harmless to organisms. However, when the body'simmunitydeclines or the virulence of *E. coli* mutates, infections and relateddiseasesmayoccur, includingurinary tract infection, foodpoisoning, and diarrhea (Enciso-Martinez *et al*., 2022; Xu *et al*., 2017; Hu *et al*., 2016).*E. coli*isalso one of the most important markers for environmental and foodsafetytesting (Heijnen *et al*., 2009).

Pathogenic *E. coli* are broadlydividedintotwocategories: extraintestinalpathogenic *E. coli* (ExPEC) and enteropathogenic*E. coli* (InPEC) (Kaperandal, 2004; Tenaillon *et al*., 2010; Dale and Woodford, 2015; Denamur *et al*., 2021). Depends on the presence of specific virulence factors, mechanism of infection, tissue tropism, interaction with host cells, and clinical symptoms.

The Taxonomic Classification of *Escherichia coli is as follow:* (Amer and Masar, 2021).

Reign: Procaryotae
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteria
Family: Enterobacteriaceae
Genus: Escherichia
Species: *Escherichia coli*

a. Habitat

Usually, *E. coli*exists as a harmless commensal bacteria in the intestinal mucosa, appendix, and colon. Gram-negative, motile bacteria have adapted their metabolismsuccessfully in penetratingthisnutritional niche and holding more than 500 otherbacterialspecies (Tenaillon*et al*., 2010).

b. Morphological, cultural, and biochemical characteristics

Escherichia coli is a non-sporulatingbacterium, measuring 2-4 μm in length and 0.4-0.6 μm in width. It appears as a slender, elongatedbacteriumwithrounded ends and exhibitsmobility due to itsperitrichous flagella. This microorganismis not fastidious, formingsmooth, glossy, and uniform colonies whencultured on standard agar media (Lobril, 1998).

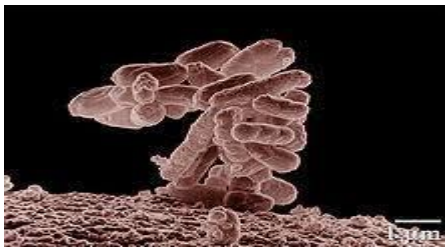
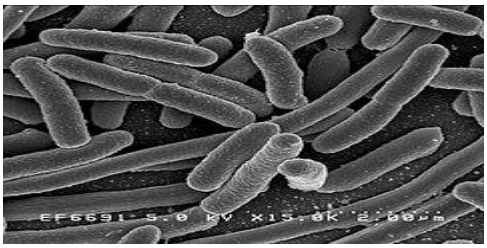



Figure 11. "Electron Micrograph of Escherichia coli" (Thorene G, 1994).

E. coli demonstrates the capability to ferment varioussugarssuch as glucose, lactose, mannitol, and sometimes sucrose, producing organicacids. Gas production accompanies glucose fermentation. An identifyingcharacteristic of *E. coli* isitsability to produce indole fromtryptophan. It isfacultativelyanaerobic, harmful for urease and tryptophandeaminase, does not yieldacetoin (adverseVoges-Proskauerreaction), and cannotutilize citrate as a carbon source. *E. coli* reduces nitrates to nitrites, lacksoxidaseactivity, and possesses catalase (Joly and Reynaud, 2002).

3.2.2. *Staphylococcus aureus*

Staphylococcus aureus remainsprominentlyimplicated in varioushumandiseases. It is a common constituent of the normal skin microbiota in animals and humans, with a prevalencerangingfrom 20 to 30% amonghealthyindividuals(Wertheim *et al*., 2005),(Hanselman *et al*., 2009). Infections caused by *S. aureus* can lead to various human ailments, includingabscesses, lung infections, bacteremia, endocarditis, and osteomyelitis(Tong *et al*., 2015).

a. Habitat and Classification

Like all staphylococci, this bacterium is ubiquitous (air, water, and soil). It is a commensal of human skin and mucous membranes. The anteriornares and the moist areas of the skin (armpits, wrists, perineum) constitute the essential reservoir site for *S. aureus* (Peacock*et al*., 2001).

Bacterial Classification is presentedaccording to Bergey'sManual of SystematicBacteriology recommendations, volume 3, second edition (2009).

Domain: Bacteria.
Phylum: Firmicutes.

Class: Bacilli.

Order: Bacillus.

Family: Staphylococcus.

Genus: Staphylococcus.

Species: *Staphylococcus aureus*

b. Morphological, biochimical and cultural characteristic

Staphylococcus aureus cellsexhibit Gram-positive characteristics and adopt a sphericalmorphology, oftenforming clusters reminiscent of bunches of grapeswhenviewedunder a light microscope following Gram staining. The term 'Staphylococcus' originatesfrom Greek, signifying 'bunch of grapes' (staphyle) and 'berry' (kokkos) (Licitra, 2013).

Scanning electronmicroscopy reveals cells with roughly spherical shapes and smooth surfaces (Greenwood and O'Grady, 1972). Thesecellstypicallymeasure 0.5 to 1.0 micrometer in diameter (Foster, 1996). Transmission electron microscopyshow cases thick cell walls, distinct cytoplasmic membranes, and amorphous scytoplasm. (Touhami*et al*., 2004).

S. aureus ischaracterized as an aerobic and facultative anaerobicorganism, formingnotably large colonies on nutrient-rich agar media, typicallyappearingyellow or white. The yellow hue of these colonies stems from the organism's production of carotenoids. The term 'aureus' originatesfrom Latin, denoting the color of gold (Liu *et al*., 2005). The organismoftenexhibitshemolyticactivity in blood agar due to the secretion of four types of hemolysins (alpha, beta, gamma, and delta) (Blair, 1958; Dinges*et al*., 2000). Nearly all *S. aureus* isolatesproduce the coagulase enzyme, a virulence factor crucial for identification and Pathogenicity (Brown *et al*., 2005). Moreover, Brown*et al*. (2005) demonstratesalttolerance and canthrive in mannitol-salt agar medium containing 7.5% sodium chloride (8). Furthermore, *S. aureus* is catalase-positive and oxidase-negative.

3.2.3. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a rod-shaped bacterium of the Pseudomonadaceae family, strict aerobe, Gram-negative, positive for cytochrome oxidase, mobile by polar flagella, producing the pigments fluorescein and pyocyanin. *P. aeruginosa* cells measure 0.5 - 1 µm × 1.5 - 4 µm. This bacterium metabolizes a wide variety of organic compounds and is resistant to several antibiotics and disinfectants (OMS, 2006).

Figure 12. Bacteria in space (Kamer, 2013).

a.Morphology

Pseudomonas aeruginosa is a Gram-negative rod-shaped bacillus with a diameter of 0.5 to 0.8 µm and a length of 1 to 3 µm. Propulsion is provided by the usually unique polar flagella, which lack spores and capsules. *Pseudomonas aeruginosa*'s wall shows Gram-negative bacteria characteristics (Sadolff and Artenstein, 1974)**.**

It consists of an outer membrane, periplasmic space, and peptidoglycan. The latter forms an asymmetric bilayer of lipopolysaccharide (LPS) and phospholipids (P.L.), which contain many proteins, such as porins, that facilitate the diffusion of various molecules across the outer membrane (Pages, 2004).

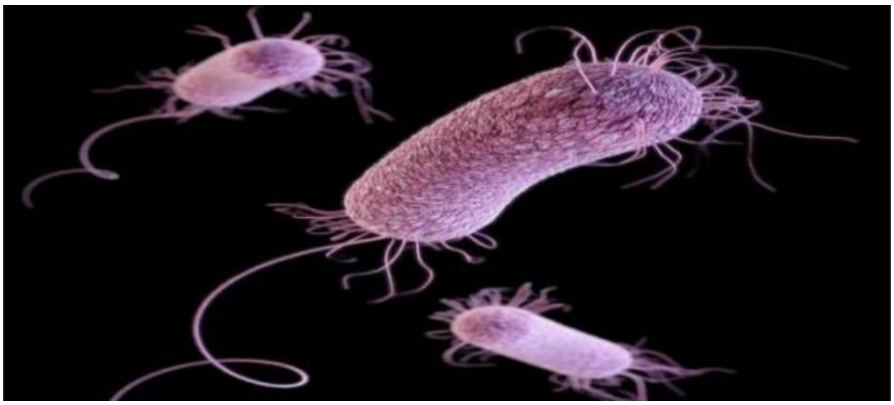


Figure 13. Three-dimensional computer-generated (3D) image of *P. aeruginosa.* (Green *et al*., 1974)

b. Habitat

Pseudomonas aeruginosa, an opportunistic pathogen, is widespread in soil, water, and diverse host environments. It is prevalent in everyday foods, particularly vegetables, and is even in small amounts in drinking water due to its varied energy metabolism. This bacterium forms biofilms on various surfaces like food packaging and medical equipment, making it vulnerable to antimicrobial agents. In drinking water systems, these biofilms may act as reservoirs, potentially contaminating water and posing health risks to humans **(**Mena and Gerba, 2009).

c.Taxonomy of *P.aeruginosa* (Balouki, 2017)

Domain: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Pseudomonales

Family: Pseudomonaceae

Gender: Pseudomonas

Species: *Pseudomonas aeruginosa*

d. Pathogenesis

For opportunistic pathogens such as *Pseudomonas aeruginosa*, the disease process begins with altering or evading the host's normal defenses. The Pathogenesis of *Pseudomonas* infections is multifactorial, as evidenced by the number and broad spectrum of bacterial virulence determinants. Various virulence determinants are expected for the various diseases it causes, including sepsis, urinary tract infection, pneumonia, chronic lung infection, endocarditis, dermatitis, and osteochondritis (Bricha *et al*., 2011)

Most Pseudomonas infections are both invasive and toxigenic. The final Pseudomonas infection consists of three distinct stages: (1) bacterial attachment and colonization, (2) local invasion, and (3) disseminated systemic disease. However, the disease process can stop at any time. Specific bacterial virulence determinants mediate each stage and ultimately lead to the characteristic syndrome accompanying the disease (Bricha *et al*., 2011; Fuentefria *et al*., 2011).

e. Biochemical and cultural characteristics

Pseudomonas is a class of chemoorganotrophic bacteria with complete respiratory metabolism. Some Pseudomonas species reduce nitrate under anaerobic conditions by synthesizing nitrate reductase. Others use oxygen as a terminal electron acceptor in aerobic organisms. They can be identified by breeding hydrocarbon substrates used in energy and carbon production. In addition to the production of o-aminoacetophenone, o-aminoacetophenone is an intermediate in tryptophan metabolism and is unrelated to the formation of tryptophan. *Pseudomonas aeruginosa* also hydrolyzes gelatin and lecithin, producing its characteristic odor Artificial orange flavor **(**Aveil *et al.*, 2000).

Pseudomonas aeruginosa is a bacterium with minimal requirements. Growing on simple synthetic media, it thrives within 24 hours at 37°C. It can grow between 5 and 42°C, with an optimum at 30°C. However, it tolerates slight variations in pH (6.5 to 7.5) with an optimal pH of 7.2. *Pseudomonas aeruginosa* is a strict aerobe but can utilize nitrates under anaerobic conditions (Souly, 2002). It is characterized by a floral odor **(**Flandrois, 1997**).** A selective medium based on cetrimide (quaternary ammonium) allows for the detection and isolation of *Pseudomonas aeruginosa* from biological samples (stools, urine, pus, cerebrospinal fluid, etc.) in medical bacteriology (Delarras, 2007**).** According to Denis (2007), three types of colonies can be simultaneously or independently observed on solid media :

- Large colonies ("la") with a diameter of 2 to 3 mm, irregularly edged, with a raised central part that exhibits metallic reflections.

- Smaller smooth colonies (‘’S’’) that are raised with regular edges.

- Mucous colonies ("M") that are raised, coalescent, and stringy, encountered in strains producing a slime of alginate polymer.

3.3.3. *Bacillus cereus*

a.Habitat

B. cereus, like many other bacilli, is common in soil. Because it does not have complex nutrient requirements, it is frequently found in soils with low nutrient levels and on rice and straw **(**Priest, 1998). Due to this property and the ability to form spores, B. cereus can spread quickly.

b. Classification

The *Bacillus cereus* sensu lato group is composed of six bacterial species, of which the three most well-known due to their Pathogenicity are *B. cereus* sensu stricto, Bacillus thuringiensis, and Bacillus anthracis. These are Gram-positive, aerobic, or facultatively anaerobic, sporulating bacteria (Fox *et al*., 2020**).**

The Classification is as follows: **(**Lechevalier, 1981**).**

Reign: Bacteria
Phylum: Firmicutes
Class: Bacilli
Family: Bacillaceae
Gender: Bacillus
Species: *B. cereus*

c. Pathogenicity of *Bacillus cereus*

The Pathogenicity of *B. cereus*, whether inside or outside the gastrointestinal (G.I.) tract, is associated with the production of exoenzymes. Among the secreted toxins are four hemolysins, three distinct phospholipases, and three pore-forming enterotoxins. The

enterotoxins that activate the nod-like receptor protein-3 (NLRP3) include hemolysin B.L. (HBL), non-hemolytic enterotoxin (NHE), and cytotoxin K (Beecher and Wong, 1994).

The enterotoxin comprises a binding component (B) and two hemolytic components, designated HBL. A three-component non-hemolytic enterotoxin, referred to as NHE, has been identified in the diarrheal form of the disease. Bacillus cereus's non-hemolytic enterotoxin (NHE) activates the inflammasome and pyroptosis of the nod-like receptor protein-3 (NLRP3). This leads to programmed cell death initiated by the activation of inflammatory caspases in the infected tissue (Fox *et al*., 2020**)**

3.2.5. Klebsiella pneumoniae

Klebsiella is a human pathogen known since the end of the 19th century when Edwin Klebs first isolated it. It is often called Friedländer pneumonia in honor of the man who identified it as a respiratory pathogen in 1882 (Simon, 1997).

Klebsiella pneumoniae is a common pathogen of nosocomial infections, occurring mainly in patients with several diseases in hospitals or long-term care facilities (Cunha, 1993; Yinnon, 1996).

a. Classification

Klebsiella pneumoniae is classified as belonging to the following groups in the second edition of Bergey's manual (Holt *et al*., 1994).

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Klebsiella
Species: *Klebsiella pneumoniae*

There are five species in the genus Klebsiella, with *K. pneumonia* being the type species (Bergogne-Berézinand, 1995).

K. pneumoniae is further divided into three subspecies: *K. pneumoniae* subsp *pneumonia*, *K.pneumonia* sub sp *ozaenae*, and *K. pneumonia* sub sp *Rhinoscleromatis*(Avril *et al*., 2000)

b. Morphological and cultural characteristics

K. pneumoniae is a species of bacteria that are Gram-negative bacilli, non-motile, usually encapsulated diplobacilli, non-sporulation, and facultative anaerobes (El Fertas-Aissani *et al*., 2012).

K. pneumoniae can grow in the presence of oxygen (aerobiosis) and in the absence of oxygen (anaerobiosis). When cultured on conventional isolation media for enterobacteria such as Drigalski, Hektoen, Mac Conkey, and EMB, the colonies of *K. pneumoniae* have a diameter of 3 to 4 mm, are round, smooth, lactose-positive, convex, shiny, mucoid, and sometimes appear stringy when picked up with a platinum loop (figure 9) (Le Minor and Véron, 1989 ; Freney, 2000).

In liquid media such as nutrient broth and peptone water, the culture of *K. pneumoniae* exhibits rapid growth (within a few hours) at temperatures of 30°C and 37°C. A mucous deposit and a viscous collar can sometimes be observed on the surface. Unlike other Klebsiella species, more than 90% of *K. pneumoniae* strains are capable of growing at 44°C in brilliant green lactose bile broth, and over 80% of them ferment lactose with the production of gas (Le Minor and Véron, 1989).

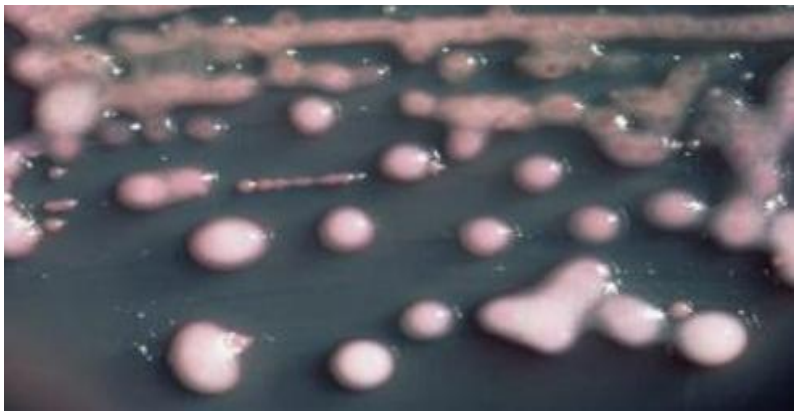


Figure 14: Appearance of *K. pneumoniae*colonies on agar medium (Gueye, 2007).

c. biochemical characters

K. pneumoniae exhibits the typical traits of enterobacteria: it can thrive in both aerobic and anaerobic conditions, ferments glucose with gas production, lacks oxidase activity but has positive catalase activity, and possesses nitrate reductase (Le Minor and Véron, 1989).

K. pneumoniae is characterized by being VP+, LDC+, ODC-, IND-, Citrate+, Urea+, ONPG+, H2S-,TDA-, and capable of reducing nitrate to nitrite (NO3+) (Le Minor and Véron, 1989).

d. Pathogenicity

K. pneumoniae is a well-known and significant cause of nosocomial infections in children, representing 19,6% of cases in China and 22,7% in neonatology and pediatric intensive care units, with a particular prevalence in Europe (Boukadida *et al*., 2002).

Klebsiella pneumonia is a prominent species associated with urinary tract infections, contributing to 6 to 17% of cases (Ben Haj Khalifa and Khedher, 2010). In the U.S., it accounts for 7.9% of all urinary infections, ranking fifth among the pathogens involved, as reported by the NHSN. However, in Tunesia, K. pneumonia is predominantly isolated from urinary infections in hospital settings, with a rate of 60,4% according to the Anti-bio Resistance Network in Tunesia (LART) (Nedjai *et al*., 2011).

Klebsiella pneumonia belongs to the KES group, essential in hospital clinical settings (Podschun and Ullmann, 1998).

Currently, *K. pneumonia* subsp. *Pneumonia* is primarily linked to nosocomial infections, including urinary tract infections caused by catheters, bacteremia, pneumonia, surgical site infections, and neonatal infections (Carpenter, 1990).

3.3. Fungus

3.3.1. *Aspergillus niger*

Aspergillus is the name of a genus of microscopic imperfect fungi (Deuteromycetes) that was initially described in 1729 by Michelle, a mycologist from Florence. This genus comprises around 180 officially recognized species, divided into 18 groups primarily based on the characteristics of their reproductive apparatus. These groups share similarities in terms of their morphology, genetics, and physiology (Gams *et al*., 1986)

a. Morphology

Aspergillus is distinguished by erect conidiophores, which have a vesicle at the top.The vesicle can either support a single row of phialides (known as uniseriate structures) or a row of underlying cells called setulae (known as biseriate structures). The conidiophore is formed

by the combination of the stipe and vesicle, while the aspergillus head is composed of the vesicle, phialides, and conidia (Leyral and Vierling, 2007 ; Masayuki and Katsuya, 2010).

The production of spores or conidia by the phialides is a defining feature of the fungus's asexualmode of reproduction. These phialospores are clustered in a plume, with their color and shape varying depending on the species (Leyral and Vierling, 2007).

b. Habitat

Aspergillus niger is cosmopolitan (Botton *et al*., 1990); although it is reported worldwide, it isslightly more common in temperate zones and sites exposed to the south. It is frequentlyfound in moldycereals, fruits and vegetables, forage, dairy products, and peanuts in the air, soil, and indoor environments (Rahul and Jha, 2014).

c. Taxonomy

The traditional taxonomy of molds relies on morphological traits such as mycelium structure, the formation of sexual spores, and the presence and mechanisms of sexual reproduction (Aguis *et al*., 2008).

Table 4: The systematic position of Aspergillus niger is summarized as follows (Koji*et al*., 2001;Chabasse *et al*., 1999).

Kingdom	Fungi
Phylum	Amastigomycota
Subphylum	Deuteromycotina
Class	Deuteromycetes
Order	Moniliales
Family	Moniliaceae
Genus	Aspergillus
Species	*Aspergillus niger*

d. Macroscopic morphology of colonies

Aspergillus niger colonies exhibit fast growth on traditional culture media such as malt agar andSabouraud agar. The ideal temperature for its growth typically falls between 25 and 30°C, although it cantolerate temperatures as high as 42°C. Initially, the colonies of *Aspergillus niger* appear white andgranular, later transitioning to a yellowish hue and finally

turning black (occasionally brown) when fullymature. The reverse side of the colonies is usually colorless or pale yellow, occasionally displayingconcentric zones (Bensmail.,2012).

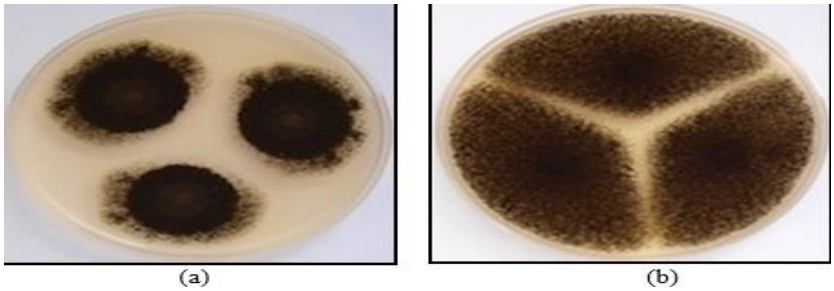


Figure 15. *Aspergillus niger* on M2 (a) and *Aspergillus niger* on M2S5 medium (b) (Samson, *et al*., 2004)

e. Microscopic morphology

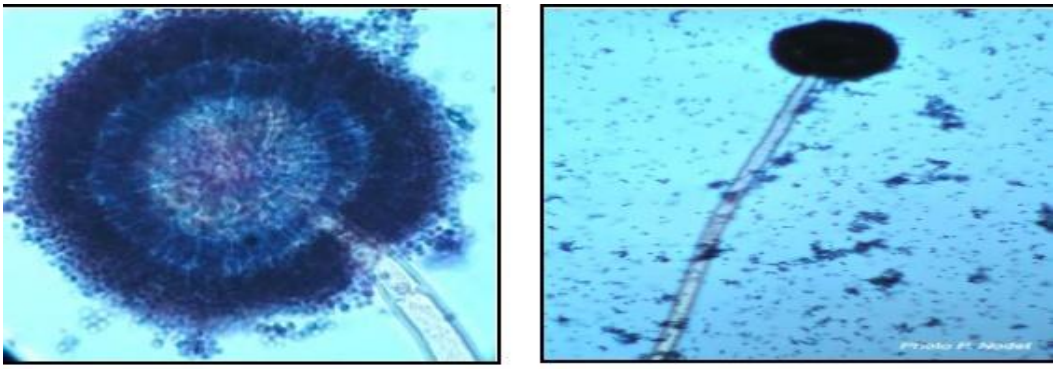
The hyphae of *Aspergillus niger* are septate and transparent. The conidial heads start as dark brown toblack and radiate when young, eventually separating into distinct columns as they mature. These conidialheads can reach a diameter of 700 to 800 µm. (Samson*et al*., 2004).

The conidiophores, which are smooth, have non-septate stipeses that are 1.5 to 2 mm long. The upper halfof the conidiophores can be either transparent or brownish. They comprise a short foot cell connected to a fertile hypha (Bensmail, 2012).

The vesicle is spherical and ranges from 25 to 75 µm. It has two rows of sterigmata covering itsentire surface (Samson*et al*., 2004).

The phialides are produced on typically brown conidiophores and measure 20-30 x 5-6µm. They are often septate and have dimensions of 7-9.5 x 3-4 µm (Abarca, 2004; Pasqualotto, 2010).

The conidia are unicellular spores produced by the phialides and are asexual. They typicallyhave a globular shape, although they can be slightly flattened, and their diameter ranges from 2 to 3 µm,occasionally reaching up to 6 µm. These conidia are black or dark brown and have a texturedsurface with spines and protrusions (called echinulate form). They are commonly found arranged inchains and are dispersed by the wind (Abarca, 2004; Pasqualotto, 2010).



(a) (b)

Figure 16. Conidial head of A. niger (× 400) (a) and conidiophores (×100) (b) (Samson*et al*., 2004).

f. Pathogenicity

Due to the presence of Aspergillus spores in the air, it is almost inevitable that they will be inhaled, leading torespiratory penetration. As a result, the bronchopulmonary system is frequently affected by Aspergillusdisease. Ahearn et al. (1997) found that *A. niger* is responsible for more than 80% of these infections.

Under certain conditions (such as when handling moldy grains or hay), if there is significantinhalation, the fungus can be detected in the sputum several days later, regardless of whether the person isill. Additionally, direct contamination (via the skin) is also possible and can result in ear infectionsand secondary infections in burn patients (Aguis*et al*., 2008)

Part Two: Experimental Study

1. Studied plants species

In this study, the stems of *Salvadora persica* and dried cloves of *Syzygium aromaticum* were purchased from a local market in the city of Khenchela (Algeria) in March 2024, identified by taxonomists, and voucher samples were deposited in the educational laboratory of the Khenchela University, Algeria. Two plant species were selected for antimicrobial testing based on literature data and data collected from traditional healers and medicinal plant dealers. These species are widely used locally and in Algeria. They are considered one of the most commonly used traditional medicinal plants due to their ease of use, accessibility, affordability, and use as antibacterial stick toothbrushes for dental hygiene and health—traditional and religious values (Chelli–Chentouf*et al*., 2012).

This research was conducted at the Khenchela University Educational Laboratory.

2. Extraction

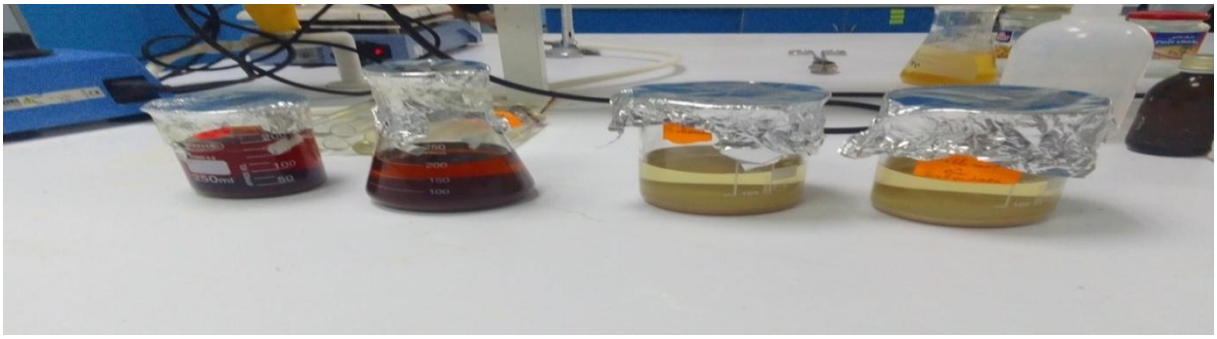
The stems of *Salvadorapersica* and *Syzygium aromaticum* (photo 1) were dried at room temperature for five days and finely ground using a coffee grinder. The liquid/solid extraction method employed in this study involved maceration with two alcoholic solvents of increasing polarity: Methanol (CH_3OH), Ethanol (C_2H_5OH), and distilled water (Cowan, 1999).

Two methods were used to prepare the aqueous extracts of *S.persica* and *S. aromaticum* : the first consisted of using 200 ml of cold distilled water and 20 g of plant powder in a beaker. After that, the mixture is stirred with a stirrer for 30 minutes, then covered with aluminum foil and left to rest for 72 hours in the refrigerator at 4 ◦c. The second: decoction was prepared by boiling a mixture of 20 g of plant material and 200 ml of distilled water for 10 minutes. After that, the mixture is stirred with a stirrer for 30 minutes, covered with aluminum foil, and left to rest for 24 hours at room temperature (Abhary and AL-Hazmi, 2015).

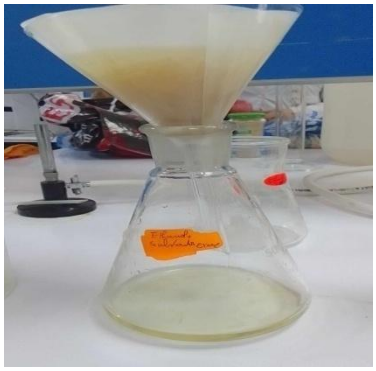
To extract using alcoholic solvents, 10 g of finely powdered plant material was soaked in a mixture of 200 ml (from a mixture of 40 ml of distilled water and 160 ml of methanol and ethanol). The mixtures were stirred for 15 minutes at room temperature and then left to rest for 24 hours at room temperature (Mau *et al*., 2001).

After being filtered twice through gauze-compressed filter paper to eliminate large plant particles, the remaining solvents were evaporated completely using a rotary evaporator at 45 ◦C. the driedextracts of *S.persica* and *S.aromaticum* were then used to create various

concentrations of each extract: 50,100,200, and 15 mg/ml with the help of dimethyl sulfoxide (DMSO) (Abhary and AL-Hazmi, 2015).



Photography1 : Maceration technique



Photography2: Filtration of extracts

Photography3: Evaporation of solvents

2.1. Yield determination

The following equation determines the extraction yield:

Extraction yield (%) = W1/W2×100;

W1 is the mass of crude extract (g), and W2 is the sample (g) mass.

3. Selected pathogenic microorganism species

The pathogenic microorganisms used in this study include *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Klebsiella pneumonia, Candida albicans* and Aspergillusniger. These microorganisms are commonly found in the oral cavity of individuals with poor dental hygiene and have been consistently identified in such environments (Vulcano *et al*., 2014; Li *et al*., 2022). The bacterial strains were supplied by Dr. Naili from the University of Khenchela, and their characteristics are summarized in the Table below.

Table 5: Characteristics of different microorganisms

Strains tested	Gram	ATCC
Escherichia coli	Bacille Gram -	25922
Pseudomonas aeriginosa	Bacille Gram -	27853
Klebsiella pneumonia	Cocci Gram -	4352
Bacillus cereus	Bacille Gram + 11778	11778
Staphylococcysaueus	Cocci Gram+ 25923	25923
Candida albicans	Fungi	
Aspergillus niger	Fungi	

4. In vitro antimicrobial activities of *S. persica* and *S. aromatic* extracts

4.1. Antibacterialactivities

The method relies on diffusing an antibacterial compound in a solid medium within a Petri dish, following a specific contact time between the product and the target microorganism. The antibacterial activity is evaluated by measuring the inhibition area based on its diameter, classifying the strain as sensitive, very sensitive, extremely sensitive, or resistant (Bouyahya*et al*., 2017).

Each antimicrobial evaluation test was conducted three times to ensure reliable and precise results. The extracts were dissolved in DMSO to prepare four concentrations for each extract: 200,100, 50, and 25 mg/ml. Antimicrobial activity was assessed using the disc agar diffusion method (Finegold and Martin, 1982; Lino and Deogracious, 2012).

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 match McFarland standard No. 0.5. These suspensions served as inoculums to test the effect of crude extracts by the agar diffusion method on Mueller Hinton agar plates (Konema*et al*., 1997).

Discs impregnated with 20 µl of various extracts were placed on the surface of these plates, which were then incubated at 37°C for 24 hours (Karou*et al*., 2005). The diameters of the inhibition zones were measured and recorded as the mean diameter (in millimeters) of the entire growth inhibition.

Photography 4: Preparing the discs

4.2. Antifungal activities

For the evaluation of antifungal activity, the method described by Yazdani and colleagues (2012) was followed. Initially, a drop of spore suspension from the fungus *Aspergillus niger* was spread on Sabouraud agar or PDA medium, followed by incubation at 25°C for seven days. Subsequently, a spore suspension with an optical density (OD) ranging between 0.15 and 0.17 at 530 nm was prepared in physiological water. Plant extracts were then diluted in DMSO at 200,100, 50, and 25 mg/ml concentrations. Inoculation onto Petri dishes containing PDA medium was carried out using swabbing. Discs of 6 mm diameter soaked with 10 or 20 µl of each concentration were placed on the surface of the PDA agar, with three repetitions performed. The plates were then incubated at 28°C for 48 to 72 hours, and the antifungal activity was assessed by measuring the zones of inhibition around the discs.

5. Phytochemical screening

The photochemical tests were performed on the aqueous and alcoholic extracts of both plants. Detection of specific compounds is carried out using methods described by Khaldi*et al*. (2012) and Vijayalakshmi*et al*. (2012).

A. Polyphenols

Add 2 mL of aqueous or alcoholic extract to a test tube and then add a drop of 2% alcoholic solution of ferric chloride. The appearance of bluish-black or greenish coloration indicates a positive test.

B. Flavonoids

The presence of flavonoids is detected by a simple and rapid test called the "Shinoda reaction" (Soulama*et al*., 2014). The test involves adding a few drops of concentrated HCl and approximately 0.5 g of metallic magnesium to 1 mL of the extract. Let it react for 3 minutes; the appearance of a red, orange, pink, or reddish-violet color indicates the presence of flavonoids.

C. Tannins

In a test tube, add 1 mL of extract with 2 mL of H_2O and 1 mL of a 2% aqueous $FeCl_3$ (ferric chloride) solution. Tannins are indicated by a greenish coloration (catechic tannins) or bluish-black (gallic tannins).

D. Terpenoids

Detection involves treating 0.5 mL of aqueous extract with 2 mL chloroform and a few drops of concentrated sulfuric acid. In the case of a positive reaction, a reddish-brown ring forms at the interface of the two liquids.

E. Saponins

We took 1 mL of the extract and added 3 mL of distilled water. The mixture is vortexed for 30 seconds and allowed to stand for 15 seconds. If the foam persists during this period, it indicates the presence of saponins.

F. Quinones

1 mL of concentrated sulfuric acid is added to 1 mL of our extract. The formation of a red color indicates a positive test.

G. Anthraquinones

Anthraquinones are detected by adding a few drops of HCl to 0.5 mL of extract. The appearance of a red precipitate indicates the presence of anthraquinones.

H. Anthocyanins

The detection reaction for flavonoids involves treating 2 mL of extract with 2 mL of sulfuric acid (H_2SO_4) and 2 mL of NH_4OH (ammonia). If the color intensifies upon acidification and turns blue in a primary environment, the presence of anthocyanins can be concluded.

Results and discussions

1. Plants extraction

The extracts were prepared from the dried *Syzygium aromaticum* and the stems of *Salvadora persica* using distilled water and two alcoholic solvents: methanol and ethanol. These solvents facilitated the separation of plant compounds based on their solubility in the extraction solvents.

Indeed, the choice of solvent employed during the extraction procedure can provide insights into the plant's active constituents. The study yielded four distinct extracts for each plant: an aqueous infusion extract, an aqueous maceration extract, an ethanolic extract, and a methanolic extract. Each extract was assessed based on its color and visual characteristics, influenced by the extracted active compounds and their weight and yield, as indicated in the Table.

Photography5: Alcoholic extracts of *S.aromaticum* and *S.persica*

Photography6: Aqueous extracts of *S.aromaticum* and *S.persica*

2. Extraction yield

After filtration, dry extracts of different weights from the stems of *S.persica* and the dried *S.aromaticum* were obtained. The results are shown in the Table below:

Table6: Dry weight of the extracts from the two plants studied

extract / plant	Methanol (48h)	Ethanol(48h)	Cold distilled water(72h)	boiled distilled water(72h)
Salvadorapersica	0.974g	0.632g	2.0931g	2.0879g
Syzygiumaromaticum	1.19g	1.4414g	2.1232g	2.7834g

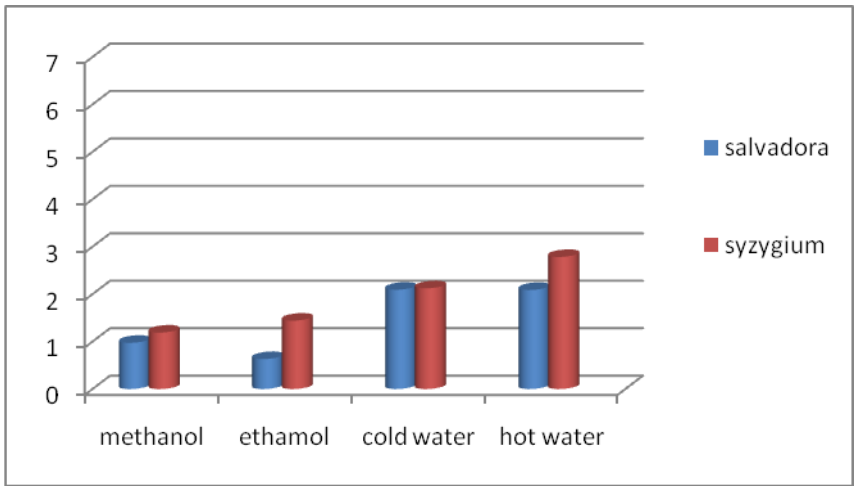


Figure 17: Comparison of the dry weight of the different extracts

2.1. Yieldcalculation

The calculation of the dry extract yield is crucial for determining the quantity and percentage obtained from an extraction. The partition coefficient, characteristics of each substance and working conditions influence this yield. It is calculated as the ratio of the weight of the dry extract (after evaporation) to the weight of the initial plant sample used in the extraction (vegetable powder), expressed as a percentage using the following formula:

Extraction of yield (%) = (weight of extract obtained/weight of plant material) x 100

Table7: Appearance, colors, and yield of the extracts of the two plants studied

Plant	Extract	Appearance	Colors	Yield(%)
Salvadorapersica	Methanol	Viscous	Yellow	9,74**%**
	Ethanol	Viscous	Yellow	6,32**%**
	Cold water(72h)	Viscous	Light yellow	10,4655**%**
	Hot water(72h)	Viscous	Yellow	10,4395**%**
Syzygiumaromaticum	Methanol	Viscous	Dark brown	11,9**%**
	Ethanol	Viscous	Brown	14,414**%**
	Cold water(72h)	Solid powdery	Brown	10,616**%**
	Hot water(72h)	Solid powdery	Dark brown	13,917**%**

The results of the *Sysygiumaromaticum*showed that the ethanolic extract had the highest extraction yield of 14.414**%**, while the Aqueous extracts(cold water) exhibited the lowest yield with 10.616**%**.

Furthermore, *Salvadorapersica* stems, the results showed that the aqueous extracts (hot and cold) took the highest yield, which are very close and similar values (10,4655**%**; 10,4395 **%**), while the ethanolic extract showed the lowest yield with 6,32**%**.

3. Antimicrobial activity

Antibacterial efficacy assessments were conducted on five bacterial strains, comprising gram-positive (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923) and gram-negative strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853), along with a fungus (*Aspergillus niger*). The experiments were replicated thrice. Dimethyl sulfoxide (DMSO) served as the negative control, while antibiotics served as the positive control. The inhibition zone diameters, measured for different concentrations of aqueous, ether extract of petroleum, and chloroform extracts of *Salvadorapersica* and *Syzygiumaromaticum* against the growth of various strains, are depicted in the ensuing tables:

Table 8: Diameters of microbial growth inhibition zones of*Syzygiumaromaticum*and *Salvadorapersica* aqueous extracts on strains identified (in mm).

	Salvadora persica								*Syzygium aromaticum*							
	Cold water				Hot water				Cold water				Hot water			
	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
Escherichia coli	13	14	14	11	15	15	12	14	15	12	14	14	18	16	15	14
Pseudomonas aeruginosa	11	14	15	10	16	15	12	11	12	11	8	9	11	14	12	11
Bacillus cereus	8	11	13	12	10	11	11	12	19	14	11	10	16	16	11	12
Klebseilla pneumonia	8	14	11	14	11	14	10	15	13	13	11	9	11	11	11	9
Staphylocus Aureus	8	12	10	9	10	11	15	18	16	16	18	11	15	14	11	15
Candida albicans	10	10	8	8	10	10	8	8	15	10	13	9	10	10	10	10
Aspergillus niger	0	0	0	0	10	12	15	20	6	6	6		11	9	6	

Table 9: Diameters of microbial growth inhibition zones of*Syzygiumaromaticum*and*Salvadorapersica* of Ethanol and Methanol extracts on the tested strains (in mm).

	Salvadora persica								*Syzygium aromaticum*							
	Ethanol				Methanol				Ethanol				Methanol			
	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
Escherichia coli	14	14	13	15	12	16	16	17	16	22	16	13	20	15	16	15
p.aeruginosa	17	18	18	18	11	12	16	16	14	12	12	10	11	11	12	14
B. cereus	12	13	13	12	11	12	11	14	9	15	13	14	11	14	17	14
Klebseilla pneumonia	11	13	15	18	12	12	20	20	10	18	19	16	9	8	16	16
Staphylocus Aureus	14	14	15	22	15	12	15	17	9	14	15	14	9	13	16	16
Candida albicans	8	8	8	8	11	8	11	9	9	11	13	13	11	10	11	11
A.niger	12	16	23	21	11	13	15	15	9	23	24		9	19	11	

C1. 25mg/ml**, C2**. 50mg/ml, **C3.** 100 mg/ml, **C4**. 200 mg/ml

The extracts exhibited differing levels of antimicrobial effectiveness against the bacteria under examination. The 200 mg/ml concentration from all extracts of the two plants (methanol, ethanol, and aqueous extracts) significantly impacted all bacterial strains.

The assessment of antimicrobial properties in *Salvadorapersica* and *Syzygiumaromaticum* revealed that the ethanolic extract exhibited superior activity compared to other extracts, displaying a diameter of 22 mm against *Staphylococcus aureus* and *Escherichia coli*. Additionally, extracts from various solvents employed for both plants under study demonstrated relatively higher effectiveness against gram-positive bacteria.

Regarding antifungal activity, the table results indicated that the aqueous extract (infusion) from *Salvadorapersica* stems displayed activity against the fungal strain, yielding an inhibition zone of 20mm. Moreover, compared to *Salvadorapersica*, the alcoholic extracts from *Syzygiumaromaticum* exhibited greater efficacy against most tested strains.

Table 10: Phytochemical screening of *Salvadora persica*and and *Syzygium aromaticum.*

	Polyphénols	Flavonoïdes	Tanins	Terpènoïdes	Saponosides	Quinones	Anthraquinones	Anthocyanes
S. persica Methanol	+++	+	-	-	-	+	-	-
S. persica Ethanol	+	+	-	-	-	-	-	-
S. persica Cold water	+	+	-	-	+	+	-	-
S. persica Hot water	+	+	-	-	+	-	-	-
S. aromaticum Methanol	+	+	++	/	-	-	+	-
S. aromaticum Ethanol	+	+	++	/	-	++	+	-
S. aromaticum Cold water	+++	+	+++	/	-	++	+	+
S. aromaticum Hot water	+++	+	+++	/	-	-	+	-

A preliminary phytochemical screening was conducted on the aqueous, ethanol, and methanol extracts of Salvadora persica and Syzygium aromaticum to identify bioactive compounds potentially responsible for their antimicrobial activity.

As presented in Table (8), the aqueous and alcoholic extracts showed the presence of polyphenols and flavonoids in both plants. At the same time, tannins were present in all extracts of Syzygium aromaticum but were absent in the extracts of Salvadora persica. Additionally, trypanoids were not present in either plant, for the saponins were absent in all Syzygium aromatic and alcoholic extracts of Salvadora persica but were present in its aqueous extracts.

Quinones were present in methanol and aqueous extracts made by maceration of Salvadora persica and ethanol, aqueous extracts made by maceration of Syzygium aromaticum and its absence in ethanol, methanol and aqueous extract made by infusion in both plants.

Salvadora persica is characterized by the non-existence of anthraquinones, unlike Syzygium aromaticum. Anthocyanins were present in an aqueous extract made by maceration and absent in the rest of the extracts (ethanol, methanol, aqueous extract made by infusion) of Syzygium aromaticum. They were absent in all extracts of Salvadora persica.

II. Discussion

Each plant yielded four distinct extracts: Methanol, Ethanol, and water, obtained via infusion and maceration methods, underscoring the importance of overseeing extraction procedures. Methanol is commonly employed to extract antibacterial compounds, while ethanol selectively extracts phenolic compounds.

These variations in extract characteristics are likely attributed to solvent polarity variances, as research indicates (Raja *et al*., 2012; Cowan, 1999; Gupta et al., 2015; Harbi *et al*., 2015). The appearance and hue of the extracts likely hinge on the organic compounds within them.

The liquid extracts from *Syzygiumaromaticum* possess a dense quality, primarily because fatty acids and oils are mainly absent, substituted instead by polyphenols, flavonoids, tannins, and saponins, with variations depending on the solubility and polarity of the solvents used for extraction (Ganesh *et al*., 2013).

The maceration method resulted in a slightly higher yield of the aqueous extract from *Salvadora persica* than the infusion technique, with yields of 10.4655% and 10.4395%, respectively. The ethanol and methanol extracts exhibited low yields of 6.32% and 9.74 %, respectively. Furthermore, the yields of the aqueous extracts obtained through infusion and maceration surpassed those reported in previous studies on the same plant, which averaged 13.75% (Aissaoui and Maamri, 2009; Niboue and Lemoussekh, 2018).

The yield of the ethanolic extract of *Syzygium aromaticum* was very high compared to the methanolic extract, with 14.414% and 11.9%. In contrast, the aqueous extract made by infusion also exhibited a high yield of 13.917%, while the maceration method yielded 10.616%, indicating relatively low extraction efficiency.

The yields of aqueous extract obtained by infusion and ethanolic extract of *Salvadora persica* were relatively higher than those of *Syzygium aromaticum*, whereas the two other extracts, aqueous made by maceration and methanol, have a low yield for *Syzygium aromaticum.*

The extraction yields of extracts derived from identical organs and species can be impacted by various factors, including the extraction technique and its associated conditions. These conditions encompass parameters such as the duration of plant material drying, the quantity of plant material subjected to extraction, extraction duration, stirring rate, temperature, and solvent polarity. Furthermore, the geographical location and prevailing climatic conditions significantly influence the secondary metabolite composition of the plant (Koné*et al*., 2017).

Duraffourd *et al*. (1990) state that an inhibition zone of less than 8 mm indicates no sensitivity, while a diameter between 8 and 14 mm suggests limited sensitivity. Medium sensitivity is characterized by a diameter between 14 and 20 mm, and a diameter equal to or greater than 20 mm signifies high sensitivity, confirming the antibiofilm activity of the extract used.

The absence of an inhibition zone around the DMSO solvent discs indicates that the extract alone is responsible for its effectiveness. In contrast, the antibiotic exhibits significantly larger inhibition diameters than our extracts against all examined strains, demonstrating its potent antibacterial action across a broad spectrum of microbial strains.

According to the findings, *Syzygium aromaticum* demonstrated superior antibacterial activity in its alcoholic extract compared to aqueous extracts. The ethanolic extract exhibited remarkable effectiveness at a concentration of 50 mg/ml against *E.coli*, resulting in an inhibition diameter of 22 mm. Similarly, it displayed significant inhibition against *Klebsiella pneumonia*, with an inhibition diameter of 19 mm, as well as *Aspergillus niger*, with inhibition diameters of 24 mm and 23 mm at a concentration of 100 mg/ml and 50 mg/ml, respectively. Our results for *K.pneumoniae* were similar to those found by Elgio Venadaginting *et al*.(2021), and *Bacillus cereus*are comparable to the results observed by m Mostaqim*et al*. (2019) with an inhibition diameter of 14 mm, whereas the results obtained for *A. niger* and *E.coli* were more significant than those from Wankhede (2015) and Amit Pandey*et al*. (2011), respectively.

The result obtained by Amit Pandey *et al*.(2011) against *S.aureus* was very close to our result with an inhibition diameter of 16 mm and 15 mm, respectively, as well as *Pseudomonas aeruginosa* with a diameter of 14 mm and their own with an inhibition diameter of 20 mm.

The methanolic extract possessed activity against *Staphylococcus aureus* with an inhibition diameter of 16 mm at a concentration of 100 mg/ml - 200 mg/ ml. It exhibited significant inhibition against *Aspergillus niger* with a diameter of 19 mm, and it was better than those obtained by Wankede TB, 2015. According to the results of Dua *et al*. (2014), the methanol extract of clove has exhibited antibacterial activity against *E.coli* with an inhibition zone measuring 24 mm at a concentration of 25mg/ml. In contrast, our result showed an inhibition zone of 20 mm. Our *Candida albicans* (10 mm-11 mm) results were not comparable to those from Mohamed TahaYassin*et al*. (2020) (16 mm).

The aqueous extract of *S.aromaticum* exhibited the lowest antibacterial activity according to the results compared to methanol and ethanol extracts. Aqueous extract produced by maceration was found to have a concentration of 25 mg/ml against *B.cereus* with an inhibition diameter of 19 mm. It differs from Faraja Gnelimali *et al*. (2018) which is 15.1 mm. Likewise, Shehu *et al*. (2023) found the highest antibacterial activity against *S. aureus* with a diameter of inhibition zone of 21 mm at 100 mg/ml concentration, whereas our result showed an inhibition zone of 18 mm. For the antifungal activity, our results showed the lowest inhibition zone of 6 mm at all the concentrations against *A.niger*, contrary to the results obtained by Wankhede (2015) with an inhibition zone of 16 mm.

The antibacterial activity results of the aqueous infusion extracts exhibited significant efficacy against *E. coli* at a low concentration, with an inhibition zone measuring 18 mm, surpassing the result given by Sbahat Saeed, Perween Tariq (2008), which was 8.73 mm.

The findings demonstrate that *Salvadora persica* affects the tested strains, with the ethanolic extract showing the highest antimicrobial activity compared to the aqueous extract. Specifically, the ethanolic extract exhibited significant efficacy at concentrations of 100 mg/ml and 200 mg/ml against *Aspergillus niger*, with inhibition diameters of 23 mm and 21 mm, respectively.

Furthermore, the aqueous extract of *Salvadora persica* also showed notable antifungal effects against Aspergillus niger, achieving an inhibition diameter of 20 mm at a concentration of 100 mg/ml. Studies by Saddiq and Alkinani (2019) and Saadabi (2006) confirmed that the aqueous extract's ability to suppress fungal growth, with *Aspergillus niger* being particularly sensitive, showing an inhibition diameter of 33 mm.

However, the cold water extract of *Salvadora persica* did not show any inhibition zone against *Aspergillus niger* at any concentration. Belaabed Zakiya and Belabed Nassira (2023) found that the methanolic extract also lacked activity against Aspergillus niger.

On the other hand, both aqueous and methanolic/ethanolic extracts of *Salvadora persica* exhibited activity against Candida albicans, with inhibition diameters ranging from 8 to 11 mm, similar to findings by Firas A. Al-Bayati and Sulaiman Khudir (2008).

The most significant inhibition zone was observed against the *Staphylococcus aureus* strain, with a 22 mm inhibition zone from the ethanolic extract of *S. persica* at 200 mg/ml. According to Baara Douni*a et al*. (2023), the ethanolic extract induced a 17 mm inhibition zone at 100 mg/ml, and the methanolic extract also showed a 17 mm zone at 200 mg/ml. This study yielded significantly better results than Firas *et al*. (2008). Likewise, the aqueous extract (boilled water) established an 18 mm inhibition diameter at 200 mg/ml, aligning with the results from Firas A. Al-Bayati and Sulaiman Khudir,2008.

The ethanolic extract of S. persica showed notable antimicrobial effects against P. aeruginosa, with an 18 mm inhibition diameter at concentrations of 50, 100, and 200 mg/ml, consistent with the findings of Baara Dounia, Merzougui Rajaa, and Zedira Dounia (2023). The methanolic extract also demonstrated promising results, with a 16 mm inhibition zone at

100 and 200 mg/ml. In contrast, Firas et al. (2008) . No activity was observedagainst the *P. aeruginosa* strain.

Moreover, the methanolic extract of S. persica showed the most antimicrobial effects against E. coli, with a 17 mm inhibition diameter at 200 mg/ml and substantial activity at 50 and 100 mg/ml with a 16 mm inhibition zone. Our study showed significantly better results than the study by Mohamed Saeed Zayed Al-Ayed *et al*. (2016).

Likewise, the ethanolic extract had a slightly lower maximum inhibition zone of 15 mm at 200 mg/ml. However, it was still similar to the findings of Baara Dounia, Merzougui et al. (2023) at their highest 100 mg/ml concentration.

Hence, both aqueous extracts of *S. persica* showed relatively high antibacterial activity ; boiled water was slightly more effective (15 mm inhibition diameter at 25 and 50 mg/ml) followed by cold water (14 mm inhibition zone at 50 and 100 mg/ml). This study established significantly better results than Merzougui *et al*. (2023).

In addition, the most important inhibition zone was observed against the *Klebsiella pneumoniae* strain, with a 20 mm inhibition zone from the methanolic extract of *S. persica* at 100 and 200 mg/ml. This work showed significantly better results than the study conducted Al-Ayed *et al*.(2016).

Besides, the ethanolic extract of *S. persica* showed the highest activity with an 18 mm inhibition diameter at 200 mg/ml, 15 mm at 100 mg/ml, and 13 mm at 50 mg/ml, similar to the findings Merzougui et al. (2023). The boiled water extract showed slightly higher activity than the cold water extract, particularly at a concentration of 200 mg/ml with a 15 mm diameter.

Furthermore, the methanolic and ethanolic extracts of miswak showed better antibacterial effects than the clove extracts. The superior antibacterial effectiveness of miswak (*Salvadora persica*) extracts can be attributed to several factors: rirstly, the methanolic extract of miswak contains a higher concentration of polyphenols (+++), which are well-known for their strong antibacterial properties. In comparison, clove's methanolic extract has a lower concentration of polyphenols (+). According to Al-Bayati and Sulaiman ,2008. They demonstrated that the polyphenols in Miswak's extracts were effective against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative).

Secondly, the presence of quinones in the methanolic extract of miswak contributes to its potent antibacterial activity by disrupting bacterial cell processes. Clove's extracts do not have quinones listed among their primary bioactive compounds. Quinones further enhance these properties, reporting their effectiveness against *Staphylococcus aureus* and *Escherichia coli* (Sofrata *et al.*, 2008).

Thirdly, combining polyphenols, flavonoids, and quinones in miswak may create a synergistic effect, enhancing its antibacterial efficacy. This synergy might be less pronounced in clove extracts, which contain a different profile of bioactive compounds such as tannins and anthraquinones.

Lastly, the unique bioactive components in miswak, such as its specific polyphenols and quinones, may be more effective in targeting and inhibiting the growth of certain oral bacteria than those found in clove.

Hence, these factors contribute to the superior antibacterial properties of miswak extracts over clove extracts.

In addition, Miswak's boilled water extract contains saponins, which are known for their antimicrobial properties, according to Al-Otaibi (2004). Clove's hot water extract, although rich in polyphenols and tannins, may not have the same synergistic effect as the combination of polyphenols, flavonoids, and saponins found in Miswak, according to Sofrata *et al*.(2008).

Likewise, Clove's cold water extract has a higher concentration of polyphenols (+++), tannins (+++), and additional compounds like anthocyanins, which provide a broad spectrum of antimicrobial activity *et al*.(2007). In contrast, miswak's cold water extract, while containing quinones, may not match the potency of clove's diverse bioactive profile, according to Al-Bayati and Sulaiman (2008).

Certain bioactive compounds are more effectively extracted at higher temperatures. The hot water extract of miswak may release more potent antimicrobial agents like saponins, which are less effectively extracted in cold water. according to Al-Otaibi, 2004.

Some compounds, such as anthocyanins in clove, are stable and highly extractable in cold water, contributing to the higher effectiveness of clove's cold water extract, according to Khoo *et al*., 2017.

Hence, the synergy between polyphenols, flavonoids, and saponins in Miswak's hot water extract may enhance its antimicrobial properties beyond the individual effects of these compounds (Sofrata *et al.*, 2008).

Likewise, Clove's combination of polyphenols, tannins, quinones, anthraquinones, and anthocyanins in its cold water extract may create a powerful synergistic antimicrobial effect that outperforms Miswak, according to Chaieb *et al. (*2007).

Conclusion

Conclusion

The search for new antimicrobial agents is an often-discussed subject in the academic community and remains a prominent area of ongoing scientific investigation. This is due to the increasing number of microbial strains that are resistant to antibiotics and antifungals. This research looked at how well different extracts from *Salvadora persica* (Miswak) and *Syzygium aromaticum* (clove) kill bacteria. These are two plants that are commonly used in traditional medicine in our country, especially for keeping teeth clean.

This study used distilled water, methanol, and ethanol to extract the stems of Salvadora persica and the clove of *Syzygium aromaticum*. Distilled water proved to be the most efficient extractor solvent for *Salvadora persica,* yielding a higher extract quantity than the other solvents, and for *S. aromaticum,* the best extractor was ethanol, which has a larger yield compared to other solvents.

Furthermore, *Salvadora persica* and *Syzygium aromaticum* extracts demonstrate significant antibacterial and antifungal activities against the tested bacteria and fungi species. Hence, Salvadora persica demonstrated particular effectiveness against pathogens like Staphylococcus aureus, commonly associated with dental plaque and cavities. Furthermore, Syzygium aromaticum extracts had strong antibacterial properties, making them useful in fighting oral bacteria.

Likewise, the hydro-alcoholic extracts were more effective against the various bacterial and fungal strains compared to aqueous extracts. The results indicated that Syzygium aromaticum extracts outperformed Salvadora persica extracts, with the aqueous extract (maceration) demonstrating the highest activity against *B. cereus.* Furtermore, *S. persica* extractsshowed the highest activity against *S. aureus,* which were more effective than *S. aromaticum* alcoholic extracts.

This study was a preliminary search for new natural antimicrobial agents, and thus, the results are considered initial findings.

From our perspective, we suggest:

- Investigate the chemical makeup and the existence of antimicrobial compounds in Persica and S. aromaticum. Likewise, it is important to do a full investigation that includes fractionation studies using a range of polar and non-polar solvents.

- We aim to investigate the function of these extracts in combination treatments with traditional antibiotics, evaluating their capacity to boost antibacterial effectiveness and curb the emergence of resistance.

- We aim to examine the impact of S. aromaticum and S. persica on the oral flora of individuals afflicted with buccal diseases.

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Appendix

Appendix1:

Culture medium(Compositionin g/1 of distilled water):

- Nutrientagar :

GelatinPeptone 5g

Beefextract..... 3g

Bactenologicalagar 15g

Distilledwater 1000ml

Ph..... 6.8

- Mueller-Hinton:

Meatextract..... 2g

CaseinAcidHydrolyzate..... 17.5g

Starch..... 1.5g

Agar 10g

Distilledwater 1000ml

Ph..... 7.4

- Sabouraud

Neopeptone..... 10g

Glucose 20g

Agar 20g

Distilledwater 1000ml

Ph……..5-5to6

- PDA (Potato

Dextrose Agar Potato 200g

Glucose 20g

Agar 20g

Distilledwaters..... 1000ml

PH 6.5

The solution

- Physiological Water

NaCl…….….. 9g

Distilled water 1000ml

PH… 7.4