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Evaluation the effect of plant extracts for *Artemisia herba alba* and T*hymus capitatus* in Zintan city on some types of bacteria resistant to antibiotics

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(Evaluation the Effect of Plant Extracts for *Artemisia Herba Alba and Thymus Capitatus* in Zintan City on Some Types of Bacteria Resistant to Antibiotics.)

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Declaration

I hereby declare that I am the sole author of this research entitled **"Evaluation the effect of plant extracts for *Artemisia herba alba* and T*hymus capitatus* in Zintan city on some types of bacteria resistant to antibiotics"**, and that no part of it has been plagiarized. I also declare that all the material submitted in this work, which is not my own, has been properly cited and referenced, and that no material is included which has been submitted for any other qualifications of other subjects or courses.

Date: 21/10/2025.

Signature: .....

Dedication

To those who continue to inspire me with positive energy, patience, and perseverance in all aspects of life

My caring mother and beloved father.

To my dear daughter (**Doaa**) who is the soul of my life.

To those who have filled my life with their kindness and love

My brothers and sisters.

To those who have provided me with continuous support

My dear relatives and friends

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List of Abbreviation

Explanation	Symbols
Artemisia herba alba	*A. herba alba*
Thymus capitatus	*T. capitatus*
Staphylococcus aureus	*S. aureus*
Staphylococcus epidermidis	*S. epidermidis*
Klebsiella pneumoniae	*K. pneumoniae*
Klebsiella oxytoca	*K. oxytoca*
Percent	%
Degrees Celsius	℃
Gram	G
Microliter	µl
Millimeter	mm
Nanometer	Nm
Kilometer	Km
Gas Chromatography- Mass Spectrometry	GC-MS
Polymerase Chain Reaction	PCR
Methicillin resistant *S. aureus*	MRSA
Base pair	Bp
potential of hydrogen	PH
Inhibition zone diameters	IZD
Cystine lactose electrolyte deficient agar	CLED agar
Mannitol salt agar	MSA
Muller hinton agar	MHA

Abstract

This study was carried out to evaluate the antibacterial activity of aqueous, alcoholic and essential oil from the leaves of *Artemisia herba alba* and *Thymus capitatus*. Was extracted with water, methanol and essential oil (hydro-distillation method). The antibacterial activity of these extracts was evaluated against bacteria *Staphylococcus aureus* (MRSA) *Staphylococcus epidermidis, Klebsiella pneumoniae, Klebsiella oxytoca,* using disc diffusion method. Concentrations of (100, 200, 300 mg/ml) were applied for the aqueous and alcoholic extracts, while the essential oil was tested in its pure form. The most effective antibacterial activity was observed for the essential oil extract and *Thymus* which was more inhibitory than *Artemisia*. The best effect was observed with *T. capitatus* essential oil, and its most effective inhibition was for *S. epidermidis* with inhibition zone (28.33±1.52 mm), *K. pneumoniae* (23.66±1.52 mm), *S. aureus* (MRSA) (23.33±1.52 mm), and *K. oxytoca* (20.66±0.57 mm). The chemical components of the essential oils were determined by GC-MS. The main compounds of *T. capitatus* were Carvacrol methyl ether (53.208%), Carvacrol (13.593%), and. The main compounds of *A. herba alba* were Camphor (30.527%). Thujone (22.471%). While the inhibitory effect of aqueous and alcoholic extracts of the *T. capitatus* plant was only on *S. epidermidis* bacteria, the effect of the aqueous extract was at a concentration of (300 mg/ml) with a diameter of inhibition (9.33±1.00 mm), while the alcoholic extract had the best effect at the same concentration with a diameter of inhibition (13.66±2.88 mm). As for the plant *A. herba alba* was the effect of its aqueous extract on *S. aureus* (MRSA) with (8.66±1.52 mm) and *S. epidermidis* with an inhibition diameter of (8.33±0.57 mm) at (300 mg/ml) concentration, the inhibitory effect of the alcoholic extract with a diameter of (10.00±0.00 mm) for *S. aureus* (MRSA) and (13.66±1.15 mm) for *S. epidermidis* at (300 mg/ml). Phytochemical of *Artemisia* and *Thymus* extracts (both aqueous and alcoholic) revealed the presence of phenols, and saponin. These findings indicate that the essential oil extract may serve as a promising source of antibacterial agents.

Keywords: *A. herba alba, K. pneumoniae, K. oxytoca, S. aureus, S. epidermidis, T. capitatus*.

Chapter 1
Introduction

Introduction

1-1 Antibiotics

Antibiotic resistance is currently the most serious global threat to the effective treatment of bacterial infections. Antibiotic resistance has been shown to negatively impact both clinical and therapeutic outcomes, with consequences ranging from treatment failure and the need for expensive, safer alternative drugs to the cost of increased morbidity and mortality, longer hospitalizations, and higher healthcare costs. The search for new antibiotics and other antimicrobials remains a pressing need in humanity's battle against bacterial infections (Chinemerem Nwobodo et al., 2022).

Antibiotics are compounds that inhibit or kill bacteria through a specific interaction with a specific target in the bacterial cell. Since antibiotics were widely introduced in the late 1940s to treat human bacterial infectious diseases, there has been a steady selection and increase in the frequency of antibiotic-resistant bacteria (Andersson & Hughes, 2017). The development of resistance is a complex process driven by the interaction of a number of biotic and abiotic factors. The main factors underlying this dynamic are the rates of emergence and persistence of resistant bacterial clones, the temporal and spatial gradients of antibiotics and other foreign substances, and the rates of transmission of infection within human populations and between humans and various other sources including animals, the environment, food, etc (Carlet et al., 2012).

Antimicrobial resistance in bacterial pathogens is a global challenge associated with high rates of morbidity and mortality (Akova, 2016). Sources indicated the appearance of the first cases of bacterial resistance to sulfonamides in 1937. In 1940, bacterial resistance to penicillin was recorded in laboratory experiments, while the first clinical case of penicillin resistance was reported in 1942. (Muteeb et al., 2023), the World Health Organization has listed antimicrobial resistance as one of the top ten public challenges, and called for urgent multilateral action (Narayana et al., 2024).

A standard international term to describe acquired resistance in bacteria has been developed by international experts from the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC). Antimicrobial resistance is divided into three categories: multidrug-resistant (MDR),

which has at least one resistant agent in three or more antibiotic classes; extensively drug-resistant (XDR), is to be an agent resistant to all antimicrobial agents except two classes or less; and pan-drug-resistant (PDR), which has resistance to all commercially available agents in all antimicrobial classes (Magiorakos et al., 2012).

It is estimated that 4.95 million antimicrobial resistance-related deaths in 2019, according to the Global Burden of Disease, as a result of 369 diseases and injuries in 204 countries, including 1.27 million deaths attributed to bacterial antimicrobial resistance (Murray et al., 2022) and by 2050, it is expected to kill 10 million people a year (Nehme et al., 2025).

Multiple drug resistance patterns in Gram-positive and Gram-negative bacteria have led to infections that are difficult to treat or even not amenable to treatment with conventional antibiotics, and the inability to make a good diagnosis of the cause leads to the use of broad-spectrum antibiotics in a large and often unnecessary way, so there is a significant increase in emerging resistance, as well as excessive use of antibiotics accelerates this process, and failure to complete the treatment period, which gives bacteria the development of resistance against these drugs (Frieri et al., 2017). The World Health Organization announced what are known as pathogens (ESKAPE) on the basis of their clinical significance and levels of resistance **E**: *Enterococcus faecium*, **S**: *Staphylococcus aureus*, **K**: *Klebsiella pneumoniae*, **C**: *Clostridioides difficile*, **A**: *Acinetobacter baumannii*, **P**: *Pseudomonas aeruginosa*, and **E**: *Enterobacteriaceae* (Muteeb et al., 2023).

The most common and dangerous antibiotic-resistant listed by the World Health Organization are methicillin-resistant *Staphylococcus aureus* (MRSA), which may cause nosocomial infections or have individual complications, carbapenem-resistant Enterobacteriaceae (CRE), and various strains of antibiotic-resistant Mycobacterium tuberculosis (Narayana et al., 2024).

1-2 Mechanism of bacterial resistance to antibiotics

1-2-1 Change in permeability barrier

Bacteria defend themselves against toxic compounds, including antibiotics and biocides, by regulating the permeability of their outer membrane. This is achieved by

altering the chemical composition of the membrane or decreasing the number of porins, which limits antibiotic entry through membrane transport systems, thereby contributing to antibiotic resistance.

1-2-2 Efflux pump

Certain bacteria possess efflux pumps, which are transport proteins capable of expelling harmful substances, including antibiotics, from the bacterial cell. This mechanism plays a significant role in contributing to antibiotic resistance. (Reygaert, 2018).

1-2-3 Change in target location

Each antibiotic targets a specific component within the bacterial cell, such as a protein, DNA, or the cell wall, to inhibit or kill the bacteria. Resistance arises through genetic mutations in the target site, which alter the binding affinity of antibiotics. This can occur by modifying protein binding sites or the bacterial ribosome, preventing antibiotic attachment and leading to resistance. For example, penicillin-binding proteins (PBPs), which are enzymatic components involved in peptidoglycan biosynthesis, serve as targets for β-lactam antibiotics. Any alteration in the structure or location of these proteins can impair the antibiotic's activity and result in bacterial resistance. (Foudraine et al., 2021).

1-2-4 Production of antibiotic-inhibiting enzymes

Bacteria can produce enzymes that directly inactivate antibiotics by either modifying the target or degrading the antibiotic molecule. In Gram-negative bacilli, the *Klebsiella pneumoniae* carbapenemase (KPC) enzyme confers broad-spectrum resistance to antibiotics including carbapenems, aminoglycosides, and penicillins (Munoz-Price et al., 2010). Similarly, Gram-positive bacteria such as penicillin G-resistant Staphylococci produce β-lactamase enzymes that inactivate antibiotics by hydrolyzing the β-lactam ring of the penicillin molecule (Foudraine et al., 2021).

To processing the increase in microbial resistance to current antibiotics, it is necessary to discover new and effective antimicrobial compounds or extracts, as medicinal and aromatic plants receive great attention in many producing countries, as they are one of

the most important sources of active substances that enter into the preparation of medicines in the form of extracts or used as raw material (Eloff, 2019).

1-3 Medicinal plants

Medicinal plants are defined as plants that possess therapeutic properties, in one or more of its various organs one or more active chemical substances are presentin low or high concentrations, and have the physiological ability to treat a specific disease or reduce the symptoms of infection with this disease by using the plant in its natural or dried form or by using the active substances extracted from it (Jamshidi-Kia et al., 2018).

According to the World Health Organization (WHO), which formulated this definition, this description distinguishes medicinal plants as those whose therapeutic properties and components have been scientifically identified (Sofowora, 2010).

Medicinal plants still meet the urgent need for treatment for many people despite the development of the modern health system. Scientists have estimated the number of medicinal plants on the surface of the earth at about 72,000 to 77,000 species (Shakil et al., 2024) which is approximately 17-18% of the plants on earth, and it is estimated that 80% of people worldwide use traditional herbal medicines (Davis & Choisy, 2024). They are the first line of defense against diseases for tens of millions of people (Pirintsos et al., 2022). They have healing properties when used in a specific dose and in a specific way (Salhi et al., 2010). This is due to their availability, ease of access, low cost, and avoidance of the negative effects resulting from the use of chemical drugs. Most medicinal plants are non-toxic, but some of them are highly toxic to both humans and animals (Okoye et al., 2014).

1-3-1 Source of medicinal plants

The source of medicinal plants is mostly wild plants as defined by the Food and Agriculture Organization (FAO). They are plants that grow spontaneously in groups that maintain themselves in ecosystems and can emerge independently away from human hands. They are edible wild plants as defined. They are characterized by being locally available, traditionally known across generations, less expensive, and have high advantages for poor populations. They are available during difficult conditions

such as periods of drought or famine and have the ability to resist climate change. They also play an important role in providing many of the important nutritional requirements for improving health and thus reducing food insecurity, scarcity, and famine (Hammoudi et al., 2022). Some are found in valleys, plains, mountains and forests, while others can be obtained through agriculture, where pharmaceutical companies and investment institutions establish special farms to produce specific varieties or types that the local or international market needs in specific quantities (Mofokeng et al., 2022). Of the 3,000 medicinal plants traded internationally, 900 are cultivated, and most of the exported biomass is harvested from the wild. Habitat loss, overharvesting, global warming, and climate change are all impacting medicinal plant populations, threatening the future existence of 15,000 species (Rao & Rajput, 2010).

1-3-2 Classification of medicinal plants

Medicinal plants are classified according to the part used, which contains the active ingredient, into the whole plant, such as datura, or just the leaves, such as aloe vera, fruits, such as chili peppers, or seeds, such as nigella sativa, or a plant whose lower parts are used, such as licorice. Or it is classified on the basis of physiology or treatment as laxative, analgesic, narcotic or stimulant (Khameneh et al., 2019).

1-3-3 Harvest time

Medicinal plants are harvested at a specific stage of their life cycle, in which the content of active substances is at the maximum, this may vary depending on the plant, but it is generally recommended to collect the aerial parts of plants during the flowering period, while the roots are during the dormant period of the plant, and the age of the plant significantly affects the harvest time (Hazrati et al., 2024). Also it does not depend on the seasons of the year only, but it may require a precise time of day, the leaves of some plants, for example, should be collected in the afternoon because it has been proven that they contain the highest percentage of active substances at this time (Silva et al., 2016).

1-3-4 The use of medicinal plants

Medicinal plants are used in two forms: raw form, as infusions, essential oils, or dye extracts, pure form in which the active ingredient (active substance) responsible

for the therapeutic effect is identified and chemically defined. Pure compounds are generally used when the active ingredients have a strong and specific effect (Kunle et al., 2012).

There are many fields for medicinal and aromatic plants, some of which are used in the manufacture of medicines as painkillers and antiseptics. Some plants contain fixed oils that are used in the composition of some medical preparations, and some of them are used in the preparation of cosmetics. Some of them are also used in the manufacture of insecticides, which depend on the toxins found in medicinal and aromatic plants that kill insects or fungi. They are also used as spices, food additives, or food fragrances (Bekro et al., 2007).

The most common juice preparation was 50% of medicinal plants for its simplicity and rapid absorption, 17% pastes were used mainly for the treatment of skin diseases, and 7% extracts (Shakil et al., 2024).

1-3-5 Extraction methods for medicinal plants

The extraction of biologically active compounds depends on the extraction solvent used and the extraction temperature. The criteria for selecting solvents include environmental impact, polarity, safety, selectivity, toxicity, etc. The advantages and limitations of solvents are compared according to their safety as well as their main physical and technological properties. The effectiveness of solvents depends on the effectiveness of the active compounds of the resulting extraction process, which have strong antioxidant activities and appears antimicrobial, antidiarrheal, antihelminthic, antiallergic, antispasmodic, and antiviral activities. Solvents are divided into green solvents (water, ethanol, glycerin, acetic acid) and other solvents (acetone, chloroform, methanol, hexane, benzene) (Shikov et al., 2022).

Traditional extraction methods are maceration, saxolite and hydrodistillation (Azmir et al., 2013). Currently, Innovative extraction techniques include Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), Enzyme-Assisted Extraction (EAE), Superfluid Extraction (SFE) and Pulsed Electric Field extraction (PEF). Devices for identifying and determining the chemical components of plant extracts and identifying their active compounds include: High-Performance Liquid Chromatography (HPLC). Gas Chromatography- Mass Spectrometry (GC-MS).

Liquid Chromatography- Mass Spectrometry (LC-MS). Nuclear Magnetic Resonance (NMR) (Pirintsos et al., 2022).

1-3-6 The biological importance of medicinal plants

Plants contain many biologically active compounds and plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years. 36% of medicines are research synthetic, while more than 50% are derived from nature (Newman & Cragg, 2016). Most of these are plant-based. These natural products show great potential for processing antibiotic resistance, especially in bacterial pathogens (Abass et al., 2024**).**

For example, artemisinin, a substance found in wormwood, is considered an anti-cancer and anti-malarial agent. The World Health Organization officially prescribes oral combination therapies based on artemisinin for malaria**.** The quercetin found in apples is a flavonoid compound with antioxidant and anti-inflammatory properties that promote cardiovascular health. The indigenous people of South America have used the bark of the cinchona tree to treat fever resulting from malaria since ancient times in the seventeenth century (Chaachouay & Zidane, 2024).

Aspirin is a widely used analgesic in the world, which was first extracted from (*Salix alba*) known as the willow tree, and underwent a synthesis process, which led to its transformation into its industrial form (Ugurlucan et al., 2012).

Many secondary derivatives, such as alkaloids, are nitrogen-containing compounds such as morphine derived from the poppy plant, quinine from the cinchona tree, and caffeine, which originates from coffee beans. These compounds have been used for their analgesic, antimalarial, and stimulant properties for centuries in traditional medicine (Chaachouay & Zidane, 2024).

The anticancer drugs (vinblastine, vincristine) are manufactured from *Catharanthus roseus* L and *Taxus brevifolia* (Pirintsos et al., 2022), while about 67% of anticancer drugs are of plant and animal origin (El-Saadony et al., 2025).

Aloin, which is found in aloe vera gel, prevents skin infections and helps moisturize the skin. Galantamine which is used to treat dementia, is extracted from snowdrops (*Galanthus* spp) (Hasan & Abdullah, 2022).

Despite its low production, it is more diverse, as its number exceeds 200,000 known secondary compounds, the most important of which are phenolic compounds, alkaloids, terpenes, and volatile oils (Monagas et al., 2022).

1-3-7 Secondary metabolites

Secondary metabolites are products of primary metabolism of the plant and are primarily used for defense and adaptation to the surrounding environment of the plant. They also contribute to the color, fragrances and specific flavors of the plant. They are stored in vacuoles within the cells or stored in the plant. Secondary metabolites are associated with environmental conditions which have an impact on the physiological and morphological characteristics of the plant (Reshi et al., 2023).

Medicinal plants are a source for the production of these biologically active compounds of pharmacological importance for the production of drugs that have therapeutic efficacy, as they are used as antioxidants, antibacterials and antivirals (Jeyasri et al., 2023).

They are classified into different groups based on functional groups and chemical structure, including phenols, alkaloids, flavonoids, saponins, tannins, terpenes, and volatile oils (Reshi et al., 2023).

1-3-7-1 Phenolic compounds

They are organic compounds that contain one or more benzene rings in their general structure linked to one or more hydroxyl groups. The structure of natural phenolic compounds varies from simple molecules to complex molecules known as polyphenols. Phenolic compounds are among the most widespread compounds in the plant kingdom, as more than 8,000 phenolic compounds have been identified (Zhang et al., 2022). Phenolics are the second largest group of secondary metabolites in plants, accounting for 45%, they are biologically active compounds (Nwozo et al., 2023). They have diverse biological activities, including anti-inflammatory, anti-

allergic, anti-cancer, anti-viral, antioxidant, and antibacterial activities (Burragoni & Jeon, 2021). Examples of phenols include Thymole, Carvacrol, and Caffeic acid, which are found in thyme and have toxic properties for microorganisms. Phenolic compounds found in Verbascum have also shown significant activity against Gram-positive bacteria, which play a significant role in inhibiting them (Donn et al., 2023).

1-3-7-2 Flavonoids compounds

They are polyphenols, phenolic compounds consisting of two benzene rings connected by a pyran ring. These compounds are the most common in plants, and they are responsible for water-soluble pigments that color many fruits and vegetables in attractive colors, ranging from yellow, red, purple, to blue (Iwashina, 2015). More than 9,000 flavonoid compounds have been identified and classified into seven subgroups based on their basic structure: flavones, flavanones, isoflavones, flavonols or catechins, and anthocyanins (Zhuang et al., 2023). Their biological activities include antioxidant, antibacterial, antifungal, antiviral, and antidiabetic activities (Salehi et al., 2019). For example, (Luteolin and Apigenin), found in *Artemisia annua*, have antioxidant and anti-inflammatory effects (Crasci et al., 2018).

1-3-7-3 Alkaloids compounds

Alkaloids are mainly derived from amino acids, resulting in a variety of chemical structures, constituting 20% of known secondary receptors (Heinrich et al., 2021). Alkaloids are found in plants in leaves, roots, seeds, fruits, and bark, or in the entire plant. They may be found in a free state or in the form of salts of some plant acids, such as citric acid and malic acid (De Luca & St Pierre, 2000). A plant may contain more than 100 different alkaloids, but their concentration does not exceed 10% of the dry weight of the plant (Muniz, 2006). Alkaloids are mostly toxic and biologically active compounds and are known for their diverse effects, such as analgesics, antihypertensives, antitumor, and antiparasitic. They are particularly known as anesthetics and anti-inflammatory agents, which include morphine, quinine, ephedrine, and nicotine. To date, approximately 12,000 alkaloids have been isolated from different genera of the plant kingdom (Dey et al., 2020). During 2020, 27,683 new alkaloids were included in the dictionary of natural products (Heinrich et al., 2021). There are three main types of alkaloids: true alkaloids, protoalkaloids, and

pseudoalkaloids. True alkaloids and protoalkaloids are produced from amino acids, while pseudoalkaloids are not derived from these compounds (Dey et al., 2020).

1-3-7-4 Saponins compounds

They are chemical compounds found in many plants, consisting of glycosides, and are characterized by producing a soapy foam when shaken with water. They are classified into alkaline soaps, steroidal soaps, and triterpene soaps (El Aziz et al., 2019). Its biological effect is a toxic physiological activity on humans and animals, as it dissolves red blood cells (Taiz & Zeiger, 2002). Some of them also have an expectorant and mucolytic effect, such as the Ivy leaves plant, which contains triterpene saponins (Sierocinski et al., 2021). Also, the *Saponaria officinalis* plant contains saponin compounds and is used to remove cholesterol and as an antidiabetic (Afzal et al., 2023).

1-3-7-5 Tannins compounds

It is a group of compounds with a complex chemical structure found in the plant and called astringent substances, which are amorphous, soluble in water, alcohol and acetone and do not dissolve in ether, benzene and chloroform, and is a mixture of polyphenolic substances that are associated with proteins or deposited on them have a large partial weight (de Frutos Fernández et al., 2004), found in all types of plants are divided into three types of decomposed tannins, condensed tannins and complex tannins, studies show that they contribute to human health through anti-inflammatory, immunoregulatory, hypoglycemic, as well as antioxidant, antiviral and antimicrobial effect (Cosme et al., 2025) as well as having antiparasitic activity at different developmental stages (Tong et al., 2022).

1-3-7-6 Terpenes compounds

Hydrocarbon compounds produced in large quantities in the plant its structural unit is the isoprene unit, which classifies turbines according to the number of isoprene units involved in the formation of the compound, the most important of which are monoterpenes. Sesquiterpenes for example α-pinene, β-pinene has a biologically active property against fungi and bacteria that is found in the *cannabis* plant (Rivas da Silva et al., 2012). And the artemisinin compound that follows sesquiterpenes, which

is found in the *Artemisia* plant and is effective in the treatment of malaria (Byakika-Kibwika et al., 2017), and the biological effect of terpenes they are used as antibiotics, anti-inflammatory, anti-some types of cancers, as well as they act as growth hormones in plants, including volatile oils, perfumes, flavorings and fat-soluble plant dyes (Wink & Schimmer, 2010) for example, Taxol diterpene was extracted from *Taxus brevifolia* anti-malignant tumors (Guerriero et al., 2018).

1-3-7-7 Essential oil

They are organic chemical compounds from the family of terpenes manufactured and stored in special formulations found in more than 3000 plants found in different parts of plants may be concentrated in leaves such as mint plant, in the stem such as cinnamon plant, flowers such as jasmine plant or in fruits and can be obtained by more than 60 families of plants (Cimino et al., 2021). Such as plants belonging to the Apiaceae family or in the peel of lemon and orange fruits, and are called essential oils or volatile oils, which are oily substances with distinctive aromatic odors and evaporate at normal temperatures without decomposing. Volatile oils are formed by the plant during the process of metabolism as a by-product of it and are collected in special vascular structures such as glandular hairs as in plants of the Lamiaceae family or in oil glands as in plants of the Rutaceae family or in oil ducts as in plants of the Apiaceae family. The percentage of volatile oils varies from one plant to another, as it may reach 16-18% or decrease to 0.02% (Dubai & Kholaidi, 2005). They may contain about 20-60 compounds, usually in quite different concentrations, two or three of which are the main components in high concentrations (20-70%) compared to other components present in trace amounts (Cimino et al., 2021). Essential oils may contain more than 300 different compounds, have a low molecular weight (Dhifi et al., 2016), are soluble in most organic solvents and insoluble in water (de Sousa et al., 2023).

Volatile oils have biological importance as they play an important role in protecting plants from harmful insects or killing them, thus preventing the destruction of flowers and leaves. They also attract beneficial insects to help them pollinate flowers, protect plants from bacteria and parasites that cause plant diseases, and are also used in the manufacture of cosmetics and perfumes. They are used in the manufacture of

medicines and are also used as disinfectants and antifungals, antiparasitics and bacteria, and as a tonic for the central nervous system (Cimino et al., 2021).

1-4 *Artemisia*

1-4-1 Botanical description

Artemisia belongs to the Asteraceae family, which includes 1,600 genera and more than 25,000 species (Nikolić & Stevović, 2015). It is a perennial wild shrub with large branches up to 40 cm tall, compound leaves, and hard erect roots. The primary leaf stalks are ovate-spherical, bipinnate, with an elongated, bipinnate leaf stalk with simple branching. The sessile terminals are decorated with 2–4 flowers each (Abou El-Hamd et al., 2010). The roots appear as a thick, woody taproot, distinct from the secondary roots, and sink into the ground like the taproot. The taproot penetrates to a depth of 40–50 cm and only branches at this depth (Hidar et al., 2024).

It is distributed worldwide, as it is found in North Africa, the Middle East, Europe, southwest Asia, and northeastern America (Watson et al., 2002). It is a plant known for thousands of years. It was described by the Greek historian Xenophon at the beginning of the fourth century BC. It was described and included in the list of medicinal plants in 1779 by the Spanish botanist Ignacio Jordan del Rio (Jiao et al., 2023). Its common name is Wormwood, its Arabic name is Chih, and its scientific name is *Artemisia*. This genus includes about 500 speciesaround the world, including *A. herba alba, A. afra, A. annua, A. judaica,* and *A. campestris* (Watson et al., 2002).

Artemisia is considered stressful for agricultural land because it greedily absorbs elements due to its rapid growth (Li et al., 2022), and the amount of oil reaches a maximum during the flowering period and then decreases after that, so the grass should be cut or mowed during the flowering season (Mohammadi et al., 2015).

Most *Artemisia* contains Artemisinin, which is the main component in the plant, and its quantity varies depending on the type of *Artemisia,* characterized by *A. annua*, which contains Artemisinin in large quantities, has been proven effective against malaria and has been used for thousands of years, Artemisinin has also shown toxicity to tested cancer cells such as leukemia, colorectal cancer, skin cancer, ovarian cancer and prostate cancer (Zeng et al., 2023).

Artemisinin is concentrated in leaves and flowering tops where an extremely high concentration of this substance 4.85-4.90% (w/w) was reported for the study conducted in the desert of Egypt (Nibras & Hasan, 2019).

1-4-2 *Artemisia herba alba*

Botanical classification for *Artemisia herba alba* (Nia, 2018).

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: *Artemisia*

Species: *herba alba*

Figure (1-1) *A. herba alba* plant.

It is a perennial wild shrub with woody, branched stems, 30-50 cm tall. Its leaves are small, with a silvery-green appearance (Rahouma et al., 2025). Its flowers are

clustered in small, oval-shaped clusters of 2–5 flowers, all of which are hermaphrodites (Zohra, 2016). It is able to adapt well to dry and harsh climatic conditions, as it has the ability to utilize soil moisture to a depth of 20 cm. Flowering usually begins in June, but the flowers develop in late summer (Matteucci & Giampietro, 2008). It is called desert wormwood or white wormwood (Tilaoui et al., 2011), and is primarily a fodder plant for livestock, characterized by a strong aromatic smell and a bitter taste (Baranová et al., 2025).

White wormwood is used by the people of North Africa to treat diabetes and high blood pressure. Chemical analysis has found that it is rich in large quantities of minerals, especially potassium and calcium, which are the predominant elements in the medicinal plant. There are also other elements such as iron, zinc, chromium, sodium, and cobalt. (Nedjimi & Beladel, 2015). In addition to containing oils, the essential oil in the date plant represents 0.33% of the plant and is rich in monoterpenes and sasquiterpenes. The most important substances are *Artemisia* Ketone 68%, Cineole 51.5%, Camphor 48%, in addition to phenolic compounds, flavonoids, tannins, saponins and alkaloids (Hassan & Hadi, 2025).

1-4-3 Biological importance of *A. herba alba*

White wormwood contains artemisinin, which is effective against malaria and has been used since ancient times in Africa. In Tunisian folk medicine, the plant is used to treat bronchitis, diabetes, diarrhea, high blood pressure, and neuralgia. In Jordan, wormwood leaves are used as an antidiabetic, antispasmodic, and anti-arthritic (Hasan et al., 2022). Snake and scorpion venoms were evaluated in Jordan to inhibit the hemolytic activity of red blood cells, where *Artemisia* extract gave 100% inhibition (Abou El-Hamd et al., 2010).

In Morocco, it is used as a treatment for high blood pressure and is also taken as a tea 2-3 times a week to treat digestive system diseases (Hamamouch, 2020). A decoction of the leaves and flowers of the plant is also used as a gargle to treat bad breath, gingivitis, toothache, bleeding gums, mouth ulcers, and oral herpes in the Sahara region of Morocco (Najem et al., 2020). In Algeria, it is used as a powder to treat diabetes, parasitic worm infections, colitis, nausea, abdominal pain, breast pain, antispasmodic, and carminative (Sarri et al., 2014). It is used in Pakistan to treat fever

and neurological problems (Zeb et al., 2019), and is considered an antioxidant and leishmaniasis killer (Akrout et al., 2009). Fresh *Artemisia* leavesare used in North Africa against the fungus *Mucor roxi*, which causes mucormycosis (Sipsas et al., 2018). The aqueous extract of the aerial parts of *Artemisia* have shown activity against the following bacteria: *Escherichia coli, Serratia mercescens, Enterobacter cloacae, Bacillus subtillis, Proteus vulgaris, staphylococcus aureus* (Hasan et al., 2022). The effectiveness of white wormwood against MRSA bacteria was shown for the methanolic extract with an inhibition diameter of 24 mm (Alghonmeen et al., 2024). In Jordan, methanolic extracts of *Artemisia*, which were tested against 32 isolates of *Mycoplasma* species, were found to be highly effective with values of 6.25-3.125 mg/ml. This plant can be considered as a readily available alternative to drugs that treat *Mycoplasma*. The aqueous extract and essential oil of *Artemisia* were also tested, with the result for the essential oil being 2 mg/ml, while the aqueous extract showed anti-leishmanial activity at a concentration of 4 mg/ml (Abou El-Hamd et al., 2010).

Erel et al. (2012) showed that several types of *Artemisia* in Turkey were tested, from which methanolic extracts and essential oils were prepared for testing on several types of bacteria, *S. aureus, Salmonella typhimurium, P. aeruginosa, Enterococcus faecalis, E. coli, E. cloacae.* The result of this study was that the essential oils effect was greater than the methanolic extracts, and the inhibition diameters were between 7 and 24 mm.

A study by Bertella et al. (2018) also indicated the effectiveness of *A. herba alba* essential oilscollected in an area in Algeria when tested on 21 types of Gram-positive and Gram-negative bacteria, with inhibition diameters ranging from 8 to 31 mm.

A study Amor et al. (2019) reported the antibacterial activity of *Artemisia* essential oils collected from a region in Morocco, prepared by hydrodistillation and their components were analyzed using GC-MS technology, which were rich in Cis-thujone, trans-thyjone, Vanilyl alcohol and tested on 20 types of Gram-negative and Gram-positive bacteria, and the effective effect was *on Bacillus clausii* 2226, *S. typhimurium* with inhibitory diameters from 17 to 24 mm.

A study Bouatrous & Mechaala (2020) in Tunisia indicated the possibility of using *Artemisia* essential oil as a milk preservative. Aqueous and alcoholic extracts and essential oils were studied on cow's milk and showed antibacterial activity against *E. coli, S. aureus,* and *Salmonella* sp. with inhibitory diameters ranging from 13 to 20 mm. In addition Mashraqi et al. (2023) revealed that the *A. herba alba* was collected from an area in Egypt and extracted using different organic solvents in addition to the aqueous extract and tested the ability of these extracts on *S. aureus, Listeria monocytogenes, P. aeruginosa, Salmonella enterica* where the most effective results were for butanol extract, chloroform extract.

Mohammed & Alhusseini (2025) mentioned that extracts of *A. herba alba* had antibacterial effect and toxic activity to cancer cells in Iraq, in which the alcoholic extracts had a greater effect than the aqueous extract on the tested bacteria, *P. aeruginosa, E. coli, S. aureus, K. pneumoniae,* which had diameters between 23 to 27 mm. Also, Rahouma et al. (2025) from Libya found that antibacterial effect of *A. herba alba* using the well diffusion method for aqueous, ethanol, and ethyl acetate extracts, where the ethanol extract had the greatest effect on *E. coli* and *S. aureus* bacteria, which had diameters between 26 and 25 mm, respectively.

1-5 *Thymus*

1-5-1 Botanical description

It is a plant in the Lamiaceae family with about 230 genera and 7100 species worldwide, this family is distributed in arid and temperate cold regions, including Europe, North Africa and Asia, the genus Thymus contains 350 species spread around the world of its species *T. zygis*, *T. capitatus*, *T. vulgaris*, *T. serpyllumis* and *T. broussonetii,* called in Arabic Zahter or Za'tar, one of its most important by - products are essential oils, where it is among the top 10 essential oils in the world used as a preservative for food purposes, the most important of which is *Thymus capitatus*, there are many environmental patterns of Thyme, which differ in their morphological characteristics and in the composition of essential oils, although all of them are characterized by a moderate aroma and sometimes a very pronounced balsamic and spicy flavor (Ballester-Costa et al., 2013). In addition to the compounds flavonoids, phenylpropanoids, tannins, organic acids, terpenoids, and phytosterols is characterized

by the presence of phenols significantly, the most of which are Carfecrol and Thymol (Li et al., 2019).

1-5-2 *Thymus capitatus*

Botanical classification for *Thymus capitatus* (Hammoudi et al., 2022).

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: *Thymus*

Species: *capitatus*

Figure (1-2) *T. capitatus* plant.

A dwarf tree with a strong aromatic odor (Goudjil et al., 2020), it is widespread in the Mediterranean region and is known for its medicinal and preventive properties, which contains a set of minerals and vitamins (Tamma et al., 2024). Its leaves are used as a seasoning in salads or as a flavoring for cooked foods and is also used in the preparation of tea (Casiglia et al., 2019), it was previously used to control the growth of microbes in processed dairy products by adding thyme to it as a natural antimicrobial (Akarca et al., 2016). It has several names *Thymus capitatus*, *Thymbra capitata*, *Coridothymus capitatus* (*L*) and *Spanish oregano* (Nehme et al., 2025), is considered endemic in the southern parts of the Green Mountain in Libya has several different colors including white flowers, white dotted, purple flowers and purple flowers and pollination by insects (Ali & Mustafa, 2021), it is characterized by erect, serrated branches 20-40 cm high (Jaouadi et al., 2018). The leaves are often small in size, simple and with a full edge of greenish-gray or silver color, the flowers are small or large in size, about 5mm long, the most important by-products of the secondary metabolism are volatile oils, especially phenolic acids and flavonoids such as caffeic acid, carvacrol and thymol, which have an important biological importance depending on the location and growing season, the ratio of these compounds, where the difference in chemical composition is due to several factors, including climatic changes, environmental stresses, genetic background and growth phase all this leads to quantitative and qualitative change of compounds leading to the presence of different chemical patterns (Tagnaout et al., 2022).

1-5-3 Biological importance of *T. capitatus*

It is used to treat many diseases such as respiratory infections including asthma, pneumonia and influenza, digestive disorders, heart disease and diabetes. It has also been used as a sedative and analgesic, as well as an antispasmodic (Benoutman et al., 2022), to treat water retention, nausea, fatigue, menstrual problems and menopause, to strengthen memory and concentration, and to treat cases of tooth decay (Salehi et al., 2018). It is used in Tunisia in traditional medicine to treat intermittent fever and rheumatism, and as an antihypertensive and blood circulation stimulant (Jaouadi et al., 2018). It is used as antimicrobial, antioxidant, antifungal, antibacterial and antiviral, anticancer (Benoutman et al., 2022), a parasite killer whose essential oils are gaining increasing attention due to their relatively safe characterization and exploitation for

possible multipurpose functional uses to increase the shelf life of food as it has various biological effects, is used as a preservative for meat and fish food thanks to its antimicrobial, antifungal and antioxidant activity and to enhance organoleptic properties (Casiglia et al., 2019), and *Thymus* oil helps in healing wounds and in cases of dermatitis (Alves-Silva et al., 2023). It is also used as a surface disinfectant and pesticide and is also used in the perfume and cosmetics industry (Salehi et al., 2018).

A study Iauk et al. (2015) on the effectiveness of alcoholic and hexane extracts of *T. capitatus* in Italy in terms of their antibacterial effect showed that the extracts were evaluated on 17 types of Gram-positive and Gram-negative bacteria related to respiratory diseases. The effectiveness of the methanol extract was particularly evident on *Streptococcus pneumonia* and *Moraxella catarrhalis*.

A study Guvenir et al. (2017) indicated the effectiveness of *T. capitatus* essential oils collected from three different regions of Cyprus and its effect on *Helicobacter pylori* bacteria and gave good results compared to several antibiotics.

A study Ben Jemaa et al. (2018) showed the effect of *T. capitatus* as a preservative on the quality of raw and pasteurized milk. *T. capitatus* essential oil was prepared using a Clevenger device at 80°C and 1 mg/ml was added per liter. The measurement was repeated every 48 hours. The results indicated that adding *Thymus* essential oil was more effective against bacterial growth in pasteurized milk than in raw milk, thus increasing its shelf life.

Salman et al. (2022) reported that the aqueous extract was more effective than the alcoholic extract in inhibiting many bacteria at different concentrations used in the study, as it showed that meat treated with thyme extract had a decrease in bacterial growth compared to the control sample. Abdelfatah et al. (2019) also indicated the effectiveness of *T. capitatus* essential oils on the microbial load in meat during drying, especially *E. coli* bacteria.

A study Zahli et al. (2023) stated that the effectiveness of *T. capitatus* essential oils on 9 strains of *Salmonella* sp bacteria, 8 of which were isolated from food sources and one was a reference strain. The essential oil was also analyzed by GC-MS and the result was that it was rich in carvacrol by 73.52%. The result was an antibacterial effect on *Salmonella* with inhibition diameters from 13 to 43 mm.

Suliman & Alnass (2025) observed that the effect of the aqueous extract of *T. capitatus* on some types of bacteria *S. aureus*, *E. coli*, where its greatest effect was on *S. aureus* bacteria using the diffusion disc method and at different concentrations (100, 150, 200 mg/ml) and the inhibitory concentration was 200 mg/ml with a diameter of 22 mm.

A study by Jaradat et al. (2025) tested samples collected from different regions in Palestine (Ramallah, Hebron, Jenin) at different altitudes above sea level. *T. capitatus* essential oils were prepared by two methods: hydro-distillation and ultrasound. Each sample was analyzed by GC-MS and the results were compared, giving different percentages between regions. The main ones were Terpinene, Carvacril, and Thymol. *T. capitatus* essential oils was tested on bacteria *K. pneumoniae, S. aureus*, MRSA, *P. vulgaris, E. coli,* and *P. aeruginosa*, which gave good results for the Hebron sample against MRSA, due to the high Thymol content of 40.35%.

1-6 *Staphylococcus aureus*

Classification of the genus *Staphylococcus aureus* (Botelho et al., 2016).

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order**:** Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species. *aureus*

Staphylococci from the Staphylococcaceae family, which includes 47 species, are Gram-positive, non-motile, non-spore-forming and facultative anaerobic bacteria, organized in clusters resembling bunches of grapes (Myles & Datta, 2012), the temperature of 37 °C is the optimum for their growth and the optimum pH for their growth ranges between 6-7, the colonies have a smooth convex shiny round (0.5-1.5

μm) opaque (Rasigade & Vandenesch, 2014), produce yellow to orange pigments and some produce white to creamy pigments on the surface of blood agar (Deepa et al., 2015).

It resists high temperatures (44-56 ºC) and high salt concentrations (Rasigade & Vandenesch, 2014). It is positive for the catalase enzyme and negative for oxidase. It is coagulase enzyme positive for Staphylococci (CoPS), and it is a complete hemolytic (β-hemolysis) on blood agar. It is positive for the urease test and the phosphatase test and reduces nitrate (No_3) to nitrite (No_2). Manitol salt agar (MSA) medium containing phenol red indicator is considered a selective and differential medium for *S. aureus* (Rasheed & Hussein, 2021).

It is a bacterium widely distributed in the environment and the normal human microbial system, found on healthy skin and mucous membranes. About 30% of humans carry *S. aureus* in their noses, pharynx, respiratory tract, armpits, and thighs (Liu et al., 2024). The danger of this bacterium lies in the infections it causes. It is a major human pathogen responsible for many community- and hospital-acquired infections. It causes many diseases from minor wound infections to more severe diseases, including pneumonia, meningitis, endocarditis, conjunctivitis, septicemia, prosthetic limb infections, and all other types of infections associated with medical devices. In addition, its toxins may cause food poisoning. This bacteria is one of the most common causes of nosocomial infections in hospitals, as it is resistant to antibiotics, especially methicillin, which is known as (MRSA) methicillin resistant *S. aureus*, and thus it has become a major disease problem (Cheung et al., 2021).

1-7 *Staphylococcus epidermidis*

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species: *epidermidis* (Botelho et al., 2016).

It is a Gram-positive, non-motile, cluster-forming, facultatively anaerobic bacterium with colonies that appear round, smooth, raised, mucoid (Flayyih, 2015), white to shiny gray, 1-2 mm in diameter, non-spore-forming, catalase-positive, coagulase enzymenegative, and non-mannitol fermenting, which are differentiating characteristics between *S. aureus* and *S. epidermidis* (Skovdal et al., 2022).

It is naturally found in the human body on the skin and mucous membranes and is an opportunistic pathogen that can cause virulence once it invades the human body (Namvar et al., 2014). It is the leading cause of hospital infections and is second only to *S. aureus* in causing infections, especially infections of implanted medical devices such as intravenous catheters and dialysis machines, and inflammation of artificial joints, which constitute 43% (Post et al., 2017), and artificial heart valves, as well as through needle punctures or surgical wounds, especially in drug users (Al-Sa'ady, 2020), keratitis resulting from contact lenses, and it also causes bacteremia, especially in newborns, and infective endocarditis (Severn & Horswill, 2023). Its danger lies in its ability to form biofilms on the surface of implanted medical devices (Flayyih, 2015). This increases antibiotic resistance, which is a serious problem in healthcare, as the mortality rate resulting from its infection can reach 24% of deaths (Chabi & Momtaz, 2019).

1-8 *Klebsiella pneumoniae* & *Klebsiella oxytoca*

Classification of *klebsiella* (Ochońska & Brzychczy-Włoch, 2024).

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacterial

Order: Enterobacterials

Family: Enterobacteriaceae

Genus: *Klebsiella*

Species: *pneumoniae, oxytoca*

These bacteria belong to the Enterobacteriaceae family. They are rod-shaped, Gram-negative, non-motile, and range in size from 0.3-1 μm in width and 0.6-6 μm in length. They are arranged singly, in pairs, or in short chains. Their colonies are large, mucous, and pink on MacConkey agar (Rawy et al., 2020). Their growth temperature range is 12-43 °C, but 37 °C is optimal. They are non-spore-forming, facultative anaerobic, lactose fermenting, oxidase-negative, and catalase-positive. They are not hemolytic (Paczosa & Mecsas, 2016). First known as Friedlander's bacilli, they were first discovered from lung isolates (Mustafa et al., 2023). They are naturally found in the intestines of humans and animals, as well as in soil, on plants, and in water and sewage (Rawy et al., 2020).

It has a mucous appearance on culture media, and this is due to its capsule, as it has 79 types of capsules, all of which differ from each other in composition, the most important of which are K1, K2, which are associated with hypervirulence, namely *K. pneumoniae and K. oxytoca*. This genus is considered an opportunistic pathogen and is common in hospital infections (Mustafa et al., 2023).

According to the World Health Organization, *K. pneumoniae* is a major global problem (Nirwati et al., 2019). The Chinese Antibiotic Monitoring Network (CHINET) has observed an increase in the resistance of this bacterium to antibiotics,

especially carbapenems, reaching 32.8%. This resistance has very limited treatments, making multidrug-resistant (MDR) infections difficult to combat. *K. pneumoniae* is a global challenge and a direct threat to humans, causing pneumonia, infections in premature neonates, liver abscesses, tissue softening, urinary tract infections, and bacteremia, which in some cases can lead to death (Li et al., 2024).

The second most common *Klebsiella* group to be linked to clinical infections in people, after *K. pneumoniae is K. oxytoca" Bacillus oxytocus perniciosus*" discovered in old milk by Flugge in 1886. Bergey renamed it *"Aerobacteroxytocum"* in 1923, while Lautrop renamed it*"Klebsiella oxytoca*" in 1956can survive in a varietyof environments, such as moist environments، unfavorable conditions such as hand soap، central venous catheters as examples of prostheticmaterials, as well as in gut flora, all of which support its ability to cause opportunistic infections inhospitals, It can be found in themouth, nose, and gastrointestinal tract in its naturalenvironments. It is an intestinal bacterium that cancause potentially fatal infections outside of the gut. (Abd Al-Hassan et al., 2023).

K. oxytoca is an opportunistic pathogen that primarily causes hospital-acquired infections, causing urinary tract infections, sepsis, and antibiotic-associated hemorrhagic colitis, and is dangerous for immune compromised patients, diabetes, alcoholism, and cancer patients (Sarma et al., 2019). More recently, it has been associated with complications of neurosurgical procedures (Jiang et al., 2024).

To differentiate between *K. pneumoniae* and *K. oxytoca*, the indole test can be employed, where *K. pneumoniae* shows a negative reaction, while *K. oxytoca* is positive. For more precise species identification, PCR-based molecular methods targeting the amplification and sequencing of the rpoB, gyrA, and parC genes were utilized. Species-specific primers for *K. pneumoniae* produced 108 base pair (bp), whereas *K. oxytoca* primers yielded a 343 bp product, enabling clear discrimination between these two species. (Rawy et al., 2020).

1-9 Aims of study

- The aim of this study is to evaluate the effectiveness of the aqueous and alcoholic extracts and essential oils of *A. herba alba* & *T. capitatus* on the activity of bacteria resistant to traditional antibiotics in the laboratory.

- Identifying active compounds capable of killing or inhibiting the growth of antibiotic resistant bacteria.

Chapter 2

Materials and Methods

Materials and Methods

2-1 Devices and materials used

2-1-1 Devices

Table (2-1) Devices, manufacturers, and country of origin.

No	Devices	Company/ origin
1	Autoclave	Hosptic, Italy
2	Digital PH meter	Verified, China
3	Electronic balance	Lachol, China
4	Incubator DNP-9052A	Zenith Lab, China
5	Electric mill	Hommer, China
6	Camera	Poco, China
7	Light microscope	Prodoti, Italy
8	Clevenger apparatus	N.K Scientific works, India
9	Vortex	Hospitec, Italy
10	Magnetic stirrer with hot plate	DLAB Scientifiic, China
11	Deep freezer (-80 ºC)	Zhejiang Heerxin Appliance Technology, China
12	MA120 apparatus	Render, China
13	Refrigerator	Schaub Lorenz, Germany/ Beko, Turkey
14	Drying oven	Hospitec, Italy
15	Biosafety cabinet	Leadthink, China
16	GC-MS device	Perkin, Uk

2-1-2 Chemical substances

Table (2-2) Chemicals, their manufacturers and country of origin**.**

No	Substances	Company/ origin
1	Methnol	LabKem, Spain
2	Ethanol	Carlo Erba Reagents, Italy
3	Gram stain kit	Liofilchem, Italy
4	Barium chloride	CC, USA
5	Glycerol	Carlo Erba, Italy
6	Normal salin	Biolyse, Algeria
7	Chloroform	Riedel-De haen, Germany
8	Hydrochloric acid	Riedel-De haen, Germany
9	Led acetate	BDH, England
10	Sodium hydroxide	Sigma, USA
11	Hexan	Riedel-De haen, Germany
12	Picric acid	Riedel-De haen, Germany
13	Sulfuric acid	Eurostar Scientific Limited, England
14	Distilled water	ALqamoudi, Libya
15	Hydrogen peroxide	Mooncid, Turkey
16	Oxidase disc	Liofilchem, Italy
17	Enterosystem 18R	Liofilchem, Italy
18	Mueller hinton agar	Liofilchem, Italy
19	Nutrient agar	Liofilchem, Italy
20	Nutrient broth	Liofilchem, Italy
21	Blood agar	Liofilchem, Italy
22	Macconkey agar	Liofilchem, Italy
23	Brain heart infusion agar	Park Scientific Limited, England
24	Brain heart infusion broth	Merck, Germany
25	CLED agar	Liofilchem, Italy
26	Mannitol salt agar	Liofilchem, Italy

2-2 Plant material

2-2-1 Plant collection

Plants were collected from the city of Zintan, which is located in the northwest of Libya, 160 km away from the Libyan capital, Tripoli, in the southwest of the capital, in the Al-jabal Al- Gharpi (Nafusa mountain), at an altitude of 650-710 m above sea level (Vakanjac et al., 2023).

Each plant (*Artemisia* and *Thymus*) was collected from different places in the city and only the aerial parts of the plant were taken. The *A. herba alba* plant was collected from the Ouled Belhol area at site (8F3JW6WV+CG) 31°56'45.8"N, 12°14'37.7"E during October 2024, as shown in the Figure (2-1) and the *T. capitatus* plant was collected from the Ouled Khalifa area at site (8F3JX747+7X) 31°57'20.5"N, 12°15'53.8"E, at the end of April and the beginning of May 2024, as shown in the Figure (2-2). The botanical identification of both plants was carried out by Dr. Abdul Hmid Giweli, Department of Ecology, Faculty of Science, University of Zintan.

Figure (2-1) Location of Ouled Belhol indicating the sampling sites of *A. herba alba*: point (31°56'45.8"N, 12°14'37.7"E).

Figure (2-2) Location of Ouled Khalifa indicating the sampling sites of *T. capitatus*: point (31°57'20.5"N, 12°15'53.8"E).

After the collection stage comes the drying stage of the *Artemisia* and *Thymus* plants, where they were washed to remove impurities, dust and damaged parts under tap water, and spread out on a clean surface (cloth) with the two plants being turned continuously until they dry naturally by placing them in a dark place away from sunlight and at room temperature, and drying continued for a period of approximately two weeks. After the drying stage of two plants, the leaf parts were collected and placed in tightly sealed plastic containers in a cool, dry place until the extraction process was carried out (Martis et al., 2021).

2-2-2 Extraction of essential oil

The method of preparing vegetable oils is through the distillation process, in which the volatile oils are separated from aromatic and medicinal plants. This is the most widely used method and has been used since ancient times.

Hydro-distillation method is used by the Clevenger apparatus, Figure (2-3) in which the plant is placed in a glass flask with water in an electric heater, in which the oil is condensed and turns from a gaseous state to a liquid that does not dissolve in water, which is easy to separate, and the lower the temperature, the more possible to obtain

oil of high quality and excellent natural and chemical properties, and from the cost point of view it is considered good and inexpensive (Fagbemi et al., 2021).

Figure (2-3) Clevenger apparatus.

2-2-2-1 Extraction of essential oil for *T. capitatus* and *A. herba alba*

50 g from leaves (*Thymus* or *Artemisia*) were weighed using a sensitive balance and placed in a Homer electric grinder in a slightly coarse manner, then placed in a 1000 ml glass beaker. 500 ml of distilled water was measured using a graduated glass cylinder and placed in a Clevenger apparatus at a temperature of 60 °C. The extraction period continued for 3 hours. We note the oil in terms of color and potential of

hydrogen (PH) measured by PH meter device. It was then placed in a sterile, opaque glass tube and stored in the refrigerator at 4°C until use. (Bruneton, 2016).

2-2-2-2 Chemical composition analysis using gas chromatography-mass spectrometry (GC-MS) of the essential oils of *T. capitatus* and *A. herba alba*

To identify phytochemicals involved in antibacterial activity, quantitative and qualitative analyzes of oils were carried out using the GC-MS device at the institute of arid zones in Medenine, Tunisia (central laboratory) according to the method (Adams, 2017) and based on the percentages of the essential oil composition, the compounds isolated from the oil were determined.

The chemical composition of the essential oil (EOs) was analyzed using gas chromatography coupled with mass spectrometry and flame ionization detection (GC-MS/FID), following well-documented analytical procedures. Each essential oil sample was diluted in absolute ethanol at a ratio of 1:99 (v/v), and 1 µL of the solution was introduced in splitless mode into an Agilent 8890/5977BMSD/FID system (Agilent Technologies, Santa Clara, CA, USA). Compound separation was carried out on an HP-INNOWax polyethylene glycol capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The injector was maintained at 250 °C, and high-purity helium (99.9995%) served as the carrier gas with a constant flow rate of 0.8 mL/min. The oven temperature began at 65 °C (held for 2 minutes), then increased to 220 °C at 3 °C/min, and was held at the final temperature for 15 minutes. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with the ion source and transfer line temperatures set to 150 °C and 250 °C, respectively.

To determine the Kovats Retention Index (RI) for each compound, a homologous series of n-alkanes (C_{10}–C_{40}; Sigma-Aldrich, St. Louis, MO, USA) was analyzed under identical conditions. Identification of constituents was based on comparing both mass spectral data and calculated RIs with reference standards, the Adams essential oil database, the NIST MS Search 2.4 library, and other relevant scientific sources. Relative abundance of each component was determined from FID peak areas, using MSD ChemStation software (F.01.03.2357, Agilent Technologies), with the FID detector maintained at 300 °C. Final results are presented as relative percentages of the total chromatographic area.

2-2-3 Preparation of plant extracts by maceration method

The mixing and soaking method was described by Khanzada et al. (2006) where 50 g from leaves (*Thymus* or *Artemisia*) using a sensitive electric balance, placed in a conical flask with a tight plastic cover, to which 1000 ml of distilled water was added. The mixture was stirred using a glass rod for 15 minutes, then the flasks were placed in a dark place at room temperature for 24 hours, after which the extract was filtered using gauze and medical cotton for two times, and the third time it was filtered using filter paper Whatman No.1 in glass cups using glass funnels. We note the filter in terms of color and PH.

The filtrate was evaporated using sterile glass Petri dishes containing a quantity of the aqueous extract. The dishes were then transferred to an oven at 37°C for 24 hours. The dishes were then scraped with a spatula, and the dry extract, was collected in a sterile, opaque glass container. The container was refrigerated at 4 °C until use. The container was labeled with the name of the plant, the extracted plant part, and the extraction date.

2-2-4 Alcoholic extract with methanol

The alcoholic extract was prepared to obtain the active ingredients in it, a ccording to the method of Hail & Jiru (2022) for the *T. capitatus* or *A. herba alba* plants using methyl alcohol at a concentration of 75%, which was prepared from 99% pure alcohol through the following equation: $C_1 V_1 = C_2 V_2$

We obtained 757.6 ml of concentrated alcohol, to which 242.4 ml of distilled water were added, thus obtaining 1000 ml of methyl alcohol with a concentration of 75%.

We prepare the plant extract by grinding the *T. capitatus* or *A. herba alba* leaves using a Hommer electric grinder, then weigh 50 g using a sensitive balance, which is placed in a conical flask with a plastic cover. Then we add 1000 ml of 75% diluted alcohol and place it on a magnetic stirrer, which stirs the extract using a magnetic stirrer for an hour. Then we place it in a dark place for 3 days, stirring from time to time at room temperature. Then, the alcoholic extract is filtered twice in a row using gauze and medical cotton, and the third time using Whatman No.1 filter paper using glass funnels and beakers with a capacity of 600 ml. The resulting filtrate is liquid in consistency. We note the filter in terms of color and PH. The extract was evaporated by

air oven, by placing a certain amount in sterile glass Petri dishes at 37°C for 24 hours, then the dry material was scraped by spatula and the weight of the extract and then it was stored in a tightly closed, opaque, sterile glass container with the extract data written on it and kept in the refrigerator at 4 °C until use, Figure (2-4).

Figure (2-4) Show the steps for preparation of alcoholic extract.

2-2-5 Preparation of concentrations of plant extracts

To prepare the basic stock solution of the aqueous and alcoholic (methanol) plant extracts of both *Thymus* and *Artemisia*, from which the following concentrations were prepared (100, 200, 300 mg/ml), where the solvent was sterile distilled water.

Crude concentration (mg/ml) = mass of the substance obtained (mg) / volume of solvent used (ml).

2.4 g of the extract mass was weighed and dissolved in 4 ml of sterile distilled water. Using the w/v ratio, we obtained a solution with a concentration of 600 mg/ml, which was mixed by placing the tube on a vortex device. From this concentration of the solution, we prepare the required dilutions using the following equation:

$C_1 V_1 = C_2 V2$. To prepare 1 ml of each concentration.

- To prepare 1 ml at a concentration of 100 mg/ml, take 166 µl of the basic solution and add 834 µl of sterile distilled water.
- To prepare 1 ml at a concentration of 200 mg/ml, take 333 µl of the basic solution and add 667 µl of sterile distilled water.
- To prepare 1 ml at a concentration of 300 mg/ml, take 500 µl of the basic solution and add 500 µl of sterile distilled water.

To mix them, we use a Vortex device, which is placed in tubes with a tight stopper until they are used for testing (Wijesundara & Rupasinghe, 2019).

2-2-6 Phytochemicals screening

2-2-6-1 Detection of Phenolic compounds (ferric chloride test)

To detect these compounds, 1% ferric chloride reagent was used, where the reagent was added to the extract in equal quantities. The appearance of a blue-green color indicates the presence of phenolic compounds.

2-2-6-2 Detection of Flavonoids compounds (sodium hydroxide test)

2 ml of the aqueous or alcoholic extract were mixed with 1 ml of sodium hydroxide. If a yellow color appeared, this indicated that the test was positive.

2-2-6-3 Detection of Alkaloids compounds (picric acid test)

We take 0.2 g of the plant extract and put it in a test tube containing 3 ml of hexane, shake it well and filter it, then take 5 ml of 2% hydrochloric acid and pour it over the plant extract and hexane, heat the tube and filter the mixture again, then put

drops of picric acid in the mixture, when a yellow precipitate is formed, it indicates the presence of alkaloids (Mulata et al., 2015).

2-2-6-4 Detection of Saponins compounds (foam test)

To detect these substances, we mix 5 ml of the extract with 20 ml of distilled water and shake it for 15 minutes. When foam forms, it indicates the presence of soaps (Aravindhan et al., 2009).

2-2-6-5 Detection of Tannins compounds (lead acetate test)

1 gram of lead acetate is dissolved in 100 ml of distilled water, at a concentration of 1%. Drops of this reagent are added to a test tube containing 0.5 ml of the extract. When a white precipitate is formed, it indicates the presence of tannins (Adedayo et al., 2001).

2-2-6-6 Detection of Terpenoids compounds (salkowiski test)

A solution of 2 ml of chloroform and 3 ml of concentrated sulfuric acid was prepared, then 5 ml of the extract was added. The appearance of a reddish-brown layer indicates the presence of terpenes (Ayoola et al., 2008).

2-3 Preparation of solutions used to diagnose bacteria

2-3-1 Normal saline solution

A ready-made solution from the Algerian company (Biolyse) was used to dilute the bacterial samples and compare them with Macfarland's solution and stored in the refrigerator (Forbes et al., 2007).

2-3-2 Macfarland turbidity standard solution

Prepare the solution according to the following method, which consists of two solutions:

Solution A: 1.175 g of aqueous barium chloride ($BaCl_2.2H_2O$) was dissolved in 100 ml of distilled water.

Solution B: is a 1% sulfuric acid solution, where you add 1 ml of concentrated sulfuric acid to 99 ml of distilled water.

Then take 0.5 ml of solution A and add to 99.5 ml of solution B in a conical flask and mix the resulting solution well, then distribute it into clean, sterile, tightly closed glass test tubes to prevent evaporation. Store the tubes in the dark at room temperature until use, and mix the tube before use using a Vortex device.

Macfarland solution is used to compare the bacterial suspension used in susceptibility testing, as the Macfarland tube density is (0.5) which is equivalent to (1.5 x 10^8cells/ml). This corresponds to an optical absorbance of (0.08-0.13) at a wavelength of 625 nm (Hudzicki, 2009).

2-3-3 Gram stain

Gram staining is one of the most crucial staining techniques in microbiology. Typically, Gram staining is the first test performed, utilizing crystal violet or methylene blue as the primary color. Organisms that retain the primary color and appear purple under a microscope are gram-positive have thick cell wall because of thick layers of peptidoglycan. In contrast, those that do not take up the primary stain and appear red under a microscope are gram-negative have thin layers of peptidoglycan. Gram stain reagents were procured from the Italian manufacturer Liofilchem, comprising crystal violet dye, iodine solution, ethyl alcohol, and safranin dye, to effectively distinguish between Gram-positive and Gram-negative bacteria. (Paray et al., 2023).

2-4 Preparation of culture media

Ready-made culture media were prepared for the purpose of diagnosing, preserving and activating bacteria during the study period. Among the media used were:

2-4-1 Nutrient agar

Typical formula (g/l): meat Extract 1.0, yeast extract 2.0, peptone 5.0, sodium chloride 5.0, agar 15.0, (PH 7.4±0.2 at 25 ºC).

Prepared by dissolving 28 g of the ready-made medium powder in a liter of distilled water, from the Italian company Liofilchem, then heating to ensure its dissolution with a magnet placed in the flask to stir the mixture and place it on the magnetic stirring device, then transfer it to the Autoclave for 15 minutes at 121 ºC.

2-4-2 Nutrient broth

Typical formula (g/l): beef extract 1.0, yeast extract 2.0, peptone 5.0, sodium chloride 5.0, (PH 6.8±0.2 at 25 ºC).

Prepared by dissolving 13 g of the prepared medium powder in a liter of distilled water, from the Italian company Liofilchem, then transferring it to an Autoclave for 15 minutes at 121 ºC.

2-4-3 Mannitol salt agar (MSA)

Typical formula (g/l): pancreatic digest of casein 5.0, peptic digest of animal tissue 5.0, beef extract 1.0, D-mannitol 10.0, phenol red 0.025, Sodium chloride 75.0, agar 15.0, (PH 7.4±0.2 at 25 ºC).

Prepared by dissolving 111 g of the ready-made medium powder in a liter of distilled water, from the Italian company Liofilchem, then heating to ensure its dissolution with a magnet placed in the flask to stir the mixture and place it on the magnetic stirring device, then transfer it to the Autoclave for 15 minutes at 121 ºC.

2-4-4 MacConkey agar

Typical formula (g/l): pancreatic digest of gelatin 17.0, peptone from meat 1.5, peptone from casein 1.5, lactose 10.0, bile salts 1.5, agar 15.0, neutral red 0.03, crystal violet 0.001, (PH 7.7±0.2 at 25 ºC).

Prepared by dissolving 51.5 g of the prepared medium powder in a liter of distilled water, from the Italian company Liofilchem, then heating to ensure its dissolution with a magnet placed in the flask to stir the mixture and place it on the magnetic stirring device, then transfer it to the Autoclave for 15 minutes at 121 ºC.

2-4-5 Blood agar

Typical formula (g/l): peptospecial 15.0, liver extract 25, yeast extract 5, sodium chloride 5, agar 15, (PH 7.4±0.2 at 25 ºC).

Prepared by dissolving 42.5 g of ready-mixed medium powder in 1 liter of distilled water, from the Italian company Liofilchem, then heating to ensure its dissolution with a magnet in the flask to stir the mixture and placing it on a magnetic stirrer, then

transferring it to an Autoclave for 15 minutes at 121 °C, then cooling it at 50 °C, adding 50 ml of horse or sheep blood and mixing well, then pouring it into Petri dishes.

2-4-6 Cystine lactose electrolyte deficient (CLED) agar

Typical formula (g/l): enzymatic digest of gelatin 4.0, enzymatic digest of casein 4.0, beef extract 3.0, lactose 10.0, bromothymol blue 0.02, agar 15.0, (PH 7.3±0.2 at 25 ºC).

Dissolve 36.1 g of the prepared medium powder in a liter of distilled water from the Italian company Liofilchem, then heat it until it is completely dissolved and then transfer it to an Autoclave for 15 minutes at 121 ºC.

2-4-7 Muller Hinton agar (MHA)

Typical formula (g/l): beef extract 2.0, acid hydrolysate of casein 17.5, starch 1.5, agar 17.0, (PH 7.3±0.2 at 25 ºC).

Prepared by dissolving 38 g of the ready-made medium powder in a liter of distilled water, from the Italian company Liofilchem, then heating to ensure its dissolution with a magnet placed in the flask to stir the mixture and place it on the magnetic stirring device, then transfer it to the Autoclave for 15 minutes at 121 ºC.

2-4-8 Brain heart infusion agar (BHA)

Typical formula (g/l): brain heart infusion 17.5, tryptose 10.0, glucose 2.0, sodium chloride 5.0, disodium hydrogen phosphate 2.5, agar 12.0, (PH 7.4±0.2 at 25 ºC).

Prepare by dissolving 49 g of the prepared medium powder in 1 liter of distilled water, from Park Scientific Limited, UK, leave for 10 minutes to soak and mix on a magnetic stirrer, then transfer to an Autoclave for 15 minutes at 121°C, cool to 47 °C, then pour into Petri dishes.

2-4-9 Brain heart infusion Broth (BHB)

Typical formula (g/l): extracts of brain and heart and peptone 27.5, D (+) glucose 2.0, sodium chloride 5.0, disodium hydrogen phosphate 2.5, (PH 7.4±0.2 at 25 ºC).

Prepare by dissolving 37 g of the prepared medium powder in a liter of distilled water, from the Italian company Liofilchem, then transferring it to an Autoclave for 15 minutes at 121 ºC.

The media were cooled after being removed from the Autoclave and poured into sterile plastic Petri dishes (one room or two rooms) and then stored in the refrigerator at 4°C until use. Broth was placed in a flask with a tightly closed stopper until use.

The work is done in a sterile area, either next to a Bunsen burner or in a cabinet, so that the media are not contaminated. To ensure that they are free of contamination, they are placed in an incubator for 24 hours at 37 °C.

2-5 Bacterial samples

2-5-1 Collecting bacterial samples

Pathological samples were collected from different clinical sources from people suffering from tonsillitis, urinary tract infection and dermatitis in Zintan city in a period of time from 1/11/2024 to 1/1/2025, with ages ranging from 6-40 years of both gender.

Samples were taken by cotton swab and preliminary diagnosis was made at the National research center for tropical and transboundary diseases (NRCTTD).

2-5-2 Bacterial sample culture

Samples were cultured on nutrient agar, mannitol salt agar (MSA), macConkey agar, blood agar, and CLED agar by cotton swabs and incubated for 24 hours at 37 °C.

2-5-3 Identification bacterial isolates

2-5-3-1 Culture diagnosis

- **MacConkey agar**

It is used for Gram-negative bacteria, and it distinguishes between lactose-fermenting bacteria, which give a pink color, and non-lactose-fermenting bacteria. It is a differential and selective medium for isolating Gram-negative bacilli.

- **Blood agar**

It is considered a mixed medium to which human blood is added according to the manufacturer's instructions. It is a differentiating medium between pathogenic bacteria that break down red blood cells.

- **Mannitol salt agar**

A selective media for isolating and identifying staphylococci by their ability to ferment mannitol sugar, which changes the medium from red to yellow.

- **CLED agar**

It is used to detect the causes of urinary tract infections, as colonies appear on this medium as a yellow balloon as a result of the fermentation of lactose sugar.

- **Muller hinton agar**

This medium is used to conduct antibiotic sensitivity tests and to test the effectiveness of plant extracts against the studied bacteria (Cheesbrough, 2005).

2-5-3-2 Microscopic examination

- When we examined the pure colonies growing on agricultural circles using microscopy, we placed a drop of distilled water on a clean glass slide, the colony was taken from a Petri dish by a lube and spread well on the slide, left to dry in the air and then fixed by passing it over the flame 3-4 times.
- Prepare the stain bath and place crystal violet on the slide for a minute, then wash it with water.
- Add iodine for a minute, then wash with water.

- Add alcohol for 15-20 seconds and wash with water.
- Add safranin for 30 seconds, then wash and leave to dry.
- Wipe the slide of water with filter paper and examine it under a microscope using a 100 lens with a drop of oil.

Based on the shape and color of the bacterial cell, if it is Gram-positive it is purple, while if it is Gram-negative it is red (Paray et al., 2023).

2-5-3-3 Biochemical tests

❖ **Oxidase reagen**t

Using tablets prepared by the Italian company Liofilchem to detect the ability of bacteria to produce the enzyme oxidase Detection of the ability of bacteria to produce the oxidase enzyme by transferring a single bacterial colony growing on media agar at 37 °C for 24 hours using a cotton swab and placing it on the disc. If the colour of the disc changes to purple or dark blue, the result is positive for the reagent test (Lontum et al., 2024).

❖ **Catalase reagent**

This detector uses a 3% hydrogen peroxide (H_2O_2) solution single. Colony of bacteria growing at 37 °C for 24 hours is taken on Nutrient agar or MacConkey agar using sterile wooden sticks placed on a drop of hydrogen peroxide solution on a clean glass slide. If bubbles appear, the result is positive for the reagent. If it is negative, the bacteria lack the production of this enzyme (Procop et al., 2020).

❖ **Coagulase reagent**

In this test, the ability of bacteria to produce the plasma coagulating enzyme is detected. Plasma was obtained from the central blood bank of Zintan city. 0.5 ml of human blood plasma is taken and placed in an eppendrove tube, then inoculated with a number of bacterial colonies and incubated at 37 °C for 24 hours. If clumping is observed, this indicates that the test is positive and that the bacteria are producing the enzyme (AL-Joda & Jasim, 2021).

❖ **Diagnosis using Enterosystem 18 R**

Used to identify (Gram-negative bacteria) using oxidase, from the Italian company Liofilchem.

Preparing bacterial suspensions for Enterosystem 18 R

- The bacteria must be growing (18-24 hours) or require growth within 48 hours.
- Before doing so, ensure they are oxidase-negative by testing them with a reagent.
- Take one or more colonies from the agar medium in which they were isolated in pure form and suspend them in 5 ml of normal saline. Compare the turbidity of a Macfarland tube, which is equivalent to 1.5×10^8 cells/ml.
- Mix the suspension well with a Vortex device.
- Take the test plate and place it at room temperature. Record the date, sample, and name of the agar from which it was taken.
- Place 200 microliters of the bacterial suspension in each well of the system, then place one drop of petroleum jelly in the following wells: 2-LDC, 3-ODC, 4-ADC, 7-UR, and 8-H2S.
- Cover the plate with its special cover and place it in the incubator at 37°C for 12-18 or 24 hours.
- After the incubation period, add 2 drops of Alaph-napthol and 1 drop of 40% NaOH to well 10 VP. Wait 15–20 minutes. If the color turns pink or red, the test is positive.
- Add 2–3 drops of Kovac's reagent to well 11 IND. Wait 1–2 minutes. If the color turns red, the test is positive.
- Check the color changes in the other wells and compare them with the accompanying test table.
- Note the results on the form with the test results and determine the 6-digit numerical code.
- Identify the tested organism using the Enterosystem 18R book or the identification code disc (Piccolomini et al., 1991).

2-6 Automated identification (ID) and antibiotic susceptibility tests (AST) system MA 120

This system is developed by the Chinese company Render using the MA 120 device in Al-Mazen Hospital in Zintan. It is an automatic identification system for bacteria and fungi based on traditional biochemical reactions. It is detected through the applicable colorimetric method that detects more than 300 types of bacteria and fungi. The antibiotic sensitivity test is according to the Clinical & Laboratory Standards Institute (CLSI) standard, in which the minimum inhibitory concentration (MIC) can be updated and upgraded annually for antibiotics. The effectiveness of the microbial identification and susceptibility testing was confirmed by comparison with 16S rDNA sequencing and the VITEK2 system (He et al., 2021). The MA 120 consists of a turbidity meter, a detector with a computer and its own test plates with 120 microwells, including 24 wells for identification and 96 wells for antibiotic sensitivity testing, which give both results simultaneously.

These panels are divided into 5 groups:

- Enterobacteriaceae panel
- Nonfrementers panel
- Staphylococcus panel
- Streptococcus / Enterococcus panel
- Fungus panel

The detection procedure commenced with microscopic examination employing Gram staining, aimed at determining the nature of the microorganism under investigation whether fungal, Gram-positive, or Gram-negative bacteria along with a precise description of its morphological characteristics. The specimens were obtained from a freshly prepared 24-hour culture, which had been cultivated on three standard media: Nutrient agar, MacConkey agar, and Blood agar, thereby ensuring optimal growth conditions and facilitating accurate identification of the organism’s morphological and physiological traits. The bacterial culture plate was retrieved from frozen storage and allowed to equilibrate at room temperature. Subsequently, a bacterial suspension was prepared, and its turbidity was quantified using a calibrated turbidity meter to

ensure standardization of inoculum density. Experimental procedures were conducted in strict accordance with the manufacturer's protocol for each designated group.

The bacterial isolates were subjected to diagnostic testing, followed by assessment of their antimicrobial susceptibility. The minimum inhibitory concentration (MIC) values were determined utilizing the MA 120 system, and the interpretation of resistance profiles was performed in compliance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI). Figure (2-5).

Figure (2-5) MA 120 apparatus**.**

2-7 Bacterial strains used in the study

The species identified by the MA 120 and against which the extracts will be tested are *S. aureus, S. epidermidis, K. pneumoniae, and K. oxytoca*.

2-8 The method of preserving bacterial isolates

2-8-1 Preservation of short-term isolates

After confirming their diagnosis, the bacterial isolates were stored in test tubes containing Nutrient agar prepared obliquely and were cultured by streaking, then incubated at 37 °C for 24 hours, then stored in the refrigerator at 4 °C. The isolates were renewed periodically monthly by activating them in Brain Heart Infusion Broth and then re-stored on Nutrient agar to ensure that they remained active during the study period (Gherna & Reddy, 2007).

2-8-2 Preservation of long-term isolates

To preserve bacterial isolates for several years on Nutrient broth medium supplemented with 20% glycerol, which was sterilized in an Autoclave, the bacterial isolates were activated in the Nutrient broth and incubated at 37°C for 24 hours. After incubation, 800 µl of the inoculated broth was pipetted in a sterile area and placed in Eppndrof tubes. 200 µl of sterile glycerol was added by pipet using new sterile tips. The isolates were then mixed well with a vortex device and transferred to a tightly closed plastic container. They were then stored at (-80 °C) in a deep freezer, with the identification data for each tube and the container in which it was stored being written (Dreesbach & Behle, 2018).

2-9 Preparation of the bacterial suspension

- The preserved bacterial isolates are activated and grown on appropriate media (*S. aureus*, *S. epidermidis*) on brain heart agar and incubated for 24 hours at 37 °C, while (*K. oxytoca*, *K. pneumonia*e) are activated on macConkey agar to ensure pure and active isolates.
- The work is done in a sterile area and under sterile conditions. After the incubation period, 3-5 bacterial colonies are taken and placed in a test tube containing 5 ml of normal saline solution. They are mixed well using a vortex meter and compared with a Macfarland tube (0.5) which corresponds to (1.5 x 10^8 cells/ml).
- The bacterial suspension should be used 15 minutes after its preparation (Hossain, 2024).

2-10 Assay of plant extracts activity by disc diffusion method

To estimate the antibacterial activity of isolated plant extracts, we used the disc diffusion method, which depends on saturating the discs with each extract. We prepared discs of filter paper (mm) with a diameter of 6 mm, which were placed in a clean, sterile Petri dish, then wrapped in aluminum foil and placed in an autoclave to be sterilized (Mohsenipour & Hassanshahian, 2015).

The filter papers are saturated with an amount ranging between 0.01-0.05 ml of plant extracts. After the incubation period, the inhibitory diameters are measured in (mm) using a graduated ruler for bacterial growth around each disc.

Taking into account the diameters of the microbial growth inhibition zones, the antimicrobial properties of plant extracts were evaluated according to the following criteria:

- Microbial inhibition zone diameterof less than 3 mm indicates weak sensitivity to the substance.
- Microbial inhibition zone diameterof 4–10 mm indicates moderate sensitivity to the substance.
- Microbial inhibition zone diameterof more than 10 mm indicates high sensitivity to the substance (Chokheli et al., 2025).

2-10-1 Antimicrobial effectiveness of essential oil

- After preparing the bacterial suspension and comparing it with the Macfarland tube (0.5) which corresponds to (1.5×10^8 cells/ml), work is done next to a Bunsen flame to make the area sterile and to sterilize the forceps.
- Transfer 100 µL of the bacterial suspension using a micropipette, which is placed on the surface of the MHA and spread evenly with a swab.
- Leave the plates to dry for 15 minutes at room temperature.
- Repeat each plate three times for each type of bacteria under the same conditions.
- Take essential oil saturated discs of *Thymus* and *Artemisi*, each in an amount of 10 µL.

- Essential oil saturated discs are placed on the surface of Mueller Hinton agar (MHA). In each plate, place a disc of pure *Thymus* oil and another of pure *Artemisi*oil, using a disc saturated with distilled water as a control or comparison factor.
- Press the disc with the tip of forceps to secure it to the agar surface.
- Sterilization is ensured each time the disc is removed.
- After placing the discs, leave the plates near a Bunsen burner for 15 minutes.
- Then, incubate them at 37°C for 24 hours.
- After the incubation period, remove the Petri dishes to measure the inhibitory diameters using a ruler in millimeters. This is the area where bacterial growth is absent around the disc (Abouzeed et al., 2013), Figure (2-6).

Figure (2-6) Antimicrobial effectiveness of essential oil.

2-10-2 Testing the effectiveness of aqueous and alcoholic extracts on bacteria

❖ After preparing the bacterial suspension, we tested the effect of the plant extracts on the bacteria.

- Filter papers are equipped with a diameter of 6 mm diameter filter papers were prepared, sterilized in an autoclave, and placed in a small glass Petri dish.
- Mueller-Hinton medium was also prepared for bacterial susceptibility testing. Three copies of the plates were prepared for each type of bacteria, labeled with the name of the bacteria and the concentration to be tested.
- A set of filter papers for each concentration of extract was placed in a small, sterile glass dish.
- We impregnated the papers with 20-50 µL of the extract and left them to dry. Then, we impregnated them with 10 µL of the extract for 5 minutes.
- Meanwhile, we placed 100 µL of the bacterial suspension onto Mueller-Hinton agar medium using a micropipette and spread it over the entire dish using a cotton swab. We left them to dry for 5 minutes.
- We sterilize the forceps with a flame and alcohol and place each tablet for each concentration in its designated place (100, 200, 300 mg/ml). We sterilize the forceps each time we remove the tablet before and after placing it in the dish. The tablet is held in place by applying pressure with the forceps.
- Each dish, in its three versions, consists of four filter paper discs: three for the tested concentrations and one as a negative control saturated with sterile distilled water, with the distance between the discs being taken into account.
- After placing the discs, the dishes are left for 15 minutes at room temperature.
- They are then incubated at 37°C for 24 hours.
- The diameters of inhibition are then measured and the results are recorded (Yaseen & Nayyef, 2021), Figure (2-7).

Figure (2-7) Testing the effectiveness of aqueous and alcoholic extracts on bacteria.

2-11 Statistical analysis

Data were analyzed with a statistical software program (SPSS 20). Was used for statistical analysis. The one-way ANOVA followed by Least Significant Difference (LSD) test. Results are expressed as mean±SD, and statistical significance was accepted $P<0.05$.

Chapter 3

Results

Results

3-1 Properties of plant extracts

Table (3-1) shows the characteristics of plant extracts in terms of the solvent used, color, pH, and the quantity or volume extracted.

Table (3-1) Properties of plant extracts.

Plant	Extract	Color	PH	Wight, volume extract
A. herba alba	Alcoholic	Dark brown	5.52	10.86 g
	Aqueous	Orange	4.86	3.65 g
	Essential oil	Light yellow	5	1 ml
T. capitatus	Alcoholic	Dark olive	6.94	5.31 g
	Aqueous	Light brown	5.3	3.72 g
	Essential oil	Golden yellow	6	1.7 ml

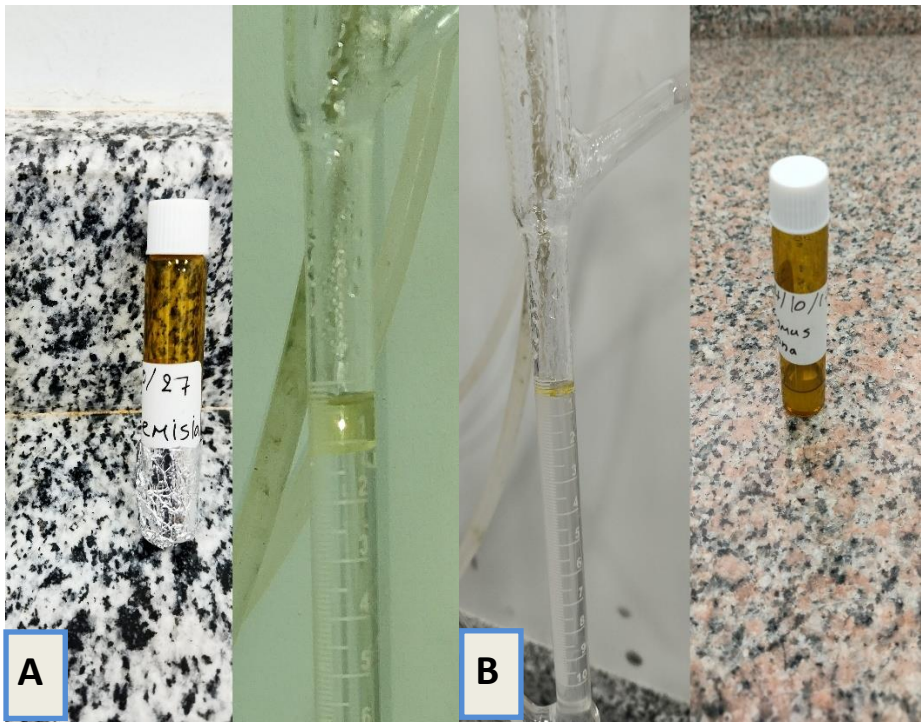

Figure (3-1) Extract of (**A**) *A. herba alba* and (**B**) *T. capitatus* essential oil by hydro-distillation using a Clevenger apparatus.

3-2 Extract yield

The results of the study showed that the yield of plant extracts from the mass of dry plant material was obtained, and the ratio for each aqueous extract, alcoholic extract, and essential oils was as shown in Table (3-2), where the ratio of *Artemisia* to the alcoholic extract was the highest, 20.76% and the lowest ratio was close between the aqueous extract of two plants, and the lowest was the extract of the *Artemisia* plant, Figure (3-2).

Percentage yield = weight extract / weight of the sample × 100

Table (3-2) Extract yield % of *T. capitatus* leaves and *A. herba alba* leaves.

Plant	Essential oil	Aqueous extract	Alcoholic extract
A. herba alba	2 %	7.3 %	20.76 %
T. capitatus	3.4 %	7.44 %	10.62 %

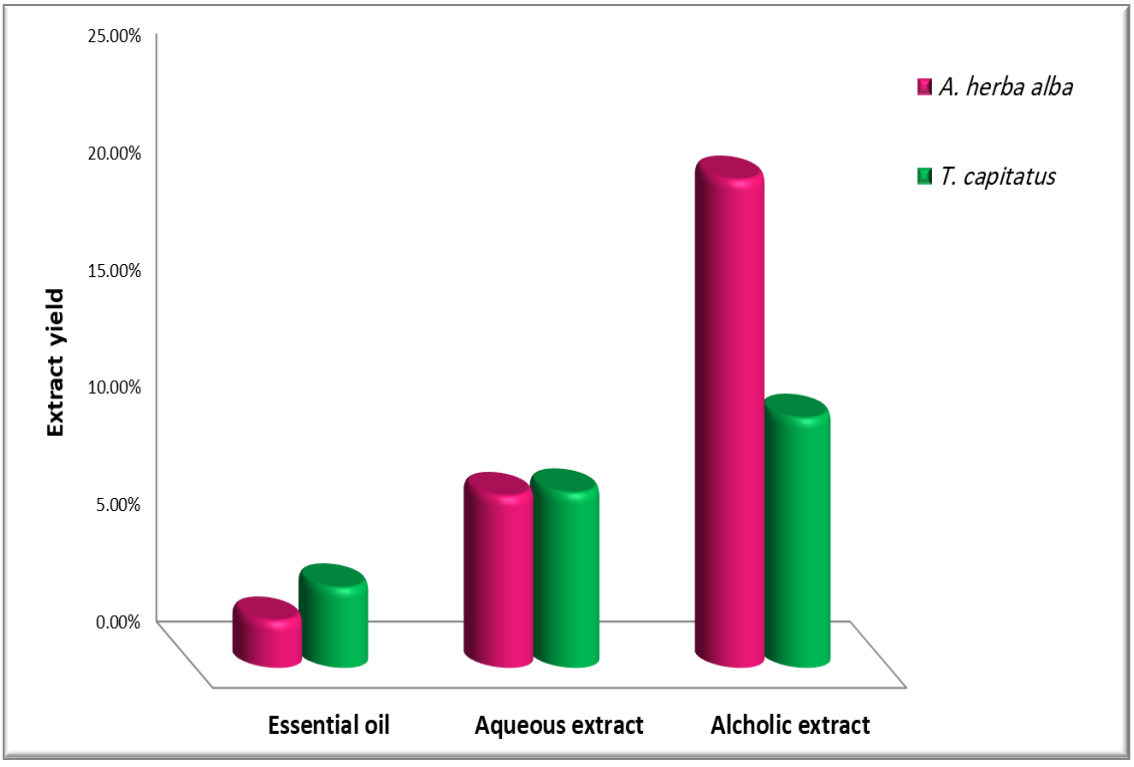

Figure (3-2) Extract yield % of *T. capitatus* leaves and *A. herba alba* leaves.

3-3 Chemical composition analysis using GC-MS of the essential oils of *T. capitatus* and *A. herba alba*

As shown in Table (3-3) approximately 18 compounds were identified from the oil mass of *T. capitatus* plant. The main compounds were Carvacrol methyl ether with a percentage of 53.208%, Carvacrol compound with a percentage of 13.593%, while Thymol compound was 9.963%. The majority of the identified compounds were from the monoterpene class, but the highest percentage was from the phenolic class.

As shown in Table (3-4), the compounds of *A. herba alba* oil were identified, which contains 24 compounds of the oil mass. The main compounds were Camphor at 30.527%, Thujone at 22.471%, Camphene at 10.291%, and 3-carene at 7.322%.

Table (3-3) Chemical composition of *T. capitatus* essential oil using GC-MS from Libya-Zintan**.**

No	Identified compound	%	RT	RI
1	Hexanal	0.863	2.559	576
2	α –Pinene	-	4.042	681
3	β- Myrcene	0.775	7.278	828
4	α –Terpinene	1.751	7.455	836
5	p- Cymene	1.099	7.82	851
6	Limonene	1.091	8.489	879
7	1,8-Cineole	3.107	8.797	892
8	Sabinene	0.241	8.887	895
9	γ- Terpinene	2.804	9.455	919
10	Carvacrol	13.593	9.655	927
11	Thymol	9.963	10.486	962
12	Linalool	0.318	11.193	991

13	Borneol	1.502	11.406	1000
14	Terpinen-4-ol	2.154	13.054	1072
15	α- Terpineol	0.153	13.597	1096
16	Methyl chavicol	-	15.564	1186
17	Methyl eugenol	4.818	15.711	1193
18	Carvacrol methyl ether	53.208	16.018	1208
19	δ –Cadinene	0.595	16.195	1216
20	β-Caryophyllene	-	17.421	1276
21	α-Humulene	1.41	18.573	1335

(-) There is no.

Table (3-4) Chemical composition of *A. herba-alba* essential oil using GC-MS from Libya-Zintan.

No	**Identified compound**	**%**	**RT**	**RI**
1	α –Pinene	0.369	2.557	932
2	3- Hexene-1-ol, (E)	0.313	5.597	857
3	Camphene	10.291	6.809	953
4	β –Pinene	2.934	7.46	976
5	3-Carene	7.322	7.836	1009
6	trans-β -Ocimene	4.791	8.418	1036
7	α –Terpinolene	0.397	9.452	1087
8	p-Cymene	0.945	9.641	1024

9	Sabinene	0.227	9.698	969
10	γ-Terpinene	0.461	9.755	1059
11	Eucalyptol	3.047	9.828	1031
12	Cyclohexanemethanol, 4-methylene	-	10.475	1123
13	Terpinen-4-ol	2.253	10.694	1176
14	2,3-Dimethylanisole	-	11.194	1198
15	Linalool	1.428	11.485	1096
16	1-Undecyne	-	11.631	1203
17	Camphor	30.527	11.964	1143
18	p-Menth-8-en-1-ol, stereoisomer	0.268	12.053	1169
19	Thujone	22.471	12.644	1428
20	Borneol	1.029	12.835	1165
21	2,4Cycloheptadien-1-one	-	12.998	1237
22	(-)-cis-Myrtanylamine	-	13.07	1242
23	Dihydrocarveo	4.238	13.329	1289
24	β –Elemene	0.168	13.452	1390
25	α –Terpineol	0.693	13.593	1189
26	cis-3-Hexenyl isovalerate	0.949	14.418	1261
27	Trimethadione	0.450	15.311	1233
28	Bornyl acetate	0.497	15.692	1314

29	γ–Elemene	-	18.012	1284
30	β–Bisabolene	0.154	19.055	1335
31	(+)-3-Bromocamphor-8-sulfonic acid	-	28.28	1509

(-) There is no.

3-4 Phytochemical screening

The results were shown in Table (3-5) for detecting the active compounds present in the studied plants through the qualitative examination of the aqueous and alcoholic extracts using the reagents specific to each group of active compounds, which form a white precipitate as in the case of alkaloids and tannins, or a change in color as in the case of phenol, which gives a blue-green color, flavonoids give a yellow color, while terpenes show the appearance of a reddish-brown layer, and soaps form a foam for the extract.

Table (3-5) Some active groups of *T. capitatus* and *A. herba alba* leaves extracts.

	Aqueous extract		**Alcoholic extract**	
Composite	***T. capitatus***	***A. herba alba***	***T. capitatus***	***A. herba alba***
Phenol	+	+	+	+
Alkaloids	+	-	-	-
Flavonoids	+	-	-	-
Tannins	-	-	-	+
Terpenoids	+	-	-	+
Saponins	+	+	+	+

(-) Negative detected, (+) Positive detected.

3-5 Collecting bacterial samples

Samples were collected from patients aged 6 to 40 years suffering from tonsillitis, urinary tract infection, and skin infection. Four bacterial species were isolated, including both Gram-positive and Gram-negative bacteria. *S. epidermidis* was isolated from tonsillitis cases, *S. aureus* from skin infection, and *K. oxytoca* and *K. pneumoniae* from urinary tract infection.

3-5-1 Culture diagnosis

Initially, all samples were cultured on blood and MacConkey medium. Based on the morphological characteristics of the colonies, some of them were hemolytic and others were not. As for MacConkey medium, the result was that some swabs had pink, large mucous colonies as a result of fermenting lactose sugar. Its colonies were gray, shiny, and mucous. When the bacteria were cultured on blood agar, they were unable to lyse blood.

3-5-2 Microscopic examination

A portion of a colony with typical characteristics was taken and stained with Gram stain solutions. After that, it was examined under a microscope and the shape and arrangement of the cells were observed. They were Gram-positive cocci, clustered in the form of clusters, while the other type had a Gram-negative rod-shaped shape, single or in short chains. To distinguish the type of these genera, we performed biochemical tests.

3-5-3 Biochemical tests

Biochemical tests were performed as shown in Table (3-6) Figures (3-3, 3-4). The result of the catalase test was positive, which distinguishes it from streptococci. The oxidase test is also used to distinguish the first from Micrococci. *S. aureus* was cultured on MSA agar and blood agar. The result was that one of the isolates was mannitol fermenting and the medium changed from red to yellow. On blood agar, it was completely hemolysis for red blood cells, which was of the β- Hemolysis type. A coagulase test was performed and the test was positive. As for the other type, it was not mannitol fermenting and not hemolysis. The confirmatory test was that it was

negative for the coagulation test, so the bacteria were *S. epidermidis*, which was confirmed by the MA 120 device.

The genus of bacilli was cultivated on CLED agar and blood agar, which gave isolates with a yellow color on CLED agar as a result of fermentation of lactose sugar in it, while on blood agar it did not give hemolysis of red blood cells. Biological tests were negative for oxidase, negative for catalase. To distinguish between them (*K. oxytoca* and *K. pneumonia*e), the Enterosystem 18 R system was used, as one of the isolates was positive for the indole test (*K. oxytoca*). To confirm the result, they were tested in the MA 120 device, and the result was two types of *Kelbsiella: K. oxytoca* and *K. pneumonia*e.

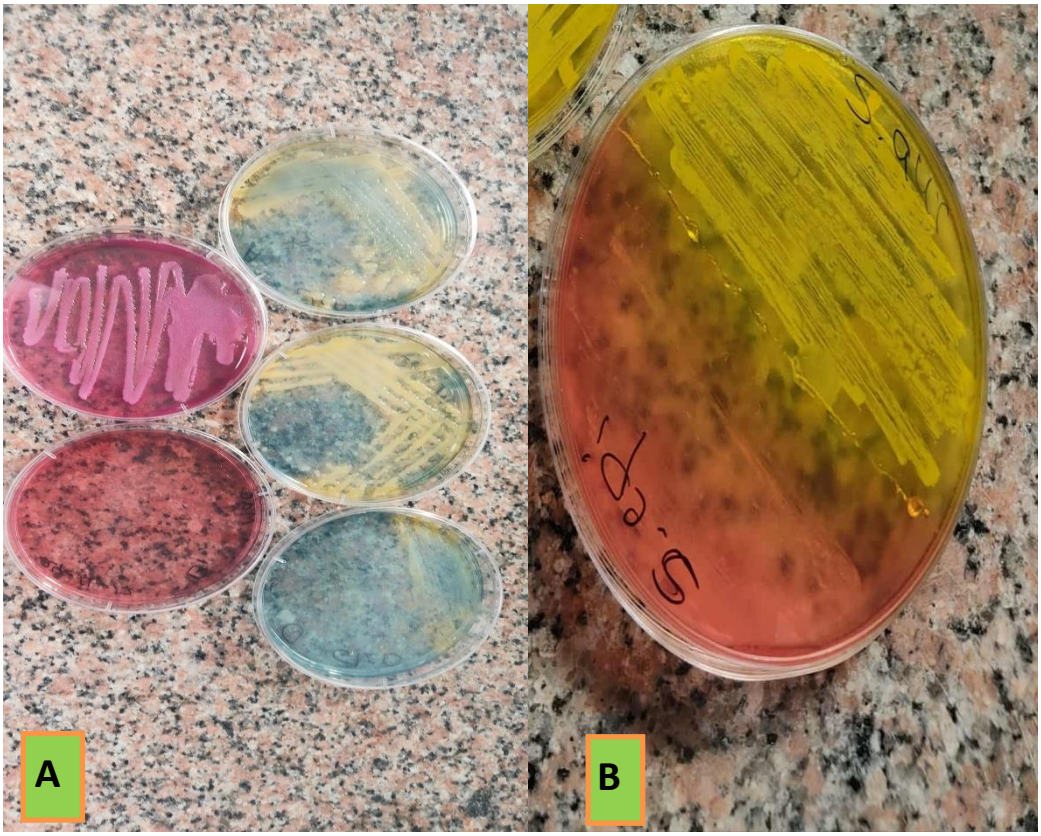

Figure (3-3) A, *Klebsiella* on MacConkey & CLED agar/ B, *Staphylococcus* on MSA agar.

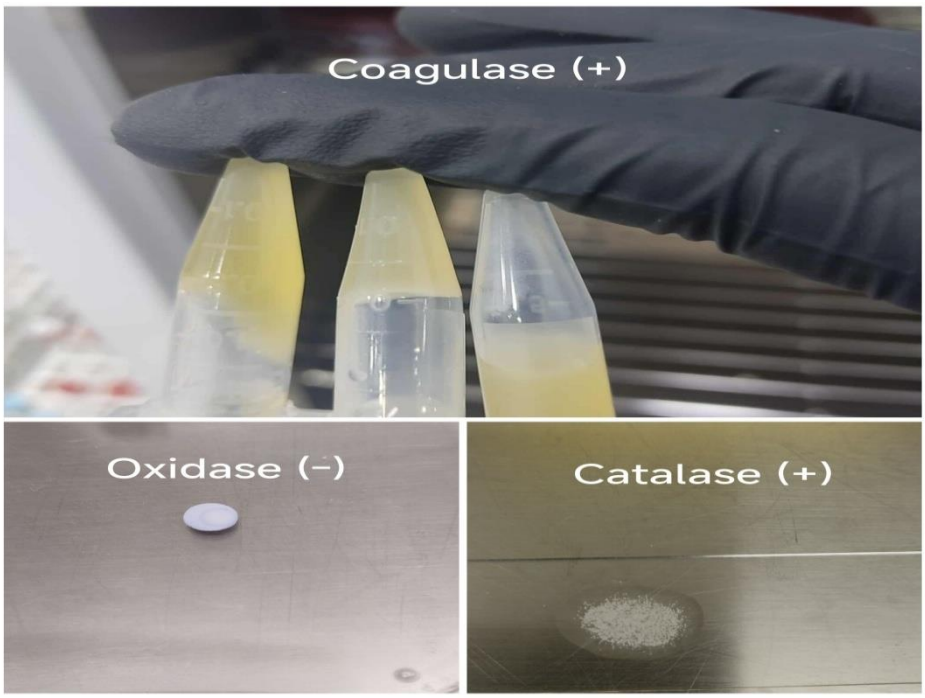

Figure (3-4) Some biochemical tests.

Table (3-6) Biochemical tests of bacteria.

Bacteria / Test	*K. pneumoniae*	*K. oxytoca*	*S. epidermidis*	*S. aureus*
Gram stain	-	-	+	+
Oxidase reagent	-	-	-	-
Catalase reagent	+	+	+	+
Coagulase test	*	*	-	+
Indole test	-	+	*	*
Hemolysis	-	-	-	+
MSA agar	*	*	-	+

(-) Negative test, (+) Positive test, *Not applied.

3-6 Bacterial resistance to antibiotics

To test the sensitivity of isolated bacterial species to antibiotics, and determine their MIC value automatically using the MA 120 device from the Chinese company

Render, located in Al-Mazn Hospital in Zintan, through which the number of antibiotics resistant to the tested bacteria was determined.

***K. pneumoni*ae:** Chloramphenicol, Moxifloxacin, Trimethoprim, Amoxicilline, Minocycline, Ciprofloxacine, Levofloxacine and Ampicillin.

***K. oxytoca*:** Cefazolin, Chloraamphenicol, Ceftazidime, Ticarcillin, Moxifloxacin, Cefuroxime, Trimethoprim, Amoxicilline, Cefotaxime, Cefoxitine and Ampicillin.

***S. aureus* (MRSA):** Azithromycin, Clarithromycin, Penicillin, Oxacillin, Erythromycin and Clindamycin.

S. epidermidis: Penicillin, Oxacillin, Tobramycin and Gentamicin.

3-7 Effect of essential oil of *A. herba alba* and *T. capitatus*

The results in Table (3-7) and Figures (3-5, 3-6) indicate the antimicrobial activity of *T. capitatus* essential oils on the tested bacteria. The average values of the inhibition zone diameters (IZD) were between 20.66±0.57 to 28.33±1.52 mm.

The results showed that the average IZD for *S. aureus* bacteria was (23.33±1.52 mm) and *K. pneumoniae* bacteria was (23.66±1.52 mm) which makes them sensitive to the *T. capitatus* essential oil compared to bacteria *K. oxytoca* which is less inhibited with an average IZD of (20.66±0.57 mm). The highest average IZD was for bacteria *S. epidermidis* (28.33±1.52 mm) compared to *K. oxytoca* bacteria which is less inhibited.

Thus, the antibacterial effects shown by *T. capitatus* essential oil were significant compared to *K. oxytoca*, which means that there are significant differences between the bacteria at the probability level ($P<0.05$).

The results in Table (3-7) indicate the antimicrobial activity of *A. herba alba* essential oil on the tested bacteria and the average inhibition zone diameters (IZD) were between 8.00±0.00 to 11.33±1.52 mm.

The results showed that the average IZD for *S. aureus* bacteria was (10.00±1.73 mm) and *K. oxytoca* bacteria was (8.66±0.57 mm) thus it was sensitive to *A. herba alba* essential oil compared to *K. pneumoniae* bacteria which was less inhibited with an average IZD of (8.00 ±0.00 mm). The highest average IZD was for *S. epidermidis*

bacteria (11.33±1.52 mm) compared to *K. pneumoniae* bacteria, which was less inhibited.

Thus, the antibacterial effects shown by *A. herba alba* essential oil were significant compared to *K. pneumoniae*, which means that there are significant differences between the bacteria at the probability level (P<0.05).

Through the results obtained for the two plants, it was shown that *T. capitatus* has a greater inhibitory effect against all types of tested bacteria than *A. herba alba*, and the greatest effect was on *S. epidermidis* bacteria (28.33±1.52 mm), and the highest effect of *A. herba alba* was on bacteria (11.33±1.52 mm), Figures (3-5, 3-6).

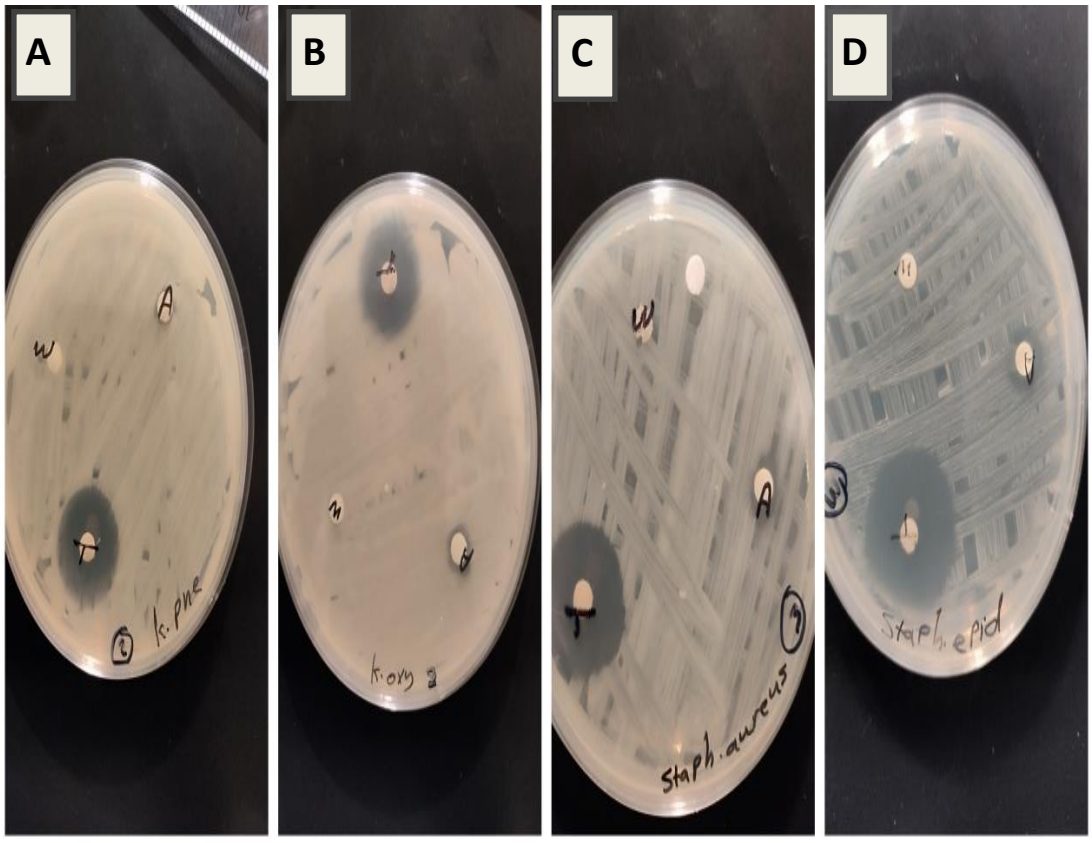

Figure (3-5) Antibacterial zone (mm) of growth inhibition of *T. capitatus* essential oil and *A. herba alba* essential oil.**A**. *K. pneumoniae*, **B**. *K. oxytoca*, **C**. *S. aureus*, **D**. *S. epidermidis*.

Table (3-7) Antibacterial zone of growth inhibition (Mean±SD, mm) of *T. capitatus* essential oil and *A. herba alba* essential oil.

Bacteria	*T. capitatus* essential oil	*A. herba alba* essential oil
K. pneumoniae	23.66±0.52*	8.00 ±0.00*
K. oxytoca	20.66±1.57*	8.66±0.57*
***S. aureus* (MRSA)**	23.33±1.52*	10.00±1.73
S. epidermidis	28.33±1.52*	11.33±1.52*

Values represent Mean±SD of IZD of triplicates, * (L.S.D) at $P<0.05$.

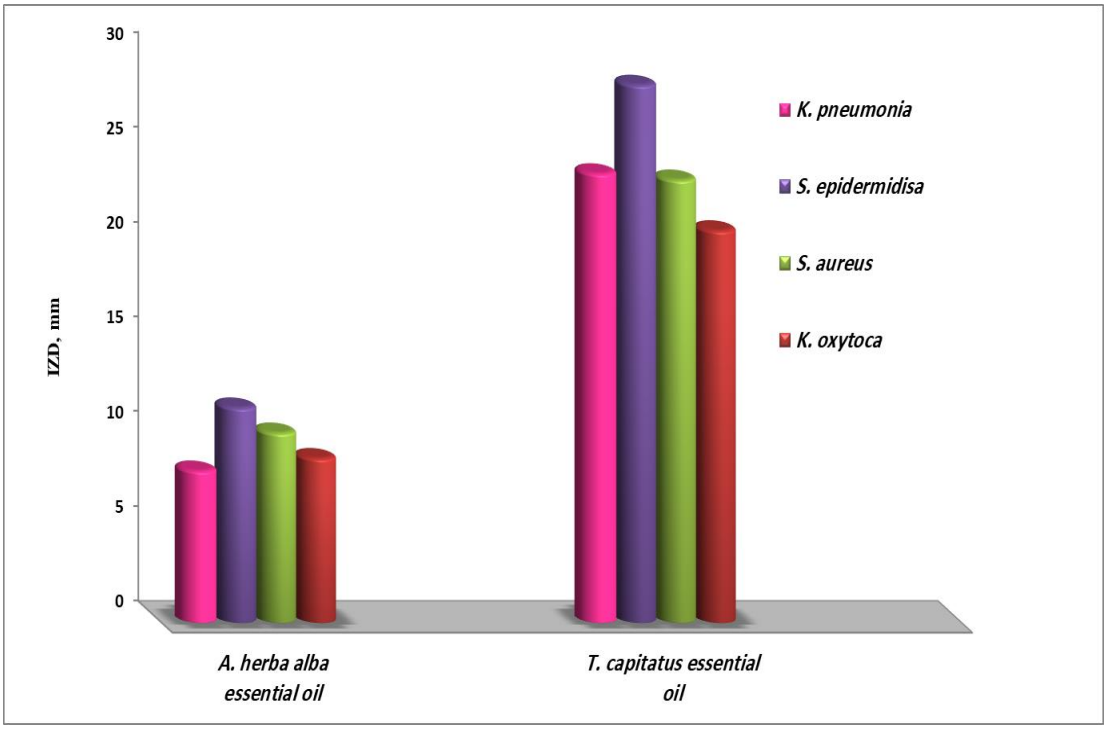

Figure (3-6) Values antibacterial zone of growth inhibition of *T. capitatus* essential oil and *A. herba alba* essential oil.

3-8 Effect of aqueous and alcoholic extract of *T. capitatus*

The results of the aqueous extract of *T. capitatus*, as shown in Table (3-8), indicates that the effect of different concentrations had no effect on each of the bacteria *K. pneumoniae, K. oxytoca, S. aureus.* As for *S. epidermdis,* its effect was at the highest concentration (300 mg/ml) with an average (IZD) of (9.00±1.00 mm). As for the concentrations (100-200 mg/ml) they had no effect on these bacteria. Therefore for the aqueous extract it had the least effect on the tested bacteria. This means that there are significant differences between the isolated bacteria at the probability level of ($P<0.05$). The results confirmed that the alcoholic extract of *T. capitatus* as shown in Table (3-8) does not have inhibitory activity against *K. pneumoniae, K. oxytoca, S. aureu*s bacteria, while the results showed that the alcoholic extract of *T. capitatus* had inhibitory activity against *S. epidermdis* bacteria at all concentrations and its effect was direct as the concentration increased the inhibition increased. At concentration (100 mg/ml) the average (IZD) was (8.00±0.00 mm) concentration (200 mg/ml) the average (IZD) was (11.00±0.00 mm), and concentration (300 mg/ml) the average (IZD) was (13.66±2.88 mm). Therefore, there are significant differences between the bacteria and the concentration of the *Thymus* extract using methanol solvent at the probability level of ($P<0.05$).

Table (3-8) Effect of aqueous and alcoholic extracts of *T. capitatus* leaves against bacteria.

Bacteria	**Aqueous extract of *T. capitatus***			**Alcoholic extract of *T. capitatus***		
Con (mg/ml)	**100**	**200**	**300**	**100**	**200**	**300**
***K. pneumonia*e**	-	-	-	-	-	-
K. oxytoca	-	-	-	-	-	-
***S. aureu*s (MRSA)**	-	-	-	-	-	-
***S. epidermidi*s**	-	-	9.00±1.00*	8.00±0.00	11.00±0.00	13.66±2.88*

Values represent Mean±SD of IZD for triplicates, * (L.S.D) at *P<0.05.* (-) No inhibition zone, (Con) Concentration.

3-9 Effect of aqueous and alcoholic extract of *A. herba alba*

The results of the aqueous extract of *A. herba alba*as shown in Table (3-9) against both *K. pneumoniae* and *K. oxytoca* bacteria showed no sensitivity to all concentrations used and did not inhibit their growth. As for *S. aureus* bacteria, the results showed that it was affected at a concentration of (300 mg/ml) and the average (IZD) was (8.66±0.52 mm) while the concentrations of (100-200 mg/ml) did not show any sensitivity towards it and did not inhibit its growth. *S. epidermdis* bacteria at the same concentration with a value of (8.33±0.57 mm). Therefore, the statistical results for this extract have significant differences at a probability level of (P<0.05), between the bacteria and the concentration of the extract. The results showed that the alcoholic extract of *A. herba alba* as shown in Table (3-9) had no inhibitory activity against *K. pneumoniae and K. oxytoca* bacteria at all concentrations used. *S. aureus* bacteria were affected at a concentration of (300 mg/ml) with an average (IZD) of (4.00±0.00 mm) while at concentrations of (100 -200 mg/ml) they showed no sensitivity and did not inhibit their growth. *S. epidermidis* bacteria were affected at a concentration of (200 mg/ml) with an average (IZD) of (13.00±0.00 mm). At a concentration of (300 mg/ml) the average (IZD) was (7.66±1.15 mm). Therefore, the results showed significant differences between the bacteria and the concentration of the thyme extract using the methanol solvent at the probability level of (P<0.05).

Table (3-9) Effect of aqueous and alcoholic extracts of *A. herba alba* leaves against bacteria.

Bacteria	**Aqueous extract of *A. herba alba***			**Alcoholic extract of *A. herba alba***		
Con (mg/ml)	**100**	**200**	**300**	**100**	**200**	**300**
***K. pneumoni*ae**	-	-	-	-	-	-
K. oxytoca	-	-	-	-	-	-
***S. aureu*s (MRSA)**	-	-	8.66±1.52*	-	-	10.00±0.00*
***S. epidermidi*s**	-	-	8.33±0.57*	-	10.00±0.00*	13.66±1.15*

Values represent Mean±SD of IZD for triplicates, * (L.S.D) at $P<0.05$. (-) No inhibition zone, (Con) Concentration.

Chapter 4

Discussion

Discussion

4-1 Discussion

Antimicrobial agents are critical in reducing the global burden of infectious diseases. However, the emergence and spread of multidrug-resistant strains of pathogenic bacteria has become a major threat to public health (Manandhar et al., 2019). Due to the increasing ineffectiveness of treatment regimens, and consequently, the increase in morbidity and mortality rates and healthcare costs, the situation is further complicated in developing countries by the lack of effective surveillance systems, laboratory diagnostics and the difficulty of obtaining appropriate antimicrobials under financial constraints. Therefore, in light of the evidence of the rapid global spread of resistant clinical isolates, the need to find new alternative antimicrobial agents is of paramount importance. If successful intervention efforts are not made in the field of research on new drugs, the death toll will rise to ten million (Kebede et al., 2021).

Medicinal plants are considered the best source for obtaining a variety of medicines and many medicinal plants have been used for their antimicrobial properties. The main strength of plant natural products lies in their rich and unique chemical diversity that is produced in the secondary metabolism of the plant, their global distribution, ease of access, diversity of antimicrobial modes of action and proven clinical efficacy of plant extracts isolated from them and found in different parts of plants (Porras et al., 2020). Rich in a wide range of secondary metabolites such as phenols, alkaloids, terpenes, tannins, saponins and other components, these substances are biologically active as innovative disease-preventing agents including antimicrobial, antiviral, antioxidant and anti-inflammatory properties (Đurović et al., 2022).

Through the results of our study of *Thymus* and *Artemisia* plants to test their effect on antibiotic-resistant bacteria, the yield of plant for *Artemisia* essential oils was 2%, which was less than the found in *Artemisia* Jordan (karak) 4.41%, and thus our results do not agree with the study Dmour et al. (2024). The aqueous extract was 7.3%, which was close to the results of Benmeziane et al. (2023) 12.2%, and the alcoholic extract was 20.76% for our study, which is not consistent with the results of Benmeziane et al. (2023) for *Artemisia* collected from (Amman) Jordan, and the

Thymus plant had a yield of 3.4% for its essential oil in our study, while in the study of Tagnaout et al. (2022) 2.6% in Morocco.The aqueous extract of *Thymus* in our study was 7.44%, which is close to the results of the study Martins et al. (2015) 12.11%, and the alcoholic extract in our study was 10.62%, which was consistent with the study Benoutman et al. (2022) 11.2%. Many factors, including environmental differences, extraction technique, and environmental conditions, may be related to the reason for the difference in the production rates of essential oil (Dmour et al., 2024). There are also differences in the extracted oil, both quantitatively and qualitatively, for different stages of the plant during vegetative growth, the beginning of flowering, full flowering, and during the fruit and seed formation stage (El-Jalel et al., 2018).

When examining the properties of the extracts, it was found that the pH value of the alcoholic extracts is higher than the aqueous extracts, and that the high pH helps to increase the solubility of the active ingredients (AL-Ani & Haleem, 2014). When chemically examining the aqueous extract of *Thymus*, which contained phenols, alkaloids, flavonoids, terpenes, and saponins, the alcoholic extract contains phenols and saponins, and the results were different from the study Rhuma et al. (2018) which lacks phenolic and saponins in both the aqueous and alcoholic extracts. The reason for the discrepancy is attributed to the difference in the dielectric constant of these solvents. As for the *Artemisia* plant, our results for the aqueous extract were phenolic and saponin compounds, while for the alcoholic extract, the compounds were phenolics, tannins, terpenes and saponins, our results were consistent with the results of Benmeziane et al. (2023).

Through the results obtained for bacteria from cultural diagnosis, microscopic examination and biological tests for the genus *Staphlyococcus,* our results were positive and consistent with the study of Rasigade & Vandenesch (2014); AL-Joda & Jasim (2021), and the genus *Klebsiella* were consistent with the study of Sarma et al. (2020); Abd Al-Hassan et al. (2023). As for the diagnosis in the MA120 device for bacterial isolates, it has been shown that it is a fast and accurate technique that must be used in the laboratory diagnosis of microorganisms. It can also diagnose the appropriate antibiotic for each pathogenic microorganism through an automatic sensitivity test, thus saving time in determining the identity and sensitivity to antibiotics at the same time.

As a result of the continuous use of antibiotics, this has led to the emergence of strains of bacteria resistant to antibiotics, which makes the infection difficult to treat despite the production of new generations of antibiotics. Antibiotics are classified into two groups in terms of their effect: either theyare bacteriostatic or bacteriocidal. They can also be classified into two categories based on their spectrum of activity: narrow-spectrum antibiotics, which affect only one group of bacteria, either Gram-negative or Gram-positive, and broad-spectrum antibiotics, which affect both Gram-positive and Gram-negative bacteria. There are four main sites of action of antibiotics: they may be inhibitors of cell wall synthesis, such as penicillins, cephalosporin, and vancomycin, or they may work to destroy the permeability of the cytoplasmic membrane, such as polymyxin, or they may be inhibitors of protein synthesis, such as amikacin and gentamicin, or they may be inhibitors of DNA synthesis, such as ciprofloxacin (Gupte, 2010).

The results of our study of the shared antibiotic resistance of both species of the genus *Klebisella* were the following antibiotics: moxifloxacin, ampicillin, amoxicilline, and trimethoprim, which were consistent with the study of Rawy et al. (2020); Mustafa et al. (2023). The reason for multi-resistance is attributed to the presence of resistance genes located on the bacterial chromosome, as well as a change in the permeability barrier, which makes it difficult for the antibiotic to pass through and reach its site of action, which is specific to Gram-negative bacteria, as the outer membrane contains protein channels called porin, which work to prevent the entry of antibiotics into the bacterial cell. The production of biofilms is one of the most important mechanisms of resistance, and thus it is a major source of concern, and often leads to therapeutic failure, as it provides protection for bacteria (Abbas et al., 2024).

Our results for the genus *Staphylococcus* were that they are resistant to antibiotics common to both species, namely oxacillin and penicillin, and thus are resistant to methicillin (MRSA). The reason for the high resistance of the genus *Staphylococcus* is attributed to the bacteria possessing beta-lactamase enzymes that degrade the penicillin group, whose genes are either chromosomal or plasmid in origin. These bacteria also produce penicillin-binding proteins (PBPs) located in the cytoplasmic membrane that is linked to the cell wall. These proteins are a target for both penicillin and cephalosporin antibiotics, as they change the target site of beta-lactam antibiotics,

which results in bacterial resistance to them (Hou et al., 2023). The results of our study are consistent with the findings of the study by Nomura et al. (2020).

During the study of the effect of *Thymus* on bacteria, the diameter of inhibition of *T. capitatus* essential oil extract for *K. oxytoca* bacteria was (20.66±0.57 mm), which is consistent with the study of Tagnaout et al. (2022), due to the presence of phenolic compounds carvacrol, thymol, carvacrol methl ether rich in the oil, while it differs from a previous study by El-Jalel et al. (2018), the inhibition was greater with a diameter of 50 mm, which was collected from Jabal Al-Akhdar, Libya. The diameter of inhibition for *K. pneumoniae* bacteria was (23.66±1.52 mm), and these results are not consistentwith the study of Tagnaout et al. (2022), which had a diameter of inhibition of 49 mm collected from Morocco. *S. epidermidis* bacteria have a large inhibition diameter (28.33±1.52 mm) due to the presence of monoterpene and phenolic compounds. These results are similar to the study of Aouadhi *et al*. (2024) and the inhibition was small compared to the study of El-Jalel et al. (2018) which had higher results with an inhibition diameter of 42 mm. While *S. arueus* bacteria have an inhibition diameter (23.33 ±1.52 mm) as the presence of phenolic compounds in large quantities leads to antibacterial activity. These results are similar to the study of Aouadhi et al. (2024) which was the effect of oil in his study by 28 mm.

When studying the effect of *Artemisia* on bacteria, the effect of *A. herba alba* essential oil was moderate compared to *T. capitatus* essential oil, which was more effective on antibiotic-resistant bacteria. The result for *K. oxytoca* bacteria (8.66±0.57 mm) was due to the presence of thujone, camphor, and camphene, close to a previous study by Sara et al. (2021) with an inhibition diameter of 9 mm, while the study Ouguirti et al. (2021) differed from our study with an inhibition diameter of 21 mm due to the difference in the concentration of active ingredients, as the α-thujone compound was 48%. The inhibition diameter for *K. pneumoniae* bacteria (8.00±0.00 mm) did not match what was indicated by Bertella et al. (2018), which wasan inhibition diameter of 31 mm, and the aerial parts were collected in October during the flowering stage from eastern Algeria. *S. epidermidis* has a large inhibition diameter (11.33±1.52 mm) in agreement with the study of Bouhouia et al. (2020), which was collected at the flowering stage in July from Souk Ahras, Algeria. While *S. arueus* has an inhibition diameter (10.00±1.73 mm) in agreement with the study of Bertella et al. (2018).

Our study showed that thyme was more effective compared to *Artemisia* and *Thymus* essential oil was confirmed to be the most effective antimicrobial against (*Staphylococcus* & *Klebsiella*) due to its effective effect in extracting active ingredients, as the results of quantitative and qualitative analysis by GC-MS of *Thymus* essential oils in our study for the compound Thymol 9.963% were close to the study of Said et al. (2016) collected from the city of Zintan, Libya, whose results were 12.29%, while inconsistent with the compound Carvacrol at 13.593%, and their results were 68.19%, while in our study the main compound of thyme was Carvacrol methyl ether at 53.208%, and in the study of Mohyeldin et al. (2023) where thyme was collected from coastal cities in Libya, Carvacrol was 1.52%, while Thymol was 89.38%.As for the *Artemisia* plant, when analyzed by a quantitative and qualitative GC-MS device, the result of the Camphor compound was 30.527% in our study close to the study Mohyeldin et al. (2023) which was 34.84%. The Thujone compound in our study had a high percentage of 22.471% compared to what was reached Bouhouia et al. (2020) which was 0.16%. while it differed with the Camphene compound in our study, which was 10.291%, while the study of Mohyeldin et al. (2023) showed a small percentage of 1.97%, while the 3-Carene compound was 7.322% which does not agree with the study of Bouhouia et al. (2020) in the city of Bejaia, Algeria, which was 0.16%, and it was close in the study of Bekka-Hadji et al. (2022) in Bejaia, Algeria, for the Camphor compound 32%, while the following compounds were at a small percentage compared to our study: Camphene 3.6% and the 3-Carene compound 0.2%.

Aqueous and alcoholic extracts of *T. capitatus* showed selective inhibitory activity against the growth of *S. epidermidis* bacteria. The result was in the aqueous extract at a concentration of (300 mg/ml) with an inhibition diameter of (9.00±1.00 mm). This is due to the presence of secondary compounds of phenols, alkaloids, flavonoids, terpenes and saponins, which have an antibacterial effect (Maniki et al., 2023). This is consistent with a study conducted by Messaoudi et al. (2019) for *T. vulgaris*. As for the alcoholic extract, inhibition was at all concentrations used, but the greatest effect was at a concentration of (300 mg/ml) with a diameter of (13.66±2.88 mm). It was found that the sensitivity of the bacteria increases with increasing concentration. This is consistent with the study of Martins et al. (2015). This is attributed to the presence of phenolic and saponin compounds in the alcoholic extract (Maniki et al., 2023).

When testing the effect of aqueous and alcoholic extracts of *A. herba alba*, no effect was shown on the growth of Gram-negative bacteria (*K. pneumonia, K. oxytoca*). This effect was consistent with the study of Abdel-Monem et al. (2021) for the aqueous extract, while in a study conducted in northeastern Algeria in which *A. herba alba* was collected in November by Saida et al. (2024) it gave an inhibition with a diameter of 6 mm. Our results also agree with the alcoholic extract, which had no effect, with Jasim & El-Zayat (2019). However when comparing our results with the study of Hafidh et al. (2022) for the methanol extract, the inhibition diameter was 11 mm, in which the plant was collected in January in Algeria. This discrepancy is due to the nature of the bacteria, the extraction method, and the concentration of active substances in the extract (Bordean et al., 2023). Regarding positive bacteria, the aqueous and alcoholic extracts of *A. herba alba* showed a clear inhibitory activity against bacteria (*S. aureus* & *S. epidermidis*). The results for *S. aureus* bacteria for the aqueous extract were weak at a concentration of (300 mg/ml) with a diameter of (8.66±0.52 mm), which was not consistent with what Hassan & Hadi (2025) indicated, as the aqueous extract contains saponins and phenols that have an antibacterial effect (Mahboub et al., 2024). As for the alcoholic extract, its highest inhibition was at a concentration of (300 mg/ml) with a diameter of (10.00±0.00 mm) due to the presence of phenols, terpenes, saponins and tannins that have an important role in inhibiting bacteria. The results were close to the study of Benmeziane et al. (2023) as the plant was collected in May from Amman, Jordan, and its inhibition was 8 mm. As for *S. epidermidis* bacteria, the highest inhibition was in the alcoholic extract at a concentration of (300 mg/ml) with a diameter of (13.66±1.15 mm). These results are agreement with what was indicated by Jasim & El-Zayat (2019).

It is noted that negative bacteria were more resistant to both the aqueous and alcoholic extracts of the studied plants. This is due to the composition of the bacterial cell wall, which is thick in negative bacteria and consists of several lipid, protein, and sugar layers that work to prevent the entry of chemicals into the cell (Suliman & Alnass, 2025). While the aqueous and alcoholic extracts had an inhibitory effect on positive bacteria by affecting the selective permeability of the membrane, which leads to destabilization of the cellular structure, weakening the integrity of the membrane, and increasing permeability, which leads to rupture of the cell membrane. It also depends on the type of chemical elements present in the extracts and their mechanism of action

on bacteria. Plant compounds affect the flow pumps of bacteria and produce substances that inhibit the work of the pumps, which facilitates the entry of active substances into the cell (Dmour et al., 2024).

The most important aspect that must betaken into account is the extraction efficiency, as it is affected by solvents, their chemical composition, concentration, polarity, pH, temperature, time, and the ratio of solvent to sample, because one solvent cannot extract all active substances reliably (Nawaz et al., 2020). The molecular affinity between the solute and the solvent may also affect the extractability. Traditional extraction methods are considered to require a long time, a large amount of solvent, low extraction yield, and reduced selectivity, they are sometimes exposed to excess oxygen, heat, and light, which leads to their subsequent deterioration (Tzima et al., 2018). Climate changes and environmental stresses such as salinity and drought also affect the active ingredients. Therefore, it can be concluded that the composition of the soil changes the pressures on the physical and chemical processes of plant compounds (Tamma et al., 2024). The quality of the active ingredients is also affected by the age of the plant, the harvest period, and the drying and extraction techniques (Tagnaout et al., 2022).

4-2 Conclusion

While studying the effect of plant extracts (*A. herba alba* and *T. capitatus*) on antibiotic-resistant bacteria, four resistant bacteria (*K. pneumoniae, K. oxytoca, S. aureus* and *S. epidermidis*) The isolated bacteria were tested using a disc diffusion method were isolated. Essential oil was extracted by by hydrodistillation using a Clevenger apparatus. The greatest antimicrobial activity was observed against *S. epidermidis*, with an inhibition zone (28.33±1.52 mm) for the essential oil of *T. capitatus*, while *A. herba alba* effect was the highest at the same bacteria showing an inhibition zone of (11.33±1.52 mm). The essential oil was also analyzed using GC-MS to determine their quantitative and qualitative components. *T. capitatus* It is Carvacrol methyl ether at a rate of 53.208%, Carvacrol compound at a rate of 13.593%, and Thymol compound at a rate of 9.963%, the primary compound, while *A. herba alba* was a compound, Camphor 30.527%, Thujone 22.471%, Camphene 10.291%, and 3-Carene 7.322%. Regarding the extracts of the *Thymus* plant, the greatest antibacterial activity against *S. epidermidis* was observed at a concentration

of (300 mg/ml), with the aqueous extract producing an inhibition zone diameter of (9.00±1.00 mm) and the alcoholic extract showing a larger inhibition zone of (13.66±2.88 mm). The *Artemisia herba alba* extracts exhibited activity primarily against Gram-positive bacteria. At the same concentration (300 mg/ml), the aqueous extract inhibited *S. aureus* with a diameter of (8.66±1.52 mm), while the alcoholic extract showed an inhibition diameter of (10.00±0.00 mm). For *S. epidermidis*, the aqueous extract had an inhibition zone of (8.33±0.57 mm) compared to (13.66±1.15 mm) for the alcoholic extracts. Phytochemicals screening of both aqueous and alcoholic extracts from the two plants revealed the presence of phenols and saponins as the active compounds.

4-3 Recommendations

- Do not use antibiotics unless the pathogens have been tested for antibiotic sensitivity.
- Future studies should focus on the biological activity and successful extraction methods of active ingredients using advanced devices for purifying active ingredients that affect bacteria, which play an important role in traditional medicine in Libya.
- Molecular investigation of virulence factors of antibiotic-resistant isolates used in this study, which were stored at the Center for Tropical and Transboundary Diseases Research.
- Conducting a study on the effect of plant extracts on pathogenic bacteria in laboratory animals.

Chapter 5

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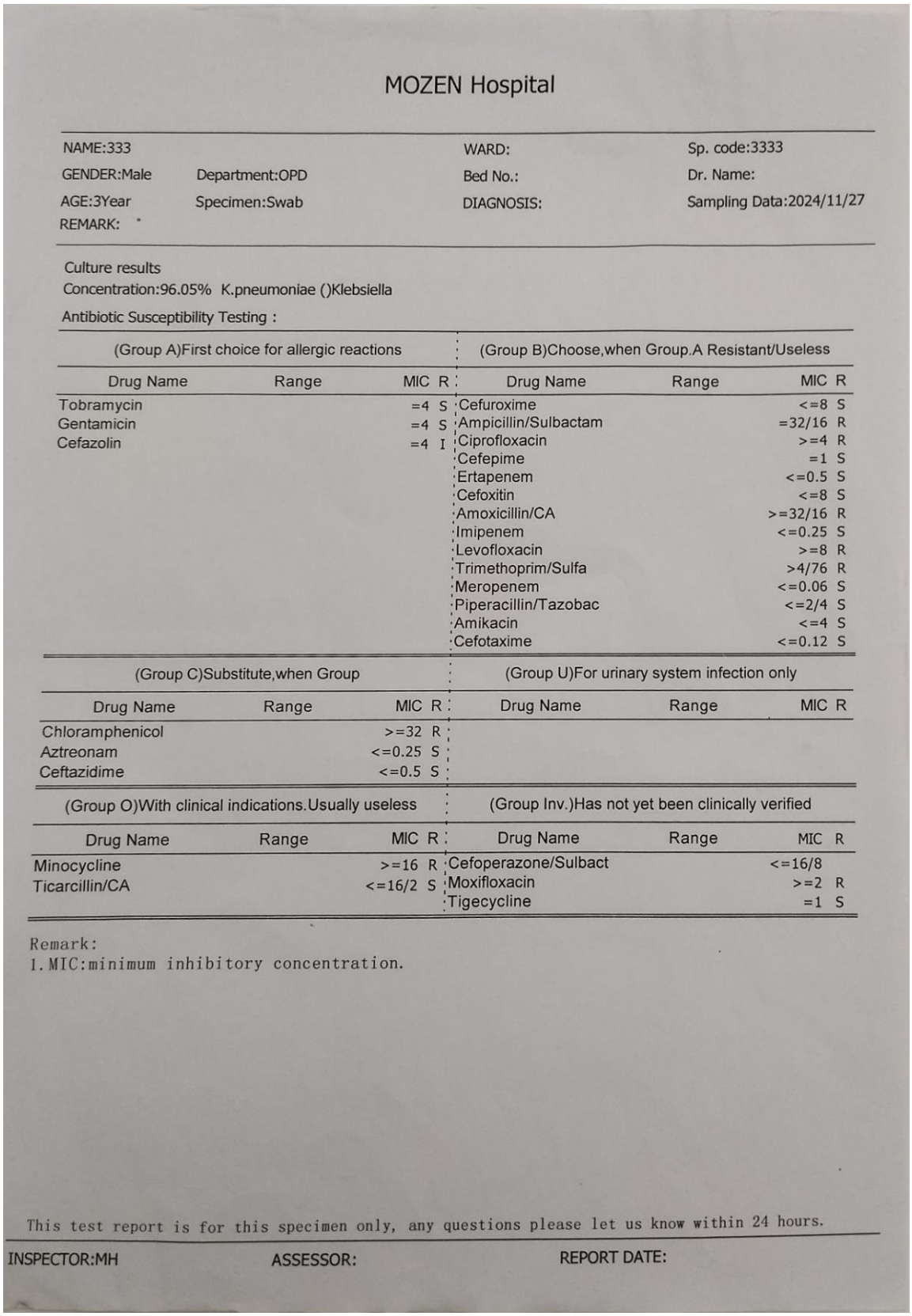
Chapter 6

Appendixes

Appendix 1



MOZEN Hospital

NAME:333 WARD: Sp. code:3333

GENDER:Male Department:OPD Bed No.: Dr. Name:

AGE:3Year Specimen:Swab DIAGNOSIS: Sampling Data:2024/11/27

REMARK:

Culture results

Concentration:96.05% K.pneumoniae ()Klebsiella

Antibiotic Susceptibility Testing :

(Group A)First choice for allergic reactions

Drug Name	Range	MIC	R
Tobramycin		=4	S
Gentamicin		=4	S
Cefazolin		=4	I

(Group B)Choose,when Group.A Resistant/Useless

Drug Name	Range	MIC	R
Cefuroxime		<=8	S
Ampicillin/Sulbactam		=32/16	R
Ciprofloxacin		>=4	R
Cefepime		=1	S
Ertapenem		<=0.5	S
Cefoxitin		<=8	S
Amoxicillin/CA		>=32/16	R
Imipenem		<=0.25	S
Levofloxacin		>=8	R
Trimethoprim/Sulfa		>4/76	R
Meropenem		<=0.06	S
Piperacillin/Tazobac		<=2/4	S
Amikacin		<=4	S
Cefotaxime		<=0.12	S

(Group C)Substitute,when Group

Drug Name	Range	MIC	R
Chloramphenicol		>=32	R
Aztreonam		<=0.25	S
Ceftazidime		<=0.5	S

(Group U)For urinary system infection only

Drug Name	Range	MIC	R

(Group O)With clinical indications.Usually useless

Drug Name	Range	MIC	R
Minocycline		>=16	R
Ticarcillin/CA		<=16/2	S

(Group Inv.)Has not yet been clinically verified

Drug Name	Range	MIC	R
Cefoperazone/Sulbact		<=16/8	
Moxifloxacin		>=2	R
Tigecycline		=1	S

Remark:

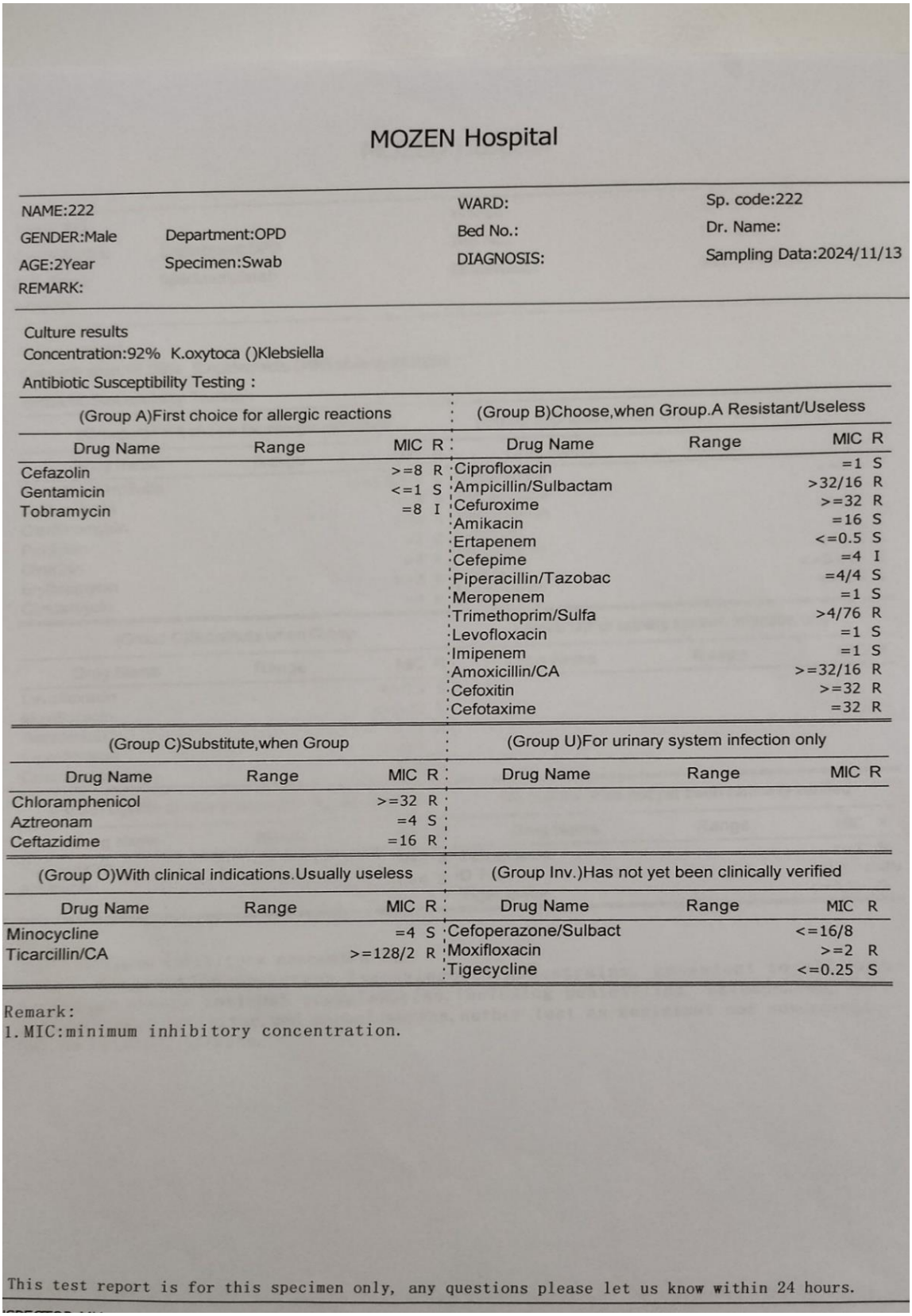
1.MIC:minimum inhibitory concentration.

This test report is for this specimen only, any questions please let us know within 24 hours.

INSPECTOR:MH ASSESSOR: REPORT DATE:

The result of testing the sensitivity of *K. pneumoniae* bacteria to antibiotics by MA120 device.

Appendix 2



MOZEN Hospital

NAME:222 | WARD: | Sp. code:222
GENDER:Male | Department:OPD | Bed No.: | Dr. Name:
AGE:2Year | Specimen:Swab | DIAGNOSIS: | Sampling Data:2024/11/13
REMARK:

Culture results
Concentration:92% K.oxytoca ()Klebsiella

Antibiotic Susceptibility Testing :

(Group A)First choice for allergic reactions

Drug Name	Range	MIC	R
Cefazolin		>=8	R
Gentamicin		<=1	S
Tobramycin		=8	I

(Group B)Choose,when Group.A Resistant/Useless

Drug Name	Range	MIC	R
Ciprofloxacin		=1	S
Ampicillin/Sulbactam		>32/16	R
Cefuroxime		>=32	R
Amikacin		=16	S
Ertapenem		<=0.5	S
Cefepime		=4	I
Piperacillin/Tazobac		=4/4	S
Meropenem		=1	S
Trimethoprim/Sulfa		>4/76	R
Levofloxacin		=1	S
Imipenem		=1	S
Amoxicillin/CA		>=32/16	R
Cefoxitin		>=32	R
Cefotaxime		=32	R

(Group C)Substitute,when Group

Drug Name	Range	MIC	R
Chloramphenicol		>=32	R
Aztreonam		=4	S
Ceftazidime		=16	R

(Group U)For urinary system infection only

Drug Name	Range	MIC	R

(Group O)With clinical indications.Usually useless

Drug Name	Range	MIC	R
Minocycline		=4	S
Ticarcillin/CA		>=128/2	R

(Group Inv.)Has not yet been clinically verified

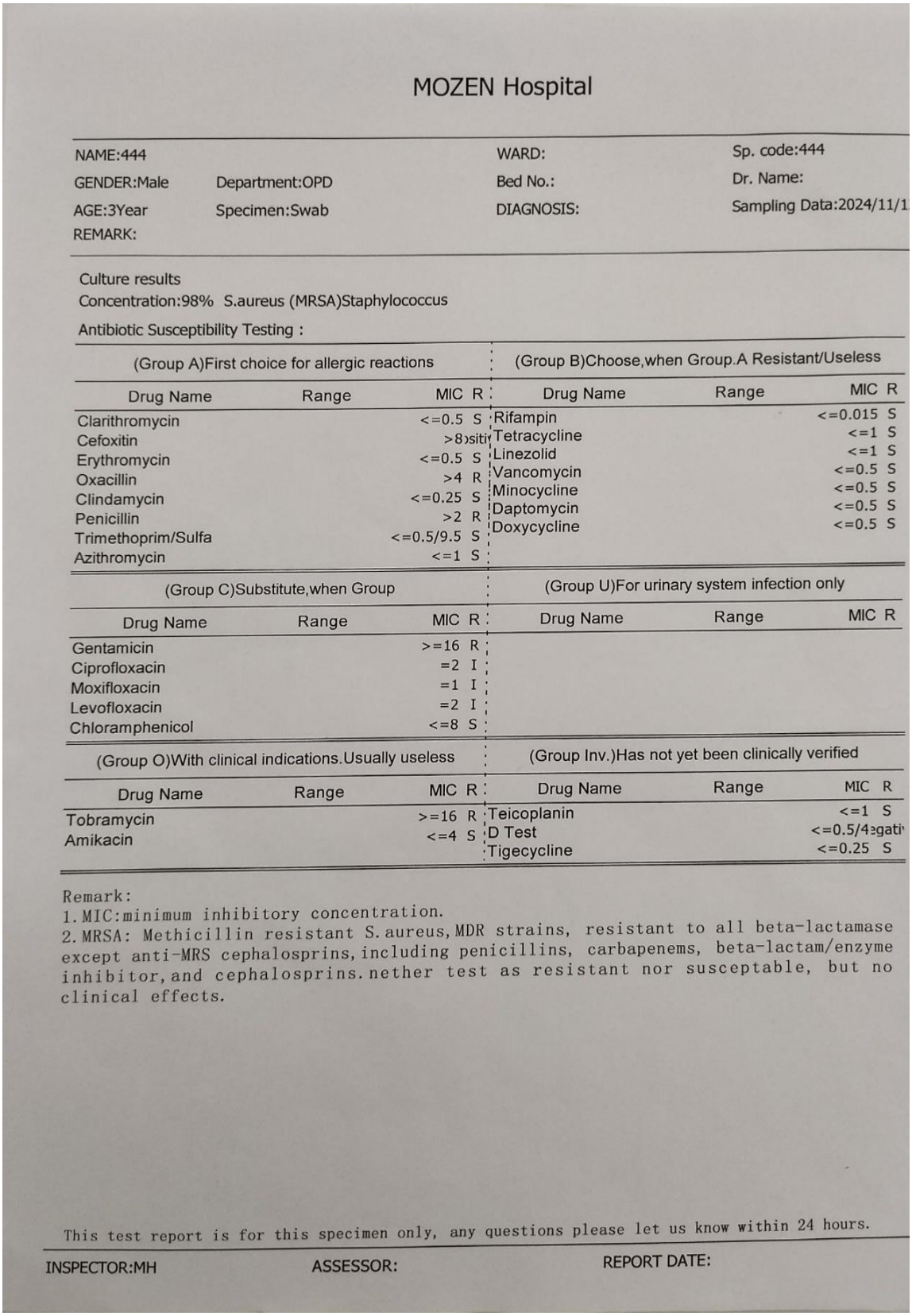
Drug Name	Range	MIC	R
Cefoperazone/Sulbact		<=16/8	
Moxifloxacin		>=2	R
Tigecycline		<=0.25	S

Remark:
1.MIC:minimum inhibitory concentration.

This test report is for this specimen only, any questions please let us know within 24 hours.

The result of testing the sensitivity of *K. oxytoca* bacteria to antibiotics by MA120 device.

Appendix 3



MOZEN Hospital

NAME:444 WARD: Sp. code:444
GENDER:Male Department:OPD Bed No.: Dr. Name:
AGE:3Year Specimen:Swab DIAGNOSIS: Sampling Data:2024/11/1
REMARK:

Culture results
Concentration:98% S.aureus (MRSA)Staphylococcus
Antibiotic Susceptibility Testing :

(Group A)First choice for allergic reactions

Drug Name	Range	MIC	R
Clarithromycin		<=0.5	S
Cefoxitin		>8	siti
Erythromycin		<=0.5	S
Oxacillin		>4	R
Clindamycin		<=0.25	S
Penicillin		>2	R
Trimethoprim/Sulfa		<=0.5/9.5	S
Azithromycin		<=1	S

(Group B)Choose,when Group.A Resistant/Useless

Drug Name	Range	MIC	R
Rifampin		<=0.015	S
Tetracycline		<=1	S
Linezolid		<=1	S
Vancomycin		<=0.5	S
Minocycline		<=0.5	S
Daptomycin		<=0.5	S
Doxycycline		<=0.5	S

(Group C)Substitute,when Group

Drug Name	Range	MIC	R
Gentamicin		>=16	R
Ciprofloxacin		=2	I
Moxifloxacin		=1	I
Levofloxacin		=2	I
Chloramphenicol		<=8	S

(Group U)For urinary system infection only

Drug Name	Range	MIC	R

(Group O)With clinical indications.Usually useless

Drug Name	Range	MIC	R
Tobramycin		>=16	R
Amikacin		<=4	S

(Group Inv.)Has not yet been clinically verified

Drug Name	Range	MIC	R
Teicoplanin		<=1	S
D Test		<=0.5/4	gati
Tigecycline		<=0.25	S

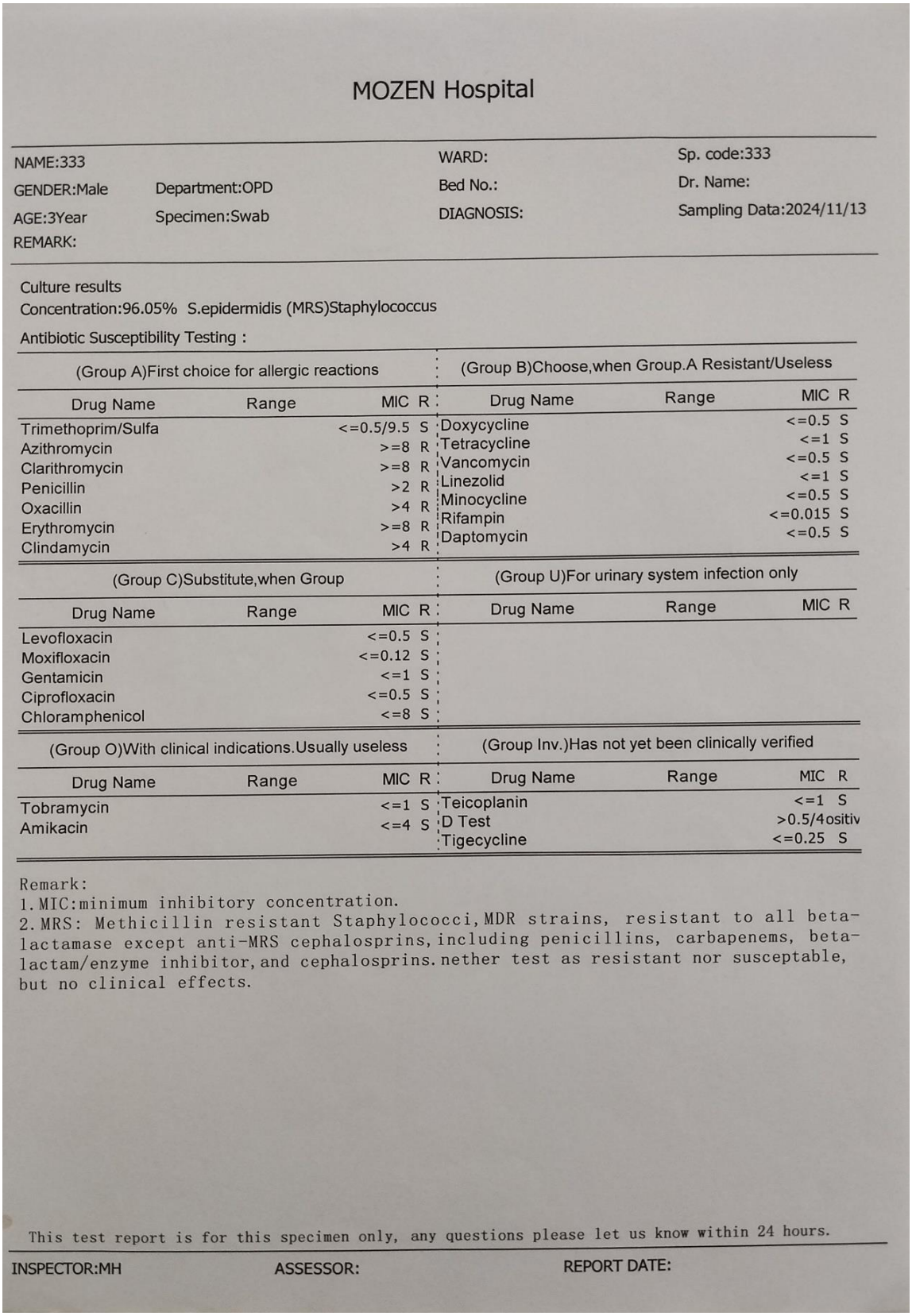
Remark:
1. MIC:minimum inhibitory concentration.
2. MRSA: Methicillin resistant S.aureus, MDR strains, resistant to all beta-lactamase except anti-MRS cephalosprins, including penicillins, carbapenems, beta-lactam/enzyme inhibitor, and cephalosprins. nether test as resistant nor susceptable, but no clinical effects.

This test report is for this specimen only, any questions please let us know within 24 hours.

INSPECTOR:MH ASSESSOR: REPORT DATE:

The result of testing the sensitivity of *S. aureus* bacteria to antibiotics by MA120 device.

Appendix 4



MOZEN Hospital

NAME:333 WARD: Sp. code:333
GENDER:Male Department:OPD Bed No.: Dr. Name:
AGE:3Year Specimen:Swab DIAGNOSIS: Sampling Data:2024/11/13
REMARK:

Culture results
Concentration:96.05% S.epidermidis (MRS)Staphylococcus

Antibiotic Susceptibility Testing :

(Group A)First choice for allergic reactions				(Group B)Choose,when Group.A Resistant/Useless			
Drug Name	Range	MIC	R	Drug Name	Range	MIC	R
Trimethoprim/Sulfa		<=0.5/9.5	S	Doxycycline		<=0.5	S
Azithromycin		>=8	R	Tetracycline		<=1	S
Clarithromycin		>=8	R	Vancomycin		<=0.5	S
Penicillin		>2	R	Linezolid		<=1	S
Oxacillin		>4	R	Minocycline		<=0.5	S
Erythromycin		>=8	R	Rifampin		<=0.015	S
Clindamycin		>4	R	Daptomycin		<=0.5	S

(Group C)Substitute,when Group				(Group U)For urinary system infection only			
Drug Name	Range	MIC	R	Drug Name	Range	MIC	R
Levofloxacin		<=0.5	S				
Moxifloxacin		<=0.12	S				
Gentamicin		<=1	S				
Ciprofloxacin		<=0.5	S				
Chloramphenicol		<=8	S				

(Group O)With clinical indications.Usually useless				(Group Inv.)Has not yet been clinically verified			
Drug Name	Range	MIC	R	Drug Name	Range	MIC	R
Tobramycin		<=1	S	Teicoplanin		<=1	S
Amikacin		<=4	S	D Test		>0.5/4	ositiv
				Tigecycline		<=0.25	S

Remark:
1. MIC:minimum inhibitory concentration.
2. MRS: Methicillin resistant Staphylococci, MDR strains, resistant to all beta-lactamase except anti-MRS cephalosprins, including penicillins, carbapenems, beta-lactam/enzyme inhibitor, and cephalosprins. nether test as resistant nor susceptable, but no clinical effects.

This test report is for this specimen only, any questions please let us know within 24 hours.

INSPECTOR:MH ASSESSOR: REPORT DATE:

The result of testing the sensitivity of *S. epidermidis* bacteria to antibiotics by MA120 device.

الملخص

هدفت هذه الدراسة إلى تقييم تأثير المستخلصات النباتية المائية، الكحولية والزيوت الأساسية لنباتي الشيح والزعتر (*Thymus capitatus* و *Artemisia herba alba*). على البكتيريا المقاومة للمضادات الحيوية، حيث تم اختبار أربعة أنواع من البكتيريا وهي: *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis, Klebsiella pneumoniae, Klebsiella oxytoca* باستخدام طريقة قرص الانتشار، وطبقت التركيزات للمستخلص المائي والكحولي (100، 200، 300 مجم/مل) ، بينما تم استخدام الزيت العطري في شكله النقي. أظهرت نتائج دراسة الفعالية الحيوية لزيوت الأساسية بأن أفضل الكفاءة في التثبيط ضد البكتيريا كان لنبات الزعتر أتجاه *S. epidermidis* الأكثر حساسية و بقطر تثبيط (28.33±52مم)، *S. aureus* (MRSA) (23.33±1.52مم)، *K. pneumoniae* (23.66±1.52مم)، *K. oxytoca* (20.66±0.57مم). تم تحديد المكونات الكيميائية للزيوت الأساسية بأستخدام جهاز GC-MS المركبات الرئيسية لنبات الزعتر كانت كارفاكرول ميثيل الأثير (53.208٪)، كارفاكرول (13.593٪)، المركبات الرئيسية لنبات الشيح كانت الكافور (30.527٪). ثوجون (22.471٪). أما بالنسبة للمستخلصات المائية و الكحولية لنبات الزعتر، كانت بكتيريا *S. epidermidis* الأكثر حساسية وبقطر بلغ (9.00±1.00مم)، (13.66±2.88مم) عند تركيز (300 مجم/مل) لكلا المستخلصين علي التوالي. أما نتائج نبات الشيح فكان تأثيره علي البكتيريا الموجبة الجرام عند نفس التركيز فكانت بكتيريا *S. aureus* (MRSA) أكثر حساسية للمستخلص المائي بقطر تثبيط (8.66±0.52مم)، و كان أفضل تأثير مثبط للمستخلص الكحولي بقطر(13.66±1.55مم) لبكتيريا *S. epidermidis*. وأظهرت نتائج الفحص الكيميائي أن كلا النباتين، في جميع أنواع المستخلصات، يحتويان على مركبات الفينولات والصابونيات ذات النشاط الفعّال. تشير هذه النتائج إلى إمكانية اعتبار مستخلص الزيت العطري مصدرًا واعدًا للمركبات ذات النشاط المضاد للبكتيريا.

كلمات المفتاح: الزعتر، الزيوت الأساسية، الشيح، المستخلص المائي، المستخلص الكحولي.

الأكاديمية الليبية للدراسات العليا

فرع الجبل الغربي-الزنتان

مدرسة: العلوم الأساسية، قسم:علوم الحياة، شعبة: الاحياء الدقيقة

تقييم تأثير المستخلصات النباتية لنباتي الشيح (*A. herba alba*) و الزعتر (*T. capitatus*) في مدينة الزنتان على بعض أنواع البكتيريا المقاومة للمضادات الحيوية

مقدم من

منى شعبان الناجح كريميد

تحت أشراف: د. إيمان ضو امحمد

رسالة مقدمة لنيل درجة الماجستير في علم الأحياء الدقيقة

خريف 2025-2026