An-Najah National University

Faculty of Graduate Studies

ANTIMICROBIAL EFFECT OF PALESTINIAN HONEY FROM DIFFERENT BOTANICAL ORIGIN AGAINST HUMAN PATHOGENIC BACTERIAL AND FUNGAL

By

Haneen Mohammed Kamel Qalalweh

Supervisors

Prof. Raed Alkowni

Prof. Hazem Sawalha

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This Thesis was Defended Successfully on 6/11/2022 and approved by

Prof. Raed Alkowni Supervisor	Signature
Prof. Hazem Sawalha Co-Supervisor	Signature
Dr. Abdalsalam Kmail External Examiner	Signature

Dedication

I dedicate my thesis,

To my husband Hazem for his support, love, patience and encouragement for me.

To my two sons, Ameer and Kareem, for their patience for being away from them because I was busy all the time.

To my parents for their constant prayers and encouragement for me.

To my beloved sister Nadine, who supported me, listened to my troubles, supported me, and gave me advice to reach this stage.

To my brothers for their love and fear for me.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

ANTIMICROBIAL EFFECT OF PALESTINIAN HONEY FROM DIFFERENT BOTANICAL ORIGIN AGAINST HUMAN PATHOGENIC BACTERIAL AND FUNGAL

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: _____

Signature: _____

Date: _____

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Abstract

Background: Because of the increased resistance of certain bacterial and fungal species to various antibiotics, uses of natural base chemicals such as honey and medicinal plants have become increasingly appealing**.** Honey is one of the most well-known natural antibacterial sources documented in ancient medicine.

Objectives: To assess the antibacterial effects against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonellae typhimurium*, and antifungal effects against *Candida albicans* of four Palestinian honey from different botanical origin and determine the antibacterial and antifungal effects of flower botanical origin for honey. The following honey samples will be assessed A. (Ziziphus) B. (Thymus vulgaris) C. (Citrus honey) D. *(*Wildflower honeys).

Methodology: The antibacterial and antifungal effects were measured through assessment of the honey and plant antibacterial effect through; the disc diffusion method which is concerned as the main qualitative test for detecting the susceptibility of bacteria and fungi to antimicrobial substances, broth microdilution assay to determine minimum inhibitory concentration (MIC) assay, which reflects the quantity needed for bacterial and fungal inhibition, and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) which reflects the quantity needed for of bacterial and fungal death respectively.

Results: The present study reveals that local Palestinian honey that differ in botanical origin such as Sidr, Thymus, Multiflowers and Citrus honeys were effective in inhibiting the in vitro growth of *Klebsiella pneumoniae, Escherichia*

coli, Staphylococcus aureus, Salmonellae typhimurium. Multiflowers honey was most potent than other honeys in inhibiting *Klebsiella pneumoniae* and *Staphylococcus aureus* growths where the mic50 values was 177.86±9.75 and 78.08±23 respectively and also Citrus honey was most potent than other honeys in inhibiting *Escherichia coli* and *Salmonellae typhimurium* growths and the mic50 values was 196.72±6.26 and 166.13±2.85 respectively. In vitro all honey samples in the different concentrations were more effective against *Staphylococcus aureus* than the other bacteria. There is no effect of the honeys on *Candida albicans.*

Conclusion: According to the present results, the antibacterial activity of Palestinian honeys depends on their botanical origin, honey can be applied as antimicrobial agent against specific pathogenic bacteria, and the proliferation of bacterial strains is inhibited by these honeys.

Keywords: Honey, Bacteria, Fungi, Botanical origin, Palestine.

Chapter One
Introduction

1. Background

Currently, the only hope for the world to eradicate infectious diseases is antimicrobial agents. However, the pattern of pathogenic microbe resistance to necessary antibiotics, particularly multidrug resistant ones, has changed, which has reduced the potency of known antibiotics. Because the increasing of resistance in the worldwide this poses a very thoughtful risk to elevation of good health. As a result, it's important to assess alternative medicinal agents with antimicrobe characteristics. Natural honey produced by bees is composed of a complex combination of sugars, including fructose and glucose. For ages, it has been utilized as medicine in numerous countries. In recent years, understanding of the use of honey as a therapeutic ingredient has grown, and it is becoming more widely accepted as a treatment for a wide range of illnesses brought on by pathogenic microorganism (Nweze et al., 2016).

1.1 Palestine and Honey

Palestine is considered to have a great biodiversity because of its geophysical features and climate (Jaradat et al., 2016). In spite of its limited area, it has an eastern Mediterranean environment which has a long, hot, dry summer and a wet winter. Its flora includes around 2780 blooming plant species, intern this floral biodiversity is critical to honey production. For a very long time, honey was the main source of sugar in Palestine. An extensive beehive industry and honey production as a source of sugar were present during the Mamluk and Ottoman periods. The Mediterranean Beekeepers Association claims that honey has significant economic and health benefits (Imtara et al., 2018b). In 2010, Palestine was found to have 51698 bee colonies producing an average of 500 tons of honey (Abdulkhaliq & Swaileh, 2017). The type of flowers from which bees collect nectar, the climatic conditions under which plants flourish, and the conditions of honey production and storage all affect the quality of honey. Additionally, toxic metals, herbicides, and medicines could contaminate honey. Monitoring honey quality criteria is crucial for consumer health because of this(Abdulkhaliq & Swaileh, 2017).

1.2 Diseases and Antibiotics

In recent years, an increase in the number of diseases and the emergence of new ones. Many types of disease-causing bacteria develop resistance to practically all antibiotics (Ventola, 2015). Because of the adverse effects and antibiotic resistance that pathogenic bacteria and fungi have evolved. Extracts and biologically active substances extracted from natural species used in herbal therapy have recently received a lot of attention (Anand et al., 2019). Alternative medicines have been characterized by the World Health Organization (WHO) as a low-cost approach to provide comprehensive health care coverage for the world's population, and have been urged to utilize plant-based alternative medicines rationally as a main source of health care traditional medicines (Mama et al., 2019).

1.3 Antimicrobial Resistance

Antimicrobial agents are chemicals that have been shown to have a therapeutic impact on microorganisms as a means of controlling, preventing, or curing microbial. They either stop microorganism from reproducing or destroy them. Antimicrobial resistance (AMR) is becoming a worldwide concern. Antimicrobials have played an important role in the fight against infectious illnesses, but their long-term usage, overuse, and abuse has resulted in the selection of resistant bacteria. AMR is one of the most serious threats to global public health, with an estimated 700,000 deaths worldwide in 2014. Furthermore, it is anticipated that if no action is made to combat it, its attributable mortality would reach 10 million by 2050. As a result, protecting the integrity of already used antimicrobials has become crucial, given that the development of new antimicrobials has halted in recent decades. Inappropriate antibiotic usage, insufficient infection prevention and control strategies, limited laboratory capability, and poor monitoring, population increase and migration, as well as inadequate sanitation, have all exacerbated the AMR problem (Sekyere & Asante, 2018).

While saving lives, antibiotics and antifungals can also help to breed resistant bacteria. When the presence of antibiotics and antifungals forces bacteria and fungi to adapt, antimicrobial resistance is increased. Some microorganisms that cause illnesses are killed by antibiotics and antifungals, but they also destroy good germs that shield our bodies from infection. The microorganisms that are resistant to antibiotics endure and

proliferate. The DNA of these resilient germs contains resistance features that they can pass on to other pathogens(Abushaheen et al., 2020).

1.3.1 Mechanisms of Antibiotics Resistance

1.3.1.1 Mechanisms of Antibiotics Resistance for bacteria

First, the changes that occur in the drug-related receptor and the location of the target regions of the antibiotic relationship are unique. The second, antibiotic inactivation by enzymes. Most bacteria produce antibiotic degrading enzymes, and enzymatic inactivation is one of the most important antibiotic resistance mechanisms, the third one is reduction in the permeability of the inner and outer membranes.

Changes in the permeability of this mechanism are the cause of internal and exterior membrane, resulting in a reduction in drug either quickly expelled from the pump or taken up by the cell and the last one is active pumps system. Antimicrobial resistance can also be caused by the creation of complex bacterial mechanisms that can expel a poisonous substance from the cell (Al-Qassemi et al., 2003).

1.3.1.2 Mechanisms of Antibiotics Resistance for fungi

Briefly, resistance frequently manifests. From various synergistic pairings of a small number of molecular processes. Among these are: modifications to the cell a plasma membrane or cell wall, resulting in a reduced absorption of efflux pumps that transport antifungals outside the body; antifungals the antifungal targets overexpressed; mutations in the antifungal target, which reduces its ability to attach; alternative routes are activated, which increases the sequestration of the antimicrobial; antifungal metabolism in organelle-like vacuoles; or modifications to the chromosome raise the essential gene's copy number. Some candida strains are resistant to polyenes due to structural alterations in the sterol content of the cell wall. Amphotericin B and nystatin's ability to attach to liposomes is diminished as a result of the absence of ergosterol and its replacement by more saturated versions(Eggimann et al., 2003).

1.4 Human pathogenic bacterial and fungal species

1.4.1 Pathogenic bacteria

Single-celled, tiny bacteria are a common type of organism in nature. They have a significant impact on human life since they are involved in natural material cycles, utilised in industrial and medical operations, and have the potential to be used in bioremediation of polluted areas. Some of them, nevertheless, are harmful and cause infectious diseases that are spread by contact with food, drink, aerosols, and other things(Li et al., 2021).

The human microbiome depends on bacteria, which colonize tissues like the skin and gastrointestinal tract. Although the majority of bacteria are benign and some even aid in digestion and fight off opportunistic pathogens, bacterial infections are among the most prevalent disorders that affect humans. A disruption to the regular coexistence of bacteria and host cells seen in healthy people is what causes bacterial pathogenesis (Deusenbery et al., 2021).

All information about pathogenic bacteria is presented in the table1.1

Table 1.1

All information about pathogenic bacteria

Type of bacteria	Abbreviation	Family of bacteria	Type of gram stain	Disease causes
Klebsiella pneumoniae	*K.pneumoniae*	*Enterobacteriaceae*	Gram-negative	• Pneumonia • Meningitis • Urinary tract infection
Staphylococcus aureus	*S. aureus*	*Staphylococcaceae*	Gram-positive	• Superficial skin lesions • Abscesses • Sepsis
Escherichia coli	*E. coli*	*Enterobacteriaceae*	Gram-negative	• Urinary tract infection • Sepsis/meningitis • Enteric/diarrheal disease
Salmonellae typhimurium	*S. typhimurium*	*Enterobacteriaceae*	Gram-negative	• gastroenteritis • enteric fever

Klebsiella spp. is a facultative anaerobic, Gram-negative, and non-motile bacterium of the *Enterobacteriaceae* family. The major reservoir for *Klebsiella pneumoniae* (*K. pneumoniae*) is humans. In the general population, 5 percent to 38% of people have the organism in their feces, and 1% to 6% have it in their nasopharynx (Ashurst & Dawson, 2018). Hospital-acquired infections are frequently caused by its, especially in intensive care units. Food and moist items are examples of environmental sources; hand-to-hand contact is typical; intestinal colonization may have place before infection. Primary urinary tract infections are more common than secondary ones. Males over 50 who have diabetes, chronic lung disease, or alcoholism are more likely to get primary *K. pneumoniae* (Von Graevenitz, 1977). Similar to community-acquired pneumonia, pneumonia brought on by *K. pneumoniae* presents similarly. A cough, fever, pleuritic chest pain, and shortness of breath are possible symptoms in patients. The sort of sputum generated is one obvious distinction between community-acquired pneumonia brought on by *Streptococcus pneumoniae* and *K. pneumoniae* and *S. pneumoniae*-infected individuals' sputum is described as "blood-tinged" or "rust-colored," whereas *K. pneumoniae*-infected individuals' sputum is described as "currant jelly." This is because *K. pneumoniae* causes considerable tissue necrosis and inflammation in the surrounding area (Ashurst & Dawson, 2018).

It is a leading source of community-acquired infections such necrotizing pneumonia, hepatic abscess, and endogenous endophthalmitis, as well as nosocomial infections of the respiratory tract, urinary tract, wounds, and circulation Infections induced by *K. pneumoniae* are typically fatal in newborns, the elderly, and immunocompromised people (Anand et al., 2020).

Staphylococcus aureus (*S. aureus*) Leading opportunistic human pathogen Staphylococcus aureus is recognized for its capacity to escape the immune system and cause a wide range of diseases. From superficial skin lesions to deep-seated abscesses to sepsis, *S. aureus* infections can take many different forms. Understanding the fundamentals of infection is difficult due to the range of disease modalities. Human commensals like *S. aureus* are most common in the nares, where they can spread illness. Iatrogenic *S. aureus* infections are prevalent, and they're frequently linked to the colonization of indwelling medical equipment. Normally during an infection, after invasion, macrophages start an immune response and release cytokines to call neutrophils. Additionally, fibrosis takes place, and many of the invasive immune cells die, resulting in the pus-filled abscesses connected to *S. aureus* infections. Additionally, S. aureus frequently escapes from initial infection sites and spreads to new locations. If it gets into the bloodstream, it can cause sepsis and invade other organs, which can result in more local infections. Infection with *S. aureus* is thus a very dynamic process with widespread diffusion and frequent metastases(Pollitt et al., 2018).is a Gram-positive commensal also regarded as a potentially harmful pathogen. About 30% of people have asymptomatic *S. aureus* colonization, but it can also cause infections ranging from minimally invasive illnesses like cutaneous abscesses and impetigo to far more serious invasive diseases including endocarditis, osteomyelitis, and necrotizing fasciitis. The diverse bacterial pathogen *S. aureus* can cause major disease burden and mortality. *S. aureus*, like other pathogens, must modify its environment to develop virulence factors in order to withstand the immunological reactions induced by infection. Up to one-third of the human population is colonized by the well-known bacterium *S. aureus*, which can also lead to fatal infections in about 15% of instances. Because of its diverse set of virulence characteristics, *S. aureus* is a frequent pathogen that causes local and systemic infections in both community and hospitalized patients. It is the most prevalent germ detected in screening samples from the throat and nasal

canals. Although the nasal cavities are thought to be the predominant location of *S. aureus* carriage, evidence reveal that the pharynx can also contribute to carrier status. *Staphylococcus* colonizing nasal or pharyngeal areas in many hospitalized patients can become virulent and cause serious and even deadly infections in situations of endocarditis, meningitis, and blood stream infections(Ungureanu et al., 2017).

Escherichia coli (*E. coli)* The most common commensal dweller of the gastrointestinal tracts of people and warm-blooded animals, *E. coli* is also one of the most significant diseases. It belongs to the bacterial family Enterobacteriaceae. It coexists with hosts in a mutually beneficial relationship as a commensal and rarely transmits illness. However, because it causes such a wide variety of illnesses, it is also among the most widespread infections in humans and animals. *E. coli* is a unique host organism for biotechnology because of its unique traits, including simplicity in handling, accessibility to the whole genome sequence, and capacity to grow in both aerobic and anaerobic environments. *E. coli* is the most commonly employed microbe in the field of recombinant DNA technology and has a wide range of uses in both the industrial and medicinal sectors(Allocati et al., 2013). It is a Gram-negative bacterium. It is present as a commensal in the human microbiome. Within hours of birth, the bacterium usually colonizes the infant's digestive system, and from that point on, both the host and *E. coli* benefit. Even common "nonpathogenic" strains of *E. coli* can cause infection in weak or immunosuppressed hosts or when gastrointestinal barriers are breached; however, *E. coli* typically remains harmlessly contained to the intestinal lumen. Pathogenic *E. coli* infections can spread throughout the body or be restricted to the mucosal surfaces(Nataro & Kaper, 1998). *E. coli* was primarily categorized on the basis of the serologic identification of O (lipopolysaccharide, LPS) and H (flagellar) antigens before the discovery of particular virulence factors in pathogenic strains. E. coli strains are divided into pathogenic types based on the type of virulence factor present and host clinical symptoms. Pathotypes are defined as a group of strains of the same species that cause a common disease. There are at least seven major pathotypes for enteric *E. coli*, while three *E. coli* pathotypes are extraintestinal strains (ExPEC). Ingestion of tainted food or water facilitates the spread of intestinal infections via the faecal-oral pathway(Allocati et al., 2013). Infection with naturally pathogenic *E. coli* strains causes

three common clinical syndromes: (i) urinary tract infection, (ii) sepsis/meningitis, and (iii) enteric/diarrheal disease (Nataro & Kaper, 1998).

However, due to the flexibility of its genome, this organism has evolved into dangerous strains capable of causing illnesses and syndromes of public health relevance in people and animals. Pathogenic *E. coli* are classified into two types based on where the illness is located: extraintestinal pathogenic *E. coli* (ExPEC) and intestinal pathogenic *E. coli* (InPEC). While ExPEC strains are mostly linked with newborn meningitis and urinary tract infections in adults, InPEC strains are associated with diarrheal illness and are classified into at least six distinct pathotypes. The gastrointestinal disorders caused by these *E. coli* strains range from self-limiting diarrhea to hemorrhagic colitis(Mirsepasi-Lauridsen et al., 2019; Rojas-Lopez et al., 2018).

Salmonellae typhimurium *(S. typhimurium)* are significant foodborne pathogens everywhere in the world. Salmonella-related mortality and morbidity place a significant cost on both developing and wealthy nations. More gravely, antibiotic resistance worsens Salmonella's effects on public health (AMR)(Sun et al., 2020). *(S. typhimurium)* are gram-negative bacteria that cause gastroenteritis and enteric fever. *Salmonella* pathogenicity necessitates the coordinated production of complex arrays of virulence factors, which allow the bacteria to avoid detection by the host's immune system. All *Salmonella* serotypes can attack the host by stimulating their own uptake into intestinal epithelial cells. Enteric (typhoid) fever and gastroenteritis are the most common clinical symptoms associated with Salmonella infection. Enteric fever is a long-term systemic sickness caused by infection with the solely human pathogens. Fever, stomach discomfort, brief diarrhea or constipation, and a maculopapular rash are among clinical symptoms. Without therapy, death rates range from 10% to 15% (Ohl & Miller, 2001). S. *typhimurium* are generally obtained by the intake of infected water or food and must survive the stomach's acid Potential of hydrogen (pH) before adhering to and entering the cells lining the intestinal epithelium. Those invading germs that are meant to cause disease Systemic illness must also live and proliferate in the circulation. The liver's macrophages and the spleen (Groisman & Ochman, 1997).

1.4.2 Pathogenic fungi

The frequency of major fungi-caused illnesses has increased in recent decades due to an increase in immunocompromised persons, such as cancer patients, organ transplant recipients, human immunodeficiency virus (HIV) infected individuals, and an aging population. Fungi infect billions of people and kill an estimated 1.5 million of them each year, which is equivalent to the death rates of more widely recognized illnesses like. Tuberculosis or malaria. *Candida*, *Aspergillus*, and *Cryptococcus* species are the most common etiological agents of systemic fungal infections, accounting for more than 90% of mycotic mortality. *Candida* species are the most prevalent cause of invasive mycotic illness in people who are extremely immunocompromised, have had invasive clinical procedures, or have suffered catastrophic trauma that necessitates prolonged treatment in critical care units. *Candida* species, particularly *Candida albicans (C. albicans)*, are the main cause of all healthcare-associated bloodstream infections (Lee et al., 2020).

More than 200 different species of Candida have been identified. Only 10% of the organisms that make up our microbiological flora are known to cause illnesses in humans. Thrush, chronic atrophic stomatitis, chronic mucocutaneous candidiasis, and vulvovaginitis are common manifestations of superficial candidiasis that are quite specific, typically self-limited in hosts who are not immunocompromised, and simple to treat with basic hygiene precautions and local treatment(Eggimann et al., 2003).

However, candida may also cause infections that are fatal and have a prognosis that is on par with septic shock combined with multiple organ failure. *C. albicans* can cause skin and mucosal surface infections, as well as systemic illness. *Candida* species have been found in up to 400,000 systemic fungal illnesses. *C.albicans* is the most prevalent causal agent of mucosal infections and systemic infection, accounting for approximately 70% of fungal infections worldwide (Talapko et al., 2021).

The human body has developed a variety of sophisticated defense systems, including host innate immunity, to lessen the harm caused by fungal infections. Two common innate immune cells—macrophages and dendritic cells—serve as the initial line of defense in several organs, working to stop the spread of fungi. These innate cells can also bridge the gap between innate and adaptive immunity by acting as particular

antigen-presenting cells that can expose naive T cells to fungal antigens. Innate immune cells are activated by pathogen-associated molecular patterns via particular pattern recognition receptors on their surface upon identification of fungal infections, allowing for additional intracellular signaling transduction(Li et al., 2019).

C. albicans develops and creates mycelia in the host's shifting surroundings, adapting to a wide range of micro-ecological settings (Chen et al., 2020).

Mucosal infections commonly result from Candida albicans. It can also result in potentially fatal bloodstream infections that spread to internal organs in some immunocompromised patients. Being able to develop in yeast, hyphal, and pseudohyphal forms makes it a polymorphic fungus. The hyphal form damages tissue and gains access to the bloodstream by penetrating epithelia and endothelia. C. albicans develops hyphae in response to cues such the 37 °C temperature, serum, CO2 and O2 tension, and neutral pH because it is incredibly sensitive to the various conditions that it encounters in the human host. The presence of bacterial cells as well as other C. albicans cells, both of which are detected by quorum sensing chemicals, also controls the morphological flip(Sudbery, 2011).

1.5 Honey

Honey is one of the most well-known natural antibacterial sources documented in ancient medicine, particularly the honey produced by the western honey bee Apismellifera (Mandal & Mandal, 2011).

Honey is a viscous saturated sugar solution that honey bees make from plant nectar and honeydew. Nectar, a watered-down mixture of sugars found in the nectaries of plants, serves as the starting point for the manufacture of "floral" honey. All nectars are mostly composed of carbohydrates; some nectars contain mostly sucrose, while others only contain glucose and fructose as sugars; various minerals and vitamins may also be present, but in small amounts. whereas "honeydew" honey is created by hives of bees that take sugar from the plant or fruit live tissues, and/or scavenge insect waste products that tap the higher plant veins (Al-Qassemi & Robinson, 2003). According to storage conditions and time, honey's composition can significantly alter, depending on its botanical and geographic origins. Floral honeys are divided into two categories based on

their botanical origins: poly or multiflora, and mono or unfloral. While monofloral honeys are generated predominantly from the nectar of one type of flower and are named for the plant from which the pollen is obtained, multiflora honeys are made from a variety of botanical sources, none of which predominate. Due to the unique nutritional, sensory, and potential medicinal properties of unifloral honey, interest in atypical forms of honey has lately increased(Bobis et al., 2020).

Honey's composition varies depending on the plant species foraged by bees, although the main elements are the same in all honeys (Wadi, 2019), honey is a collection of nectar and is made up of sugar (75–79%), water (20%), vitamins, proteins, minerals, and antioxidants(Matzen et al., 2018).Honey has been utilized in the Greek medical system to cure scars, gout, baldness, acute fevers, contraception, eye ailments, and cough and sore throats .Honey was cited by the great Islamic and Iranian physician Avicenna as one of the greatest treatments for tuberculosis(Benlyas et al., 2016). Honey has been identified as a food having antibacterial properties since 1892.The potential uses of honey as an antibacterial agent are expanding in the modern era as a result of the widespread development of antibiotic-resistant microbial strains.

S. aureus, E. coli, Bacillus subtilis, Pseudomonas aeruginosa, *Paenibacillus larvae*, as well as some Streptococcus spp., Salmonella spp., Shigella spp., and Proteus spp., are among the numerous bacteria that are sensitive to honey. The majority of these pathogenic bacteria are gram-positive.

Honey has been shown to have bacteriostatic and bactericidal effects when used as an antibacterial, with fresh and unheated honeys having the strongest benefits (Machado De-Melo et al., 2018). According to laboratory and clinical studies, honey exhibits antimicrobial, antiviral, antifungal, anti-inflammatory, antioxidant, and anticarcinogenic properties. Oral administration of honey also appears to protect against cardiovascular and gastrointestinal tract diseases, according to numerous studies(Benlyas et al., 2016). Honey from various geographical and floral origins may have varying antibacterial effects, which might be due to variations in chemical makeup (Irish et al., 2011). The major contributor to the antimicrobial activity of honey is hydrogen peroxide and the varying antimicrobial activity in different types of honey is because of the different concentrations of hydrogen peroxide, the antimicrobial activity of honey is also

attributed by some physical factors such as acidity (low pH) and high osmolarity (Mandal & Mandal, 2011; Saba et al., 2013).

1.5.1 Honeys nutritional benefits

Natural honey is a wholesome, easily digestible, full-flavored, and high-energy food. It includes all of the essential elements of a diet, including carbs, proteins, fats, water, vitamins, and minerals. In nutritional terms, it is actually best referred to as a complete meal.(Ajibola & Science, 2015)

From ancient times to the present, honey has been one of the most popular sweetening ingredients for dishes. It is a significant source of carbohydrates and the only commonly accessible sweetener that gives our bodies with power and energy. Honey's carbohydrates are quickly digested and rapidly carried into the bloodstream, where they are used by the human body for energy. Honey's glucose is rapidly absorbed by the body and provides an instant energy boost, whereas fructose is taken more slowly and provides long-term energy. Honey has also been proven to maintain blood sugar levels relatively stable when compared to other kinds of sugar. When ingested daily in large quantities (70-95 g), honey can substitute for a full meal because it provides all six types of nutrients in a balanced diet in the appropriate proportions. This results from its beneficial therapeutic effects on almost all human illnesses as well as its functional influence on the body's essential systems and activities. The multiple health benefits of honey outweigh those of other functional foods, in addition to the nutritional enrichment that this natural substance provides(Al-Qassemi & Robinson, 2003).

1.5.2 Medicinal value of honey

The idea of utilizing honey as medicine dates back at least 6,000 years. Early writings on papyri and Sumerian clay tablets show unequivocally that Egyptians utilized honey as medicinal before 1900–1250 BC. Aristotle, a scientist and philosopher, wrote around 350 BC about the benefits of honey as a treatment for painful eyes and wounds, while Dioscorides wrote in 50 AD about the benefits of honey for all rotting and hollow ulcers, sunburn (spots on the face), coughs, throat irritation, and tonsils. Honey was used as a remedy for weariness by the ancient Greeks. Before important sporting competitions, their athletes would drink a concoction of honey and water.Every religious text and work of literature mentions honey as a beneficial food and medicine.

Though it has a long history in medicine, it has only recently been acknowledged as a medicinal agent in modern science. It is because we don't fully grasp its mechanism and scope of activity. More study is being done on the therapeutic uses of honey in the twenty-first century(Khan et al., 2018).

1.5.3 Life cycle of the honey bee

A honey bee colony has an eternal existence. There are three types of honey bees: queens, which lay eggs, drones, which are males, and workers, which are females without the ability to reproduce. In the comb's cells, the queen lays her eggs one at a time. In three to four days, larvae emerge from eggs. After that, worker bees feed them as they grow in the cells through several stages. When the larva pupates, worker bees cap the cells. Tens of thousands of people are often found in a colony. Queens, workers, and drones all have different stages of development from egg to emerging bee. A queen typically lives for three to four years. Drones typically perish after mating(Ediriweera & Premarathna, 2012).

1.5.4 Development of bee's honey

Bees gather nectar from flowers, a clear liquid that contains almost 80% water and complex carbohydrates, and use it to make honey. The nectar is repeatedly swallowed and regurgitated by the bees in the hive until it is half digested. They keep doing this until the product is of the desired quality. The honeycomb is left open after the last regurgitation. This raw honey has undergone some processing, but it still contains a lot of water and natural yeast that, if left unchecked, would ferment. The uncooked honey is then kept in the honeycomb cells to dry. As the process goes on, bees inside the hive spread their wings to create a strong draft across the honeycomb that accelerates the evaporation of roughly 80% of the water from the honeycomb. To keep the honey fresh, the honeycomb's cells are wax-capped after they have dried. When properly packed, ripe honey that has been taken from the hive by a beekeeper has a long shelf life and won't ferment(Ediriweera & Premarathna, 2012).

1.5.5 Physical properties of honey

Different physical characteristics of honey, including its color, pH, enzyme activity, ash content, electrical conductivity, and even flavor, vary depending on the type of honeybee, where it was produced, and the presence of contaminants. Depending on the plant source, honey can range in color from pale yellow to deep red to black. Temperature changes are the main cause of the darkness. The trait of honey that sets it apart from other sweeteners is its propensity for granule development. The pH of honey, like other physical factors, shows its purity or crudeness, but this depends on the local topography. Honey's pH ranges from roughly 2.4 to 4.7. The most significant factor affecting honey firmness is moisture. Another unique quality of honey is its high moisture content, which typically varies from 13 to 20 percent. The viscosity of honey is temperature and moisture content dependent, just like other Newtonian fluids. At 24 °C with 18.9% moisture. In addition to all of these qualities, honey is spoiled resistance because to its high sugar concentration. As a result, it served as a preservative for other food ingredients(Khan et al., 2018).

1.5.6 Composition of honey

Honey is a supersaturated sugar solution created by honeybees from the nectar or sweet liquid of various sections of flowering plants. It contains minerals, enzymes, vitamins, flavorful chemical compounds, free amino acids, and many volatile compounds.

In addition to floral origin, additional elements like as geographical and climatic features, as well as processing and storage conditions, may influence the ultimate quality of honey (Imtara et al., 2018b).

Honey is suitable for long-term storage due to its distinctive composition and chemical properties, but some compositional variations are frequently caused by various chemical and biochemical processes, such as fermentation, oxidation, or dehydration of sugars, which change the acidic content and result in the formation of compounds like 5-hydroxymethylfurfural, which alter the sensorial properties and lower the quality of honey.(Santos-Buelga & González-Paramás, 2017)

Sugar and water make up the majority of the honey. Honey's dry matter is made up of 95 to 99 percent sugar. Simple sugars, such as fructose (38.2%) and glucose (31.3%),

account for 85-95 percent of total sugars. These are “simple” sugars, 6-carbon sugars that the body may easily absorb. Disaccharides like maltose, sucrose, and isomaltose are also found, other sugars trisaccharide also found in honey like a melezitose and raffinose.(Saba et al., 2013)

Due to the influence of temperature and enzymes, honey's sugar composition changes while it is stored. Some of the sugars, particularly glucose and fructose, may crystallize when the honey is held at temperatures lower than those in the hive. The proportions of the primary sugars in honey have a significant impact on this phenomenon(Santos-Buelga & González-Paramás, 2017).

The second most essential component of honey is water or the moisture in honey. Honey moisture is crucial since it influences honey storage. During production, various environmental elements including as weather and humidity inside the hives, as well as nectar conditions and honey treatment during extraction and storage, all influence the final water content.(Saba et al., 2013)

In general, mature honey has a moisture level of less than 20%. Proteins, enzymes, and non-essential amino acids make up the rest of the honey (Mama et al., 2019). Organic acids make up to 0.57 percent of honey including gluconic acid, a byproduct of enzymatic glucose breakdown and aspartic acid, glutamine, histidine, glycine, threonine, β-alanine, arginine, α-alanine, γ-aminobutyric acid, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, tryptophan, phenylalanine, ornithine and lysine. Honey's acidity is due to organic acids, which also contribute significantly to its distinct flavor. Honey contains extremely tiny amounts of minerals (0.17 percent), with potassium being the most prevalent. Calcium, copper, iron, manganese, and phosphorus are among the others. Nitrogenous substances, including enzymes derived from worker honeybee salivary secretions, are also found. They play a crucial part in the production of honey. Invertase (saccharase), the enzyme that transforms sucrose to fructose and glucose, diastase (amylase) which decomposes starch or glycogen into smaller sugars units of dextrin and maltose, and glucose oxidase in the presence of water, glucose oxidase generates hydrogen peroxide and gluconic acid from glucose. These are the three major enzymes found in honey. Honey includes a variety of vitamins in different quantities such phylloquinone (K), thiamin (B1), riboflavin (B2), niacin (B5) and

pyridoxine (B6) as well as vitamins A, E and C have been reported to be present in honey (Saba et al., 2013; Wadi, 2019).

1.5.7 Antimicrobial activities of honey

Honey's antibacterial action has been related to osmolarlity, pH, hydrogen peroxide (H2O2) generation, flavonoids, phenolic compounds, and the existence of additional phytochemical components (Matzen et al., 2018). Honey's antibacterial action differs from that of antibiotics, which damage the cell membrane of bacteria or block intracellular metabolic processes (Elamine et al., 2021).

Honey's antibacterial action is linked to four characteristics.

First, Low water content and high sugar content. Water activity refers to the amount of unbound water molecules in a sample that is proportional to the amount of bacterial contamination and falls below the limit that has been proven to completely block bacterial development. Therefore, honey has a very low water content, that aids in the proliferation of microorganisms(Molan, 1992).

Honey has high sugar concentrations (70–80%), which when given to live cells, can cause osmosis. being given to living cells, which can cause osmosis. honey absorbs moisture from the surroundings, dehydrating bacteria. Honey's sugar level is high enough to stop bacteria from growing, because honey contains a lot of sugar, osmosis is brought on. Due to its sugar content, which exerts osmotic pressure on bacterial cells and causes water to flow out of the bacterial cells via osmosis, pure, undiluted honey prevents the growth of bacteria. Dehydration causes the cells to shrink and make them incapable of surviving in the hypertonic sugar solution. But the sugar content isn't the only explanation for honey's antibacterial effects.

Second, honey has a pH range of 3.2 to 4.5, which is low enough to prevent most bacteria from growing. Certain significant organic acids, particularly gluconic acid, which is present at a concentration of around 0.5 percent (w/v), are the cause of this acidity. A natural glucose oxidase enzyme produces glycogenic acid, an incredibly strong antibacterial agent, from the oxidation of glucose(Hossain et al., 2022).

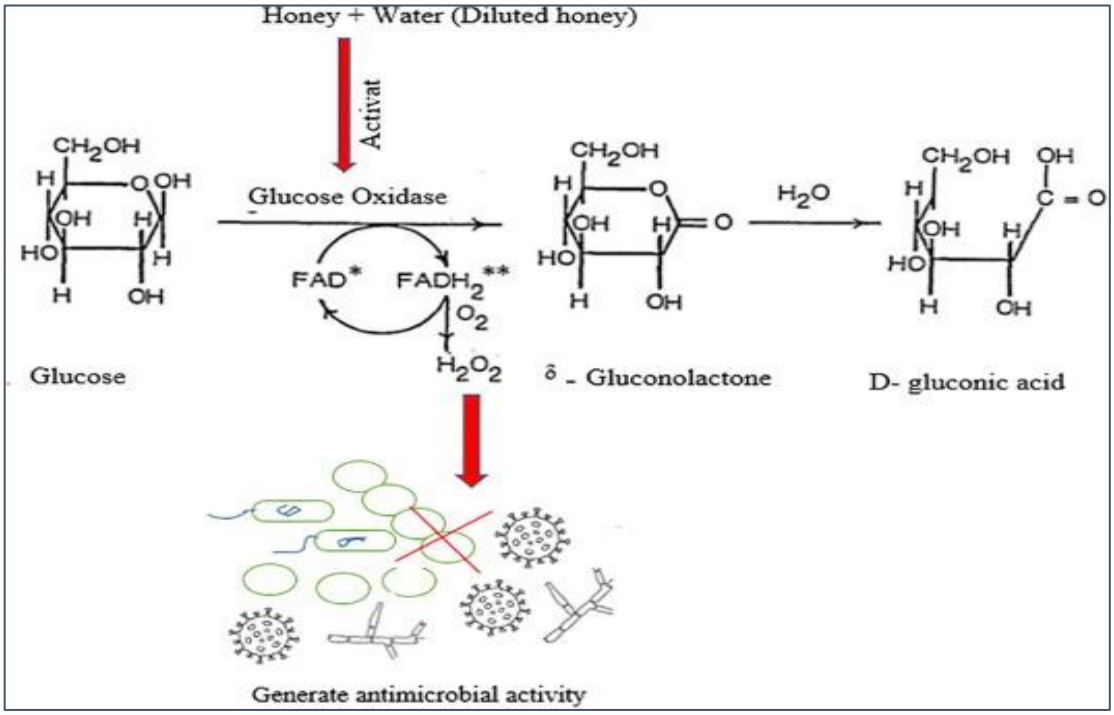
The third and most significant antibacterial component is hydrogen peroxide, which is generated by the glucose oxidase. Activated glucose oxidase reacts with endogenous glucose to create see figure1.1.

H2O2 is a potent oxidizing agent and a disinfectant. It is created enzymatically and gives honey its antibacterial activity. Due to the low pH levels in honey, the enzyme glucose oxidase is naturally present but inactive. Glucose oxidase is triggered and reacts with endogenous glucose to make H2O2 when honey is diluted (Almasaudi, 2021).

Finally, numerous antimicrobial phytochemical components have been discovered in honey. Plant secondary metabolites are called polyphenols. They are a varied category of compounds, including flavonoids and phenolic acids, that are distinguished by their phenolic structures. These biological substances are transferred from nectar to honey and have a significant role in the health-promoting qualities of honey. In honey, phenolic chemicals are abundant and may help explain its antibacterial properties. Geographical location and the plant from which the nectar was derived both have an impact on the amount of phenolic acid in honey (Mandal & Mandal, 2011).

Figure 1.1

Producing hydrogen peroxide(H2O2) is catalyzed by glucose oxidase.



1.6 Herbal plant

Among smallholder farmers with limited resources, herbal remedies have historically been used as a kind of therapy The use of alternative medicines is, however, seldom documented because many academics and medical professionals consider them to be archaic. Herbal plants need to be documented since they are anticipated to become increasingly significant in the future, especially given the rising costs of pharmaceuticals and the emphasis on organic products in the majority of developing nations. Additionally, as herbs typically have a broad spectrum of effects, alternative therapy may be the best option as infections acquire drug resistance(Mwale et al., 2005).

The content and effectiveness of honey are significantly influenced by the plants the bees consume. The four plants the study will focus on are categorized as such by the Arab American University. ***Zizyphusspina-christi***, (*Z. spina-Christi*)

Often known as Sidr, is a member of the Rhamnaceae plant family the genus is Ziziphus Mill. The spiny shrub or small tree *Z. spina-Christi* is very resistant to heat and drought. The plant often develops into a tree form, typically, the tree's name appears on the fruit in Arabic, but in the case of *Z. spina-Christi*, the tree's name is sidr and the fruit is nabag. Today's wild *Z. spina-Christi* trees can be found in Jordan, Palestine, Egypt, and some regions of Africa. Alternative medicine practitioners have treated fever, pain, dandruff, wounds and ulcers, inflammatory illnesses, asthma, and eye diseases with *Z. spina-Christi*. Recent studies have demonstrated the antibacterial, antifungal, antioxidant, antihyperglycemic, and antinociceptive properties of *Z. spina-Christi*. It has been employed in traditional medicine as an astringent, demulcent, depurative, anodyne, emolient, stomachic, and mouthwash. Fruits are used for diarrhea, while bark and fresh fruits are used to speed the healing of recent wounds and as a body wash. The fruits are also used to treat TB, coughs, and bronchitis(Nazif, 2002).

The Sidr tree is primarily grown for its nutritious fruits and honey production. The blooms are vital for the production of wild unifloral bee honey, which is greatly sought after by Palestinians for its therapeutic properties as well as its outstanding taste and pleasant scent. Antibacterial, anticancer, antidiabetic, anti-nociceptive, antihypertensive, antidiarrheal, and central nervous system effects have been described for the plant (Ilonga, 2012).

Thyme (Thymus vulgaris,* TV*) is a Lamiaceae family and genus. Thymus plant used for human nutrition. It was grown in numerous nations, including Iran, Spain, France, Greece, Portugal, and Italy. Its leaves, fresh or dried, can be used as a spice, and its essential oils can be found in cosmetics and foods additives.TV has long been used to cure coughs, headaches, diarrhea, worms, constipation, renal dysfunction, and cancer. Furthermore, it possesses antiseptic, antispasmodic, antioxidant, bactericide, and anthelmintic properties. These effects are due to the presence of carvacrol, flavonoids, eugenol, thymol, phenols, and luteolin among its components(Abu-Serie & Habashy, 2018). Thyme's incredible nutritional richness is what accounts for all of its health advantages. Thyme's nutrients have the ability to both prevent and improve health. This fragrant herb is abundant in phytonutrients, essential minerals, and vitamins for excellent health.(Dauqan et al., 2017)

TV is 5 to 30 cm tall, with a thin, fibrous root and short, narrow-edged, greenish-gray leaves. It has multiple stiff, branched stems that are 10 to 20 cm tall, and it blooms from May to September with flowers that may be white or purple in color and have a distinct aroma. It is extensively grown as a culinary herb because to its potent scent, which is caused by thymol (Javed et al., 2013). The many herb variations of the Thymus species, all of which are indigenous to Europe and Asia, are together referred to as thyme.The most common variety, known as common or garden thyme, is used commercially for flowering and decorative purposes. Thyme is a native of the southernmost Italian region of the Western Mediterranean(Dauqan et al., 2017).

Citrus limonium fruits are acidic fruits with a high nutritional value that is beneficial to the body. It is a fantastic source of vitamin C as well as a wide range of other critical nutrients. There are a variety of citrus species Limonium (lemon) is one of the most frequent. (*Citrus Limonium*) is a useful medicinal plant in the Rutaceae family. It is farmed primarily for alkaloids, which have anticancer and antibacterial properties in crude extracts of various sections (Unnisa et al., 2012). The acidity of lemon juice is induced by the presence of citric and vitamin C, which act as an acid catalyst in organic transformation (El-Saghier et al., 2019).See figure 1.2 of Sider flower, Thymus flower and Citrus flower

Figure 1.2

Flowring plant

(a) (b)

(c)

(a) Sider flower, (b) Thyme flower, (c) Citrus flower

Wildflower, according to (Yadzir et al., 2011) is a flower that blooms naturally, not as a result of artificial planting or seeding and wildflower honey, sometimes referred to as "multifloral" or "mixed floral," is created from the nectar of a variety of flowers, including the following:

Anemone coronaria. Cormous geophyte Anemone coronaria L. is native to the Mediterranean region, and various commercial variants of ornamental value have been developed their geophyte Anemone coronaria L. blooms profusely and attractively throughout the winter and early spring; it is widespread in open Mediterranean settings

that are often used for grazing animals. Red flowers are the most prevalent color in A. coronaria, however white, pink, blue, and violet flowers can also be found in the same or separate populations(Dafni et al., 2020).

Anemone is a genus containing more thaPn 150 species of flowering plants that are endemic to the temperate regions of both the Northern and Southern hemispheres and belong to the Ranunculaceae family . Triterpenoids, saponins, steroids, lactones, lipids and oils, saccharides, and alkaloids are just a few of the identified Anemone chemicals. Anemone contains large amounts of oleanolic acid. Coumarins and flavonoids are also found in anemone(Hao et al., 2017).

Bellis perennis. Also known as the common daisy or English daisy, is a small herb in the Asteraceae family. The species has a very long flowering season, with flowers appearing primarily between March and November. In traditional medicine, the common daisy is used as an expectorant, diuretic, and anti-inflammatory. The flowers and young leaves are used as food additives .Flavonoids and phenolic acids are among the phenolic constituents of Bellis perennis (Siatka & Kašparová, 2010).The Middle Ages saw the usage of common daisies by Crusaders as a traditional wound herb for the healing of bruises, broken bones, and wounds. In traditional medicine, B. perennis has also been used to treat conditions like dermatitis, boils on the skin, gastritis, enteritis, diarrhea, bleeding, rheumatism, inflammation, and infections of the upper respiratory tract. Astringent, expectorant, diuretic, stimulant, purgative, and diaphoretic qualities are also present(Karakaş et al., 2012).

Chrysanthemum coronarium L. (Garland), mostly recognized as a weed in cereals. It is herbaceous annual with 100 cm-long stems, branching, oblong leaves, and yellow flowers It was noted that *Chrysanthemum coronarium* establishes quickly, may be grazed early in the growing season, and endures where other pasture species may perish(Sulas et al., 1999).

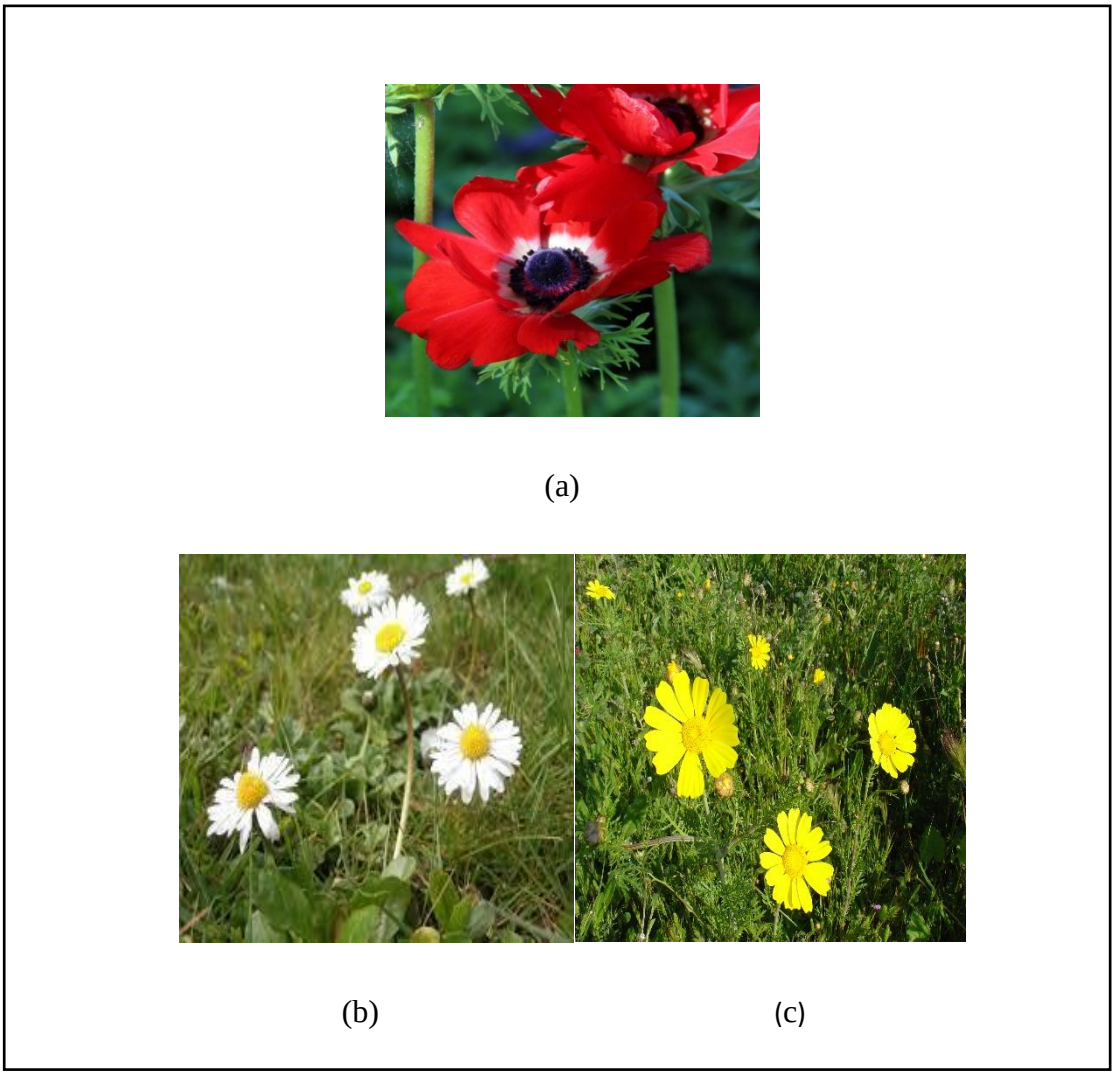
Chrysanthemum coronarium L. a member of the Asteraceae family, is a perennial herbaceous weed that grows naturally in the Mediterranean Asian Region Because of its extensive array of bioactive components, including minerals, pigments, and dietary fibers, Garland is recognized as a rich source of phenolic compounds. These

components also give the plant antioxidant, antibacterial, antifungal, and anticancerous properties(Abdelgaleil et al., 2020; Hosni et al., 2013).

East Asia has long recognized the garland as a healthy food because its edible parts, such as the leaf and stem, are rich in vitamins and minerals. Iron, potassium, calcium, â-carotene, and dietary fiber. Garland is thought to include certain chemicals that are responsible for the chemoprevention of cancer and other disorders in addition to these typical nutrients (Chuda et al., 1996). See figure 1.3 three type of wild flowering plant *Anemone coronaria, Bellis perennis and Chrysanthemum coronarium L.*

Figure 1.3

Wildflowers



(a)

(b)

(c)

(a) Anemone coronaria (b) Bellis perennis (c) Chrysanthemum coronarium L.

The general aim of this work

The goal of the study is to evaluate the antibacterial and antifungal properties of four types of Palestinian honey from four distinct botanical origins and also assess the antibacterial and antifungal effect of flower botanical origin for honey against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonellae typhimurium*, and other bacteria. These honey samples will be evaluated: A. (Ziziphus) B. (*Thymus vulgaris*) C. (Citrus honey) D. (Wildflower honeys).

The antibacterial and antifungal effects will be measured through the assessment of the honey and plant antibacterial effect though; the disc diffusion method which is concerned as the main qualitative test for detecting the susceptibility of bacteria and fungi to antimicrobial substances, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) which reflects the quantity needed for bacterial and fungal inhibition and bacterial and fungal death respectively.

The main problem of research

People are afflicted by several prevalent illnesses. It is mostly brought on by bacteria and fungi that may be resistant to the majority of antibiotics. Since alternative medicine has been suggested as a cure for microbial disorders, the current study examined the antibacterial properties of honey gathered from various sources against a variety of human pathogenic bacteria and fungi.

Research question

Is there a relation between the honey's botanical origin and its well-known antibacterial properties?

Hypothesis

Palestinian honey represents a cheap and effective remedy in the management of microbial infection. The chemical composition of various Palestinian honey samples are dependents of the botanical origin of the honey. Here we assume that the antibacterial efficacy of honey samples depends on the plant origin and composition of the honey samples.

Chapter two

Materials and Methods

2.1 Materials and instrumentation

The chemicals and instruments were used in this study is presented in the table 2.1

Table 2.1

Chemicals and instrumination

Chemicals	
Mueller Hinton agar	Sigma, Germany
Mueller Hinton broth	Sigma, Germany
Sabouraud Dextrose agar	Sigma, Germany
RPMI1640	Sigma, Germany
Ethanol (99%)	Sigma, Germany
Sodium chloride	Sigma, Germany
Instruments	
Autoclave	Tuttnauer
Incubator	Memmert
Balance	Nimbus
Microliter Pipettes	Eppendorf
Water bath	Memmert
Microtiter plate reader	Thermo Fisher

2.2 Field survey and samples collection

Field surveys were carried out in different regions where honeybees are commonly reared. Samples were collected from mono-floral and multi-floral honeys from the bee keepers of Mnahel Ard Al Taebat in the West bank, Palestine.

Based on the abundance of flora for harvesting nectar, the location of the apiary, and other factors, the beekeepers identified the floral source of the honey. Most samples were monofloral honeys. According to their botanical origins and one samples of honey from the local market that have been marketed commercially and the other is hand made. Honey samples were stored in the dark at room temperature 22–24 Celsius degrees (°C) in air tight plastic containers to prevent photo degradation until being used and labelled based on the commercial container's descriptions.

All information about honey collection including scientific name, common name, and source is presented in the table 2.2

Table 2.2

All information about honey collection

Samples	Scientific name	Arabic name	English Name	Source	Time of collected
A	*Ziziphusspina-christi*	السدر	Ziziphus	Geftlk AL-Ghor region	End of November
B	*Thymus vulgaris*	الزعتر	Thyme	Sa'ir Al-Khalil	End of August
C	*Citruslimonium*	الليمون	Citrus	The north of AL_Ghor	End of April and Beginning of May
D	*Multifloral* *Anemone coronaria,* *Bellis perennis,* *Chrysanthemum coronarium L)*	شقائق النعمان زهرة الؤلؤ البسباس	Poppy anemone, common daisy, Garland)	Tulkarm	May and June
E	-	عسل من السوق المحلي	Marketed commercially	Jenin	April
F	-	صنع يدويا	Hand made		April

2.3 Preparation of honey extract

Different concentrations of each honey sample were prepared using distilled water. Ten grams (g) of each honey were weighed by using an electric balance then 10 milliliters of water were added to make 1000 milligram/ milliliter (mg/mL) (Ghramh et al., 2019), in order to facilitate dissolving, honey was placed in a 37°C water bath (Ekhtelat et al., 2016).

2.4 Collection of plant flower

The flowers of origin of the honey plants were dried in the shade at room temperature until all the flower parts became well dried. After drying, the plant materials were crushed well by using grinder and placed into a container with a tight lid (Shawarb et al., 2017).

2.5 Preparation of flower extracts

The selected plants were extracted according to(Kadan et al., 2013), with ethanol (99%). 10 g of grinded plant material then added to 50 mL of the solvent and heated for 60 minutes at 60◦C under stirring. Extract supernatants obtained were passed through a 0.2 Micrometers(μm) filter and stored in aliquots at−80◦C for further experimental work.

2.6 Antimicrobial tests

2.6.1 Preparation of test microorganisms and growth media

Mueller Hinton agar, Sabouraud Dextrose and Mueller Hinton broth were used in the experiment and prepared according to the instructions of the manufacturer.

Mueller-Hinton broth was made by adding 22g of broth powder to 1 liter(L) of distilled water, heating it while stirring gently until it appeared clear, pouring it to test tubes, capping them, and autoclaving them.

Mueller-Hinton agar was prepared by addition of 38g of broth powder to 1L distilled water, heating and stirring until boiling, capping them, sterilizing the mixture in an autoclave, and then pouring the mixture into sterile Petri plates in a 20ml volume. It was utilized in the Disc diffusion method and minimum bactericidal concentration (MBC).

Sabouraud Dextrose agar was prepared by adding 65g of powder to 1L of distilled water, heated and gently stirred until boiling, covered, and autoclaved. It was utilized in the disc diffusion methods and minimum fungicidal concentration (MFC).

Blood agar 28 g of nutritious agar powder was dissolved in 1 liter of purified water stirring while heating that help all of the ingredients dissolve completely the dissolved mixture then autoclaved for 15 minutes at 121 degrees Celsius allow the nutritional agar to cool but do not allowed it to solidify once it was autoclaved. added 5% (vol/vol) sterile defibrinated blood that warmed to room temperature to the agar once it was cooled to 45–50 °C and stir gently but thoroughly eliminated air bubbles while still liquid, dispense into sterile plates

The bacterial and fungal strains used in the current investigation were obtained from the American Type Culture Collection (ATCC).

a) Bacterial strain :The strain includes Gram negative bacteria *E. coli* (ATCC25922), *K. pneumonia* (ATCC43816), *S. typhimurium* (ATCC14028) and Gram-Positive bacteria *S. aureus* (ATCC25923). All bacteria were sub-cultured on Mueller Hinton agar.

b) Fungal strain : One fungal strain is *C. albicans* (ATCC 90028). This strain was sub-cultured on Sabouraud Dextrose.

The Bacterial and fungi used in this study were obtained from the Microbiological laboratory, Department of Biology, Faculty of Science, An-Najah National University.

Preparation of the 0.5 McFarland standard

With continual stirring, add 0.5 mL of 0.048 M BaCl2 (1.17% w/v BaCl22H2O) to 99.5 mL of 0.18 M H2SO4 (1% v/v). Make sure the McFarland standard is uniformly suspended by thoroughly mixing it. Measure the optical density in a spectrophotometer at 625 nm using matched cuvettes with a 1 cm light path and water as a blank standard. The standard's acceptable range is 0.08 to 0.13. Put the standard into screw-cap tubes of the same size and capacity as those for the test inoculum. The tubes must be firmly sealed to stop loss due to evaporation. Store at room temperature and shielded from light(Andrews, 2007).

2.6.2 Preparation of microbial inoculum

The inocula suspension was obtained by taking colonies from 24hr cultures. The colonies were suspended in sterile saline (0.9% NaCl) and shacked for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1–5 $\times 10^8$colone forming units per milliliter (CFU /mL) for bacteria (Imtara et al., 2018a), and 1–5 × 10^6 CFU/mL for candida (Dantas-Medeiros et al., 2021).

2.7 Antimicrobial assay

2.7.1 Disc diffusion method

The disc-diffusion test of samples was done using the method described by Chandrasekaran et al 2005 (Chandrasekaran & Venkatesalu, 2004)with slight modification. See figure 2.7.1 Briefly 250 microliter (µL) of the test microbial (turbidity of a 0.5 McFarland standard) were spread over the plates containing Mueller Hinton Agar for bacteria and Sabouraud Dextrose Agar for fungi. The paper disc impregnated with 15 µLof different diluted samples were placed on the surface of the media. Then the diameter of an inhibition zone was measured around the discs after incubation of Petri dishes at 37°C for 24 hr (bacteria), 28°C for 48 hr fungi.

One other Petri dish was used as a negative control contains the bacterial cell suspension without the test extract Each test in this experiment were carried out three times(Benlyas et al., 2016).

Figure 2.7.1

Disc diffusion method

2.7.2 Minimum inhibitory concentration (MIC) assay

The assessment of antimicrobial activity was performed according to (Rayan et al., 2020) with slight modification. To determine the MIC50, a broth micro dilution method was used.

a) Determination of anti-bacterial activity

The test was done in 96-well, flat-bottomed microtitration plates. See figure 2.7.2 The cell suspension was prepared in Mueller Hinton broth with an optical density equivalent to the 0.5 McFarland standard and diluted 1:100 in Mueller Hinton broth to obtain a final concentration of $5 \times 1\ 0^5$ (CFU/ml). Controls of broth only and broth with bacteria without any of the antibacterial agents were also included in each plate. Serial dilution of each sample of honey was prepared in sterile tubes with concentration ranging from 1000 to 125mg/ml. Then, 50 µL of each concentration was added into each well containing 50 µL of Mueller–Hinton broth to give final concentrations of 250-31.25 mg/mL. For flower extract 7 µL of each dilution varying from 300 mg/mL to 37.5 mg/mL was combined with Mueller Hinton broth 93 µL to give final concentrations of 10.5-1.31 mg/mL and 100µL of the bacterial suspension (5×10^5 CFU/mL) for honey and flower extract in final volume of 200 µL. The ethanol content in each well was less than 3.5% in an overall amount of 200 µL. For the negative control, the same percentage of ethanol was used. The plates for honey and flower extract were incubated at 37°C for 24 hr. After 24 hr, visual inspection was done. Bacteria growths were assessed by turbidity in the wells and were compared to the positive and negative controls. Absorbance was measured by using the microtiter plate reader at 570 nanometer (nm). The MIC 50 was determined by using the following formula

Percentage inhibition = 1 − (optical density (OD)test/OD control) × 100,,,,,,,,,(Benlyas et al., 2016).

b) Determination of anti-yeast activity

The same procedure of broth microdilution assay that used for bacterial strain was also used for yeast *C. albicans* with minor modification.

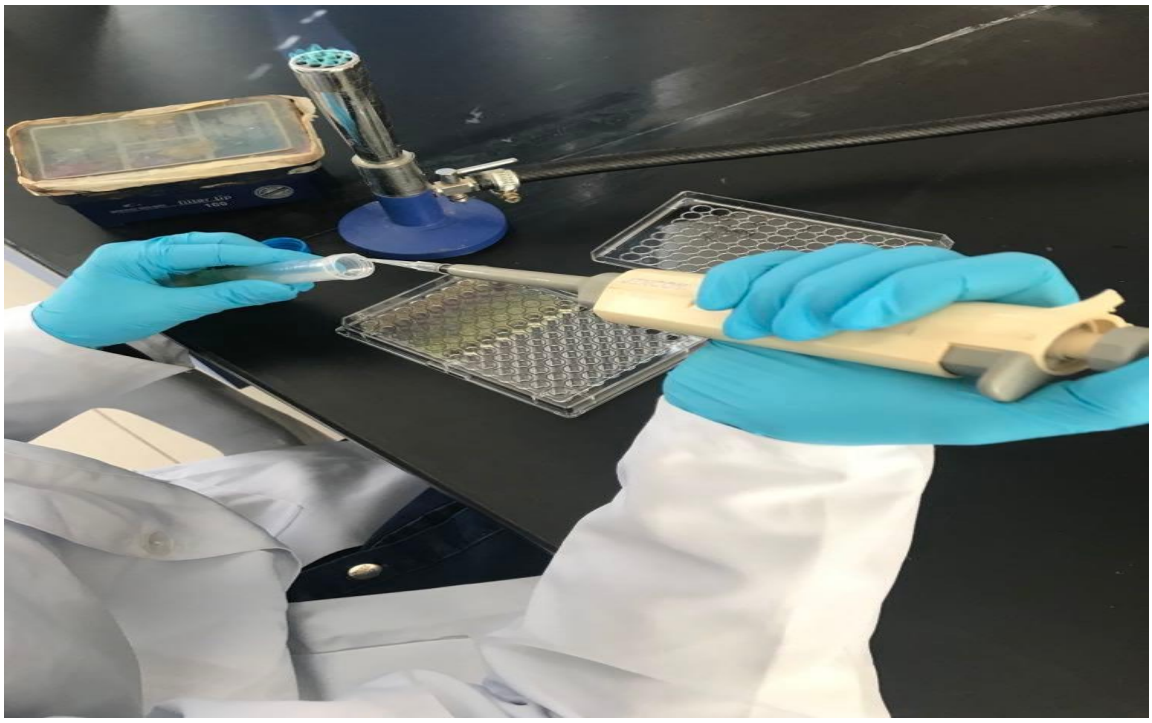
Conidial suspensions were prepared in RPMI-1640. Subsequently, two dilutions were made, the first in saline solution (1:100), and the second in RPMI 1640 (1:20), in order

to obtain the final concentration of 10^3 conidia/ml. Two-fold serial dilutions of the different honey and flower extract were prepared in RPMI-1640(Dantas-Medeiros et al., 2021).

Then all plates were incubated at 28°C for 24 hr. Growth was observed by visual inspection and OD measurement at 530 nm using microtiter plate reader (Rodríguez-Tudela et al., 2001).

Figure 2.7.2

(MIC) assay



2.7.3 Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Following the determination of the MIC values, 10 µL suspension samples were collected using a micropipette from each well of a 96-well microplate and transferred in sequence to Mueller Hinton Agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) plates. Following these treatments, the Petri plates were incubated for 24 hr at 37 °C (bacteria) and 28 ◦C for 24 hr (fungi). The lowest concentration that did not produce any bacterial and fungal colony growth on the appropriate agar plate after the period of incubation were recognized as MBC/MFC (Karlıdağ et al., 2021). The susceptibility test for each species was replicated 3 times.

Statistical analysis

The statistical analysis was performed by ANOVA through the SPSS v.26 program and using the Tukey's post hoc test at $p < 0.05$ was considered significant.

Chapter Three

Results

3.1 Disc diffusion method

In our investigation, honey and its botanical sources were tested using the disc diffusion method against *C. albican*, Gram-positive and Gram-negative bacterial strains.

Preliminary antibacterial screening of Multiflower and Citrus honeys showed that they were effective against four different bacterial strain but the Sider and Thymus honeys showed that they were effective against *S. aureus* and *S. typhimurium*.

The development of clear zones revealed that all of the honey has a strong antibacterial effect against this particular bacterium types, figure 3.1 and table 3.1.

The zones of inhibition produced by the various honey and flower strains utilized to combat *S. auras* strains range in diameter from 21.45 mm to 30.75 mm for honey; there are no significant variations between the Citrus honey ZID of 30.75 mm and the Multiflower honey ZID of 30.4 mm. There are no significant changes between the Multiflower ZID 19.5 6.21 mm and Citrus flower ZID 22.4 mm for flowers with ZIDs ranging from 12.58 mm to 22.4 mm.

For *E. coli* a diameter ranging from 23.33±1.25 mm to 24.66±1.3mm for honey and the Citrus honey generated ZID24.66±.76 mm, Multiflower honey ZID 23.33±1.25 mm there's no significant differences. For flower ZID ranged from 9.09±.08 mm to 13.3± 2.67 mm and the Citrus flower ZID 13.3 ± 2.67 mm.

For *S. typhimurium* a diameter ranging from 14.87±.12mm to 20±.5 mm for honey, Citrus honey generated ZID 20±.5 mm Multiflower honey ZID18.66 ±18.6 mm there's no significant differences. For flower zone inhibition diameters ranged from 10.25± 1.09 mm to18.9 ± 0.14 mm and the Citrus flower ZID 18.9 ± 0.14 mm.

For *K. pneumonia* a diameter ranging from 14.4±.38mm to 15.75±.66mm for honey, Multiflower honey generated ZID 15.75±.66mm. For flower zone inhibition diameters ranged from.7.12± 0.13 mm to 11.00 ± 0.25 mm high ZID is obtained by the multiflower.

For *C. albicans* no inhibition zone was detected for the different types of honeys and its flower.

Table 3.1

Diameters of the inhibition zones (mm) produced by honey against different bacterial and fungi strains.

Type of honey	DISC DIFFUSION METHODS MEAN OF ZONE INHIBITION OF HONEY				
	Klebsiella pneumonia	***Escherichia coli***	***Staphylococcus aureus***	***Salmonella typhimurium***	***Candida albican***
Sider honey	ND	ND	21.45 ±.39c	14.78 ±.12c	ND
Thymus honey	ND	ND	24.84±.13 b	17 b	ND
Multiflower honey	15.75±.66 a	23.33±1.25 a	30.4±.38 a	18.66 ±18.6a	ND
Citrus honey	14.4 ±.38b	24.66±.76 a	30.75±.25 a	20±.5 a	ND
Super honey	ND	ND	ND	ND	ND
House made honey	ND	ND	ND	ND	ND

Notes: ND: No inhibition was detected. (mean value ±standard deviation).
Values in the same column followed by the same letter are not significant different ($p < 0.05$) by the Tukey's multiple range test.

Table 3.2

Diameters of the inhibition zones (mm) produced by botanical original flowers for honey against different bacterial and fungi strains.

Type of flower	DISC DIFFUSION METHODS MEAN OF ZONE INHIBITION OF FLOWERS				
	Klebsiella pneumonia	***Escherichia coli***	***Staphylococcus aureus***	***Salmonilla typhimurium***	***Candida albican***
Sider flower	8.11 ± 0.19 c	9.09 ± 0.08 b	12.58 ± 0.52 b	10.43 ± 0.39 c	ND
Thymus flower	7.12 ± 0.13 d	10.59 ± 0.52 b	17.58 ± 0.52 ab	10.25 ± 1.09 c	ND
Multiflower	11.00 ± 0.25 a	9.3 ± 0.29 b	19.5 ± 6.21 a	17 ± 0.25 b	ND
Citrus flower	9.58 ± 0.52 b	13.3 ± 2.67 a	22.4 ± 0.38 a	18.9 ± 0.14 a	ND

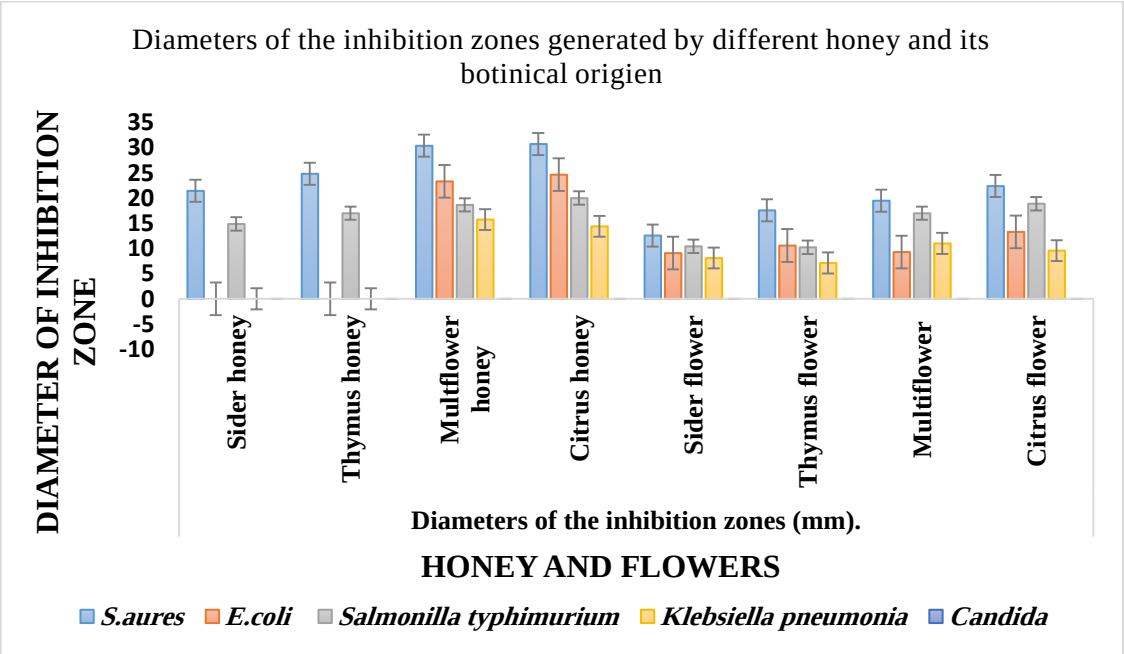
Notes: (mean value ±standard deviation).

ND: No inhibition was detected.

Values in the same column followed by the same letter are not significant different ($p < 0.05$) by the Tukey's multiple range test.

Figure 3.1

Diameters of the inhibition zones (mm) produced by different honey and its botanical original flowers against different bacterial and fungi species



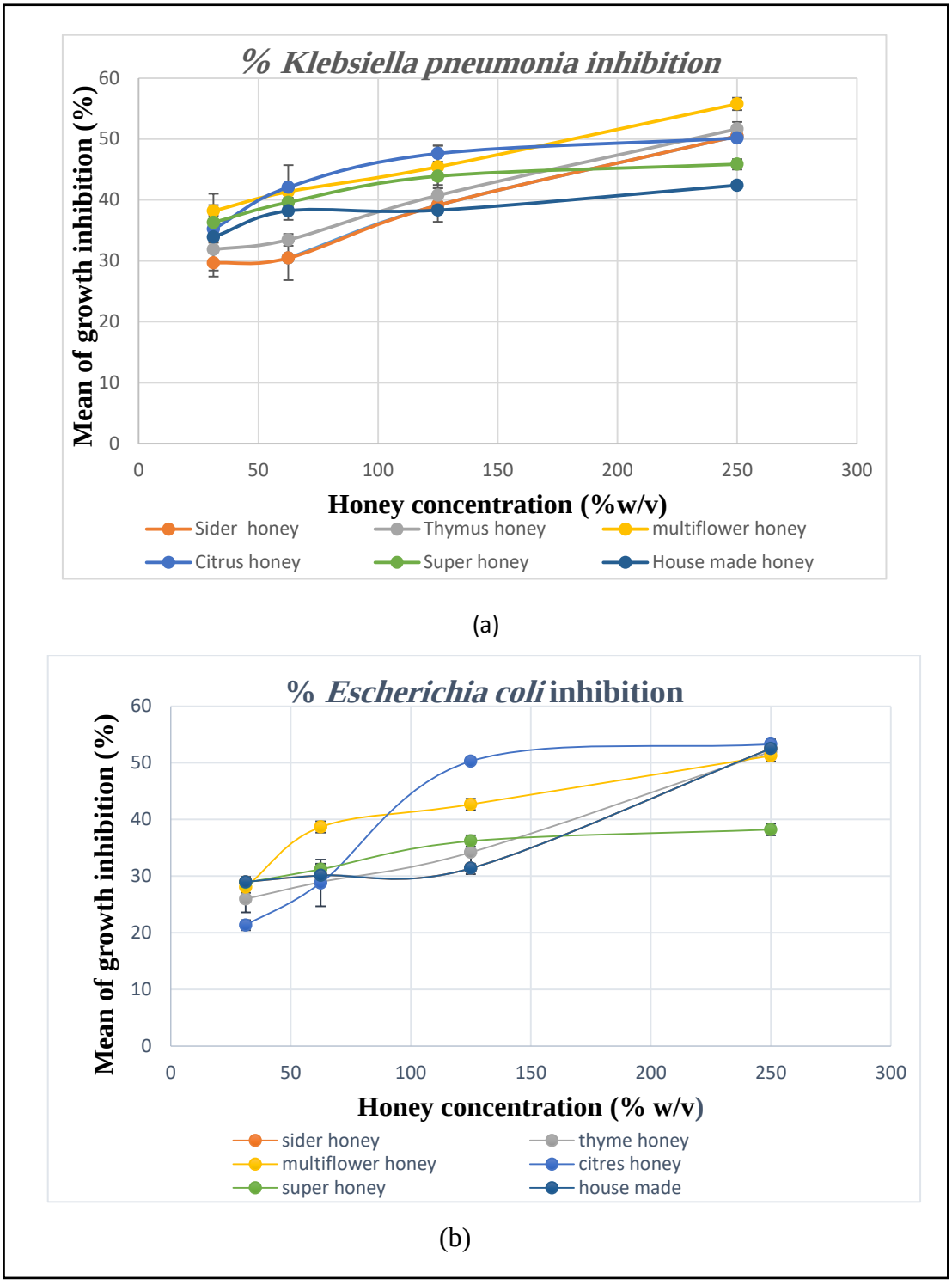
3.2 Broth microdilution methods (Minimum inhibitory concentration (MIC) assay)

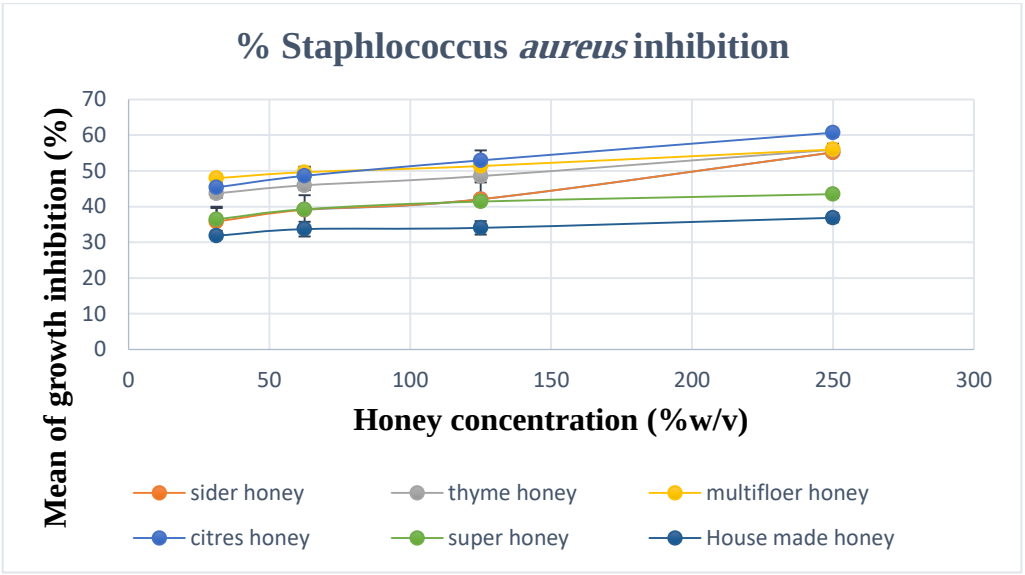
The antibacterial activity of honeys was accomplished by the MICs assay. Minimum Concentration required to inhibit the growth of 50% of organisms (MIC50). Details of the bacterial and fungi growth reaction to Palestinian honeys in the MIC test are shown in Figure 3.2, All microorganisms exhibited dose-response activity at varying degrees.

For *K. pneumonia* the lower MIC50 was Multiflower honey 177.86±9.75, and for *E. coli* it was Citrus honey 196.72±6.26 was lower MIC50, *S. aureus*, was Multiflower honey with lower MIC50 78.08±23 and *S.tyhimurium* the lower MIC50 was Citrus honey166.13±2.85 , no MIC50 for *C. albicans.*

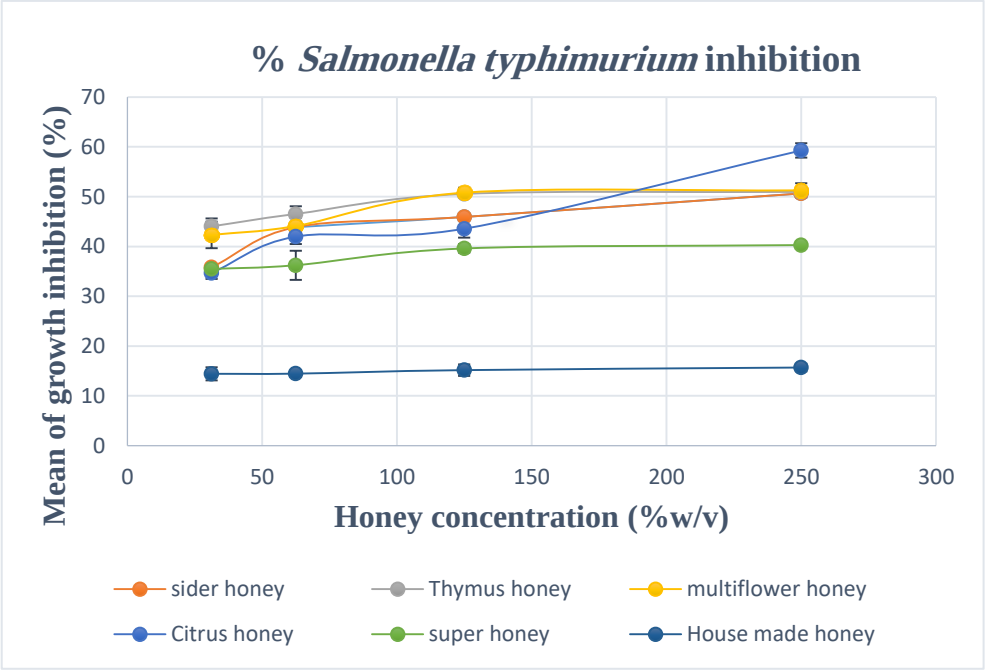
Figure 3.2

Percentage of bacteria and fungi growth inhibition by Palestinian honey

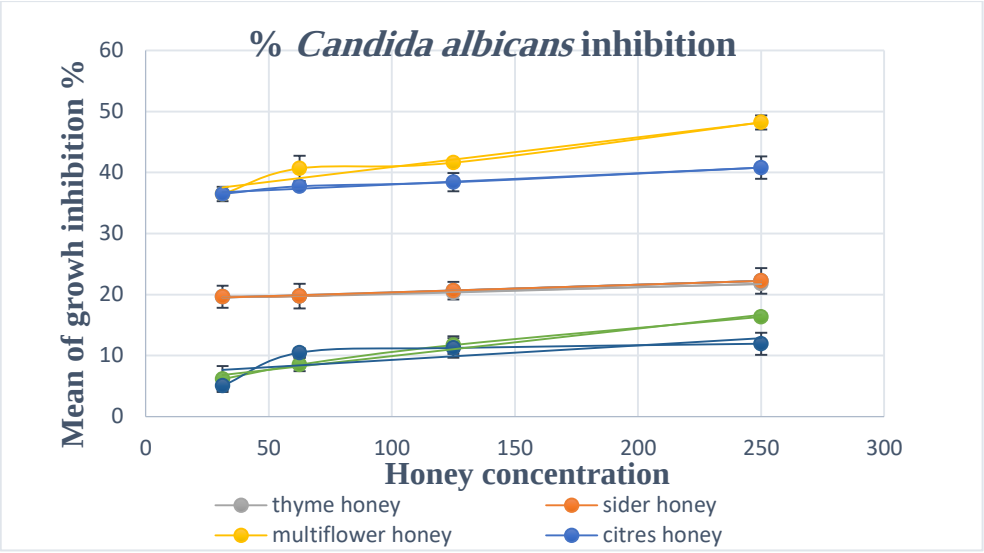



% Staphlococcus aureus inhibition
Mean of growth inhibition (%)
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Honey concentration (%w/v)
sider honey
thyme honey
multifloer honey
citres honey
super honey
House made honey

(c)


% Salmonella typhimurium inhibition
Mean of growth inhibition (%)
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Honey concentration (%w/v)
sider honey
Thymus honey
multiflower honey
Citrus honey
super honey
House made honey

(d)



(e)

(a) *Klebsiella pneumonia* (b) *Escherichia coli* (c) *Staphylococcus aureus* (d) *Salmonella typhimurium* (e) *Candida albicans* Sider honey (orange), Thyme honey (grey) , Multi flower honey (yellow), Citrus honey (light blue), Super market honey (green), House made honey (dark blue).

The MIC50 was expressed as mg/ml using the standard curve equation: Y = mX +B

Where:

Y-Mean of growth inhibition (%)

X-Honey concentration

From the equation MIC50 =(50-m)/B

All honey samples revealed a positive result against the test pathogens. Table 3.3 Summarizes the data of MIC50 of each honey sample against the test bacterial strain. The MIC 50values of honeys ranged from 78.08mg/ml to 241.35mg/ml on four strains amongst the tested bacterial strains, *S. aureus* was the most sensitive as shown figure 3.3.1 ,3.3.2

K. pneumonia was the most sensitive for Multiflower honey, *E. coli* was the most sensitive for citrus and *S. aureus* was the most sensitive for Multiflower honey and for S. *typhimurium* was citrus honey.

The lower MIC value indicates the higher antibacterial activity of the extract on the tested bacterial species. The findings also revealed that none of the extract inhibited *C. albicans* growth figure 6a,6b

Table 3.3

Minimum inhibitory concentration values (mg/ml) for different honey extract against selected pathogens (bacteria and fungi)

	MIC50 FOR HONEY EXTRACT				
Type of honey	***Klebsiella pneumonia***	***Escherichia coli***	***Staphylococcus aureus***	***Salmonella typhimurium***	***Candida albican***
Sider honey	241.35±3.64 a	240.66±4.9 a	197.23±26.12 a	233.32±10.86 a	>250
Thymus honey	230±12.1 a	238.37±6.24 a	143.3±14 ab	177.4±21.47 ab	>250
Multiflower honey	177.86±9.75 b	232.077±13.45 a	78.08±23bc	177.9±30.24 ab	>250
Citrus honey	230.75±10.1a	196.72±6.26b	82.13±31.8 c	166.13±2.85 b	>250
Super market	>250	>250	>250	>250	>250
House made honey	>250	>250	>250	>250	>250

Notes: (mean value ±standard deviation)

Values in the same column followed by the same letter are not significant different ($p < 0.05$) by the Tukey's multiple range test.

Figure 3.3.1

Minimum inhibitory concentration values (mg/ml) for one type of honey on different bacteria and fungi± standard deviation.

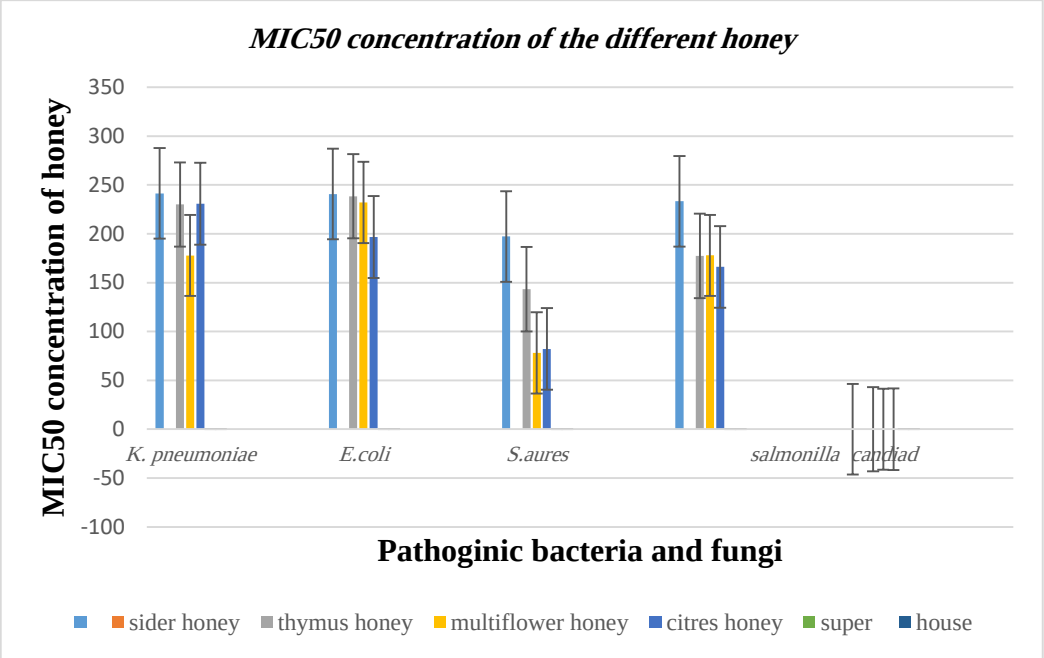
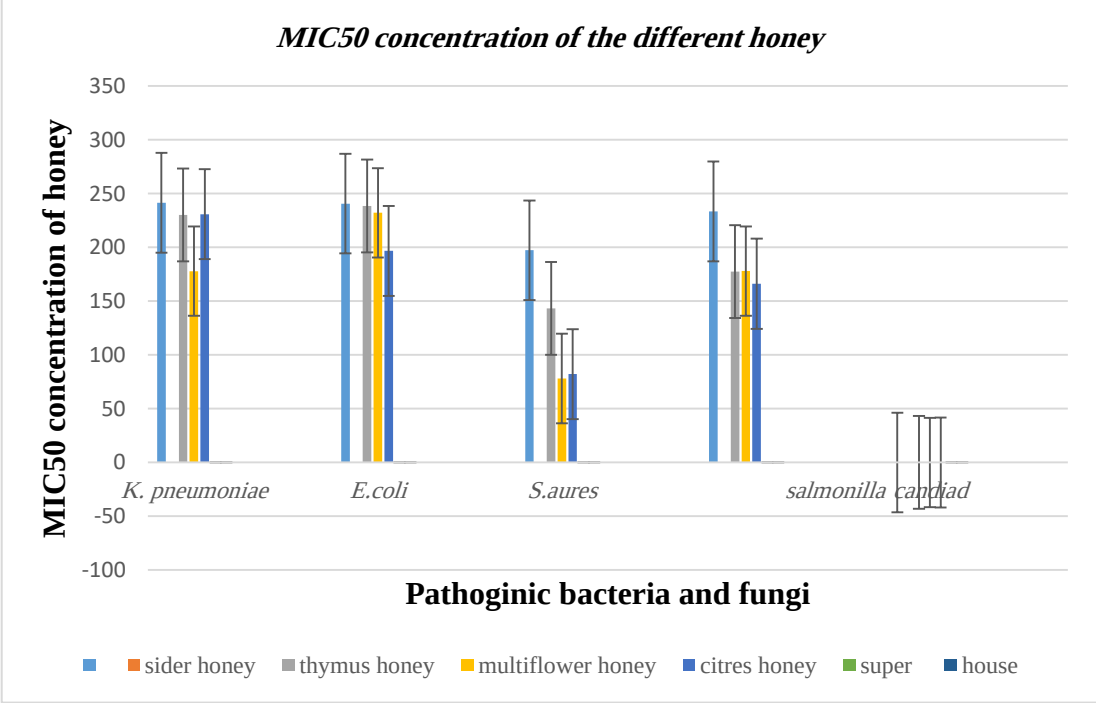


Figure 3.3.2

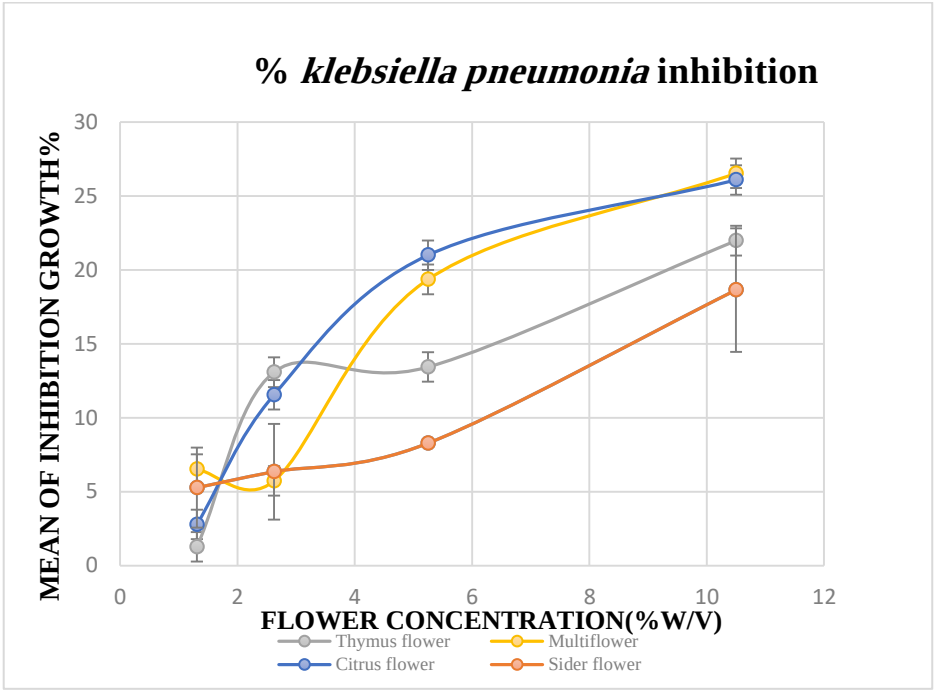
Effect of different honey on one pathogenic bacteria and fungi



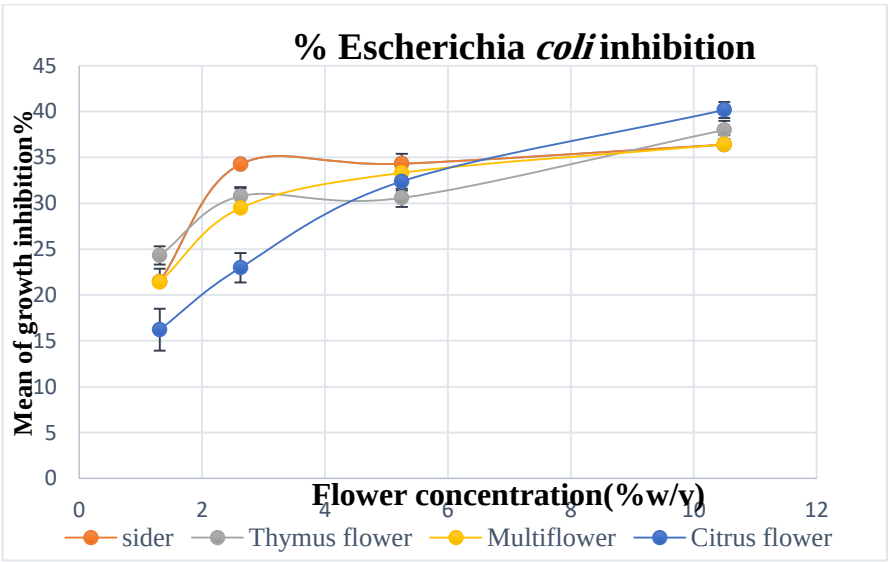
The antibacterial activity of flower extract was accomplished by the broth microdilution assay. All flower extract samples revealed a negative result for MIC50 against the test pathogens (bacterial strain and *C. albican*). Figure4.4 the highest concentration of flower extract was 10.5 mg/ml and the result of inhibition at these concentrations show at the table 3.4.

Figure 3.4

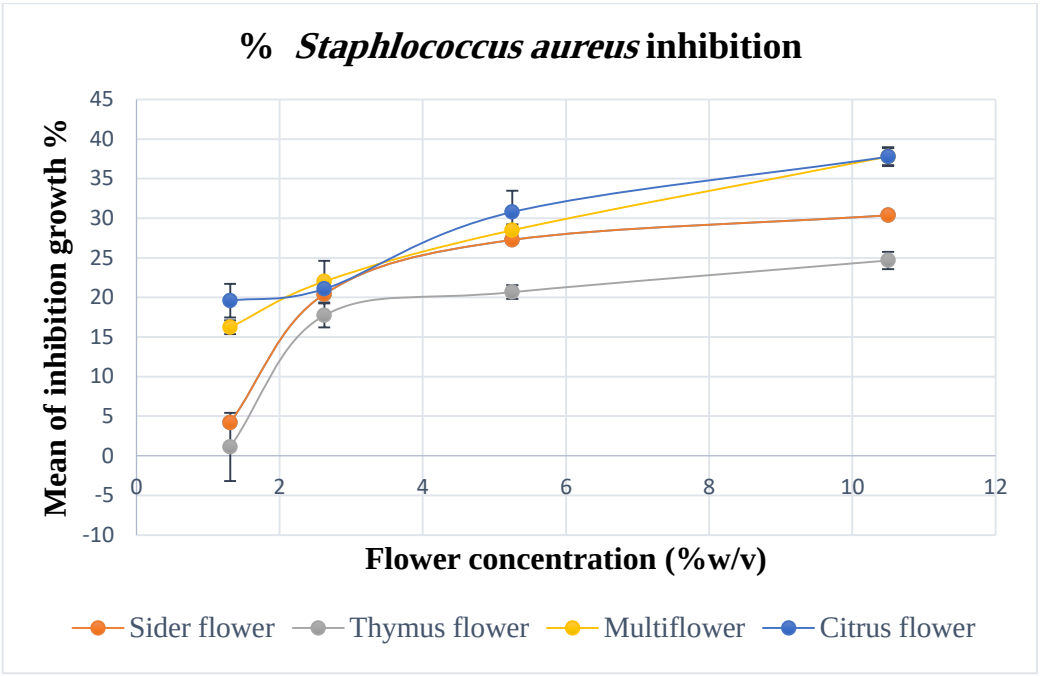
Percentage of bacteria and fungi growth inhibition by the origin of the honey.



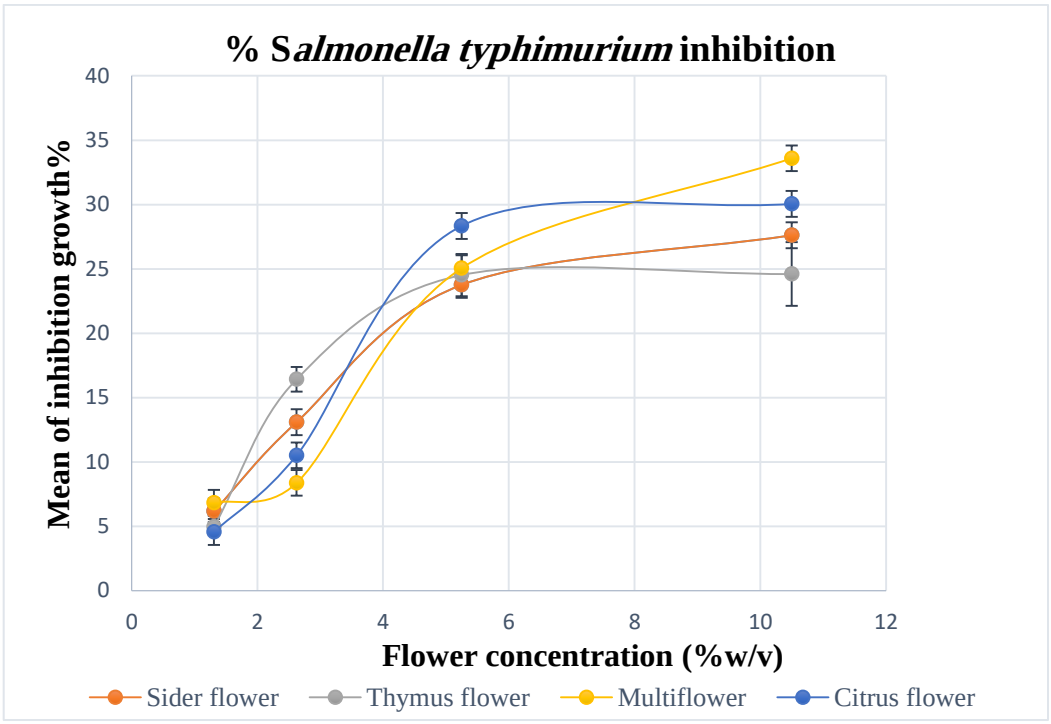
(a)



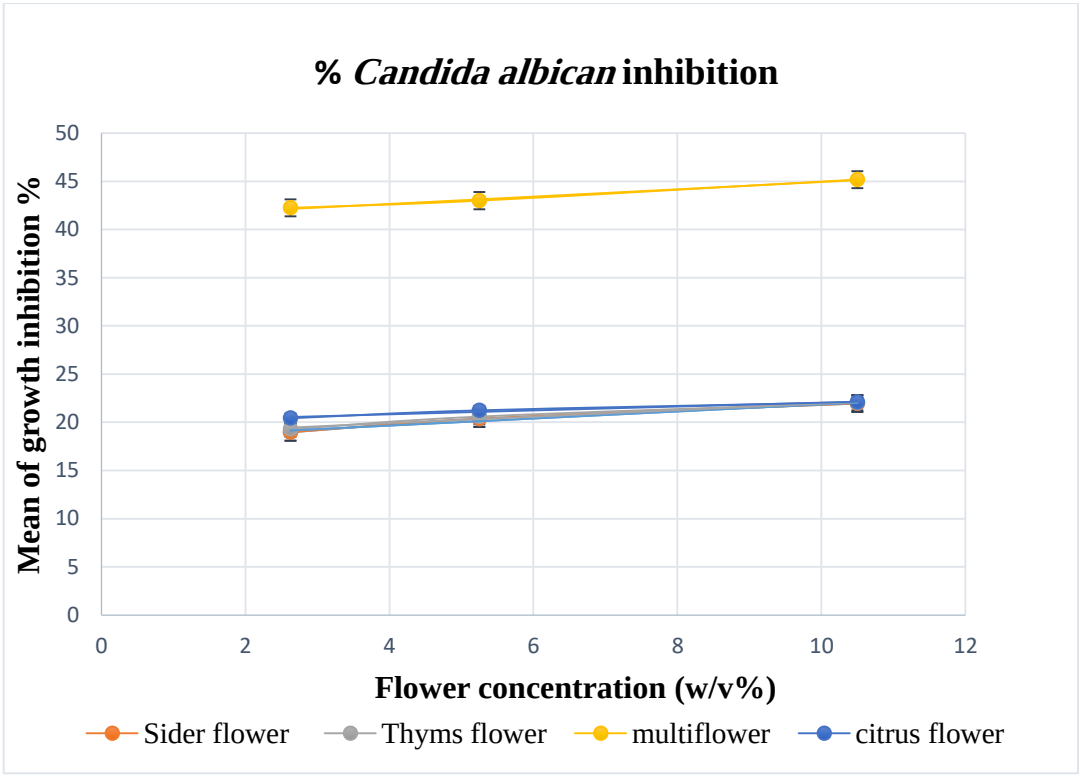
(b)


% Staphlococcus aureus inhibition
Mean of inhibition growth %
45
40
35
30
25
20
15
10
5
0
-5
-10
0
2
4
6
8
10
12
Flower concentration (%w/v)
Sider flower
Thymus flower
Multiflower
Citrus flower

(c)


% Salmonella typhimurium inhibition
Mean of inhibition growth%
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
Flower concentration (%w/v)
Sider flower
Thymus flower
Multiflower
Citrus flower

(d)



(e)

(a) *Klebsiella pneumonia* (b) *Escherichia coli* (c) *Staphylococcus aureus* (d) *Salmonella typhimurium* (e) *Candida albicans*

Sider flower (orange), Thyme flower (grey) , Multi flower (yellow), Citrus flower (blue).

Table 3.4

The mean of inhibition at 10.5 mg/ml for different flower extract against selected pathogens (bacteria and fungi)

Type of flower extract	MEAN OF INHIBITION AT 10.5 mg/ml				
	Klebsiella pneumonia	***Escherichia coli***	***Staphylococcus aureus***	***Salmonella typhimurium***	***Candida albican***
Sider flower	18.64±4.18 a	36.38±.01 a	30.37±1.75 b	27.6±2.12 ab	45.19±2.52 a
Thymus flower	21.98±1.76 a	37.99±2.06 a	24.66±1.1 c	24.59±2.47 b	43±1.11 a
Multiflower	26.53±3.82 a	36.40±1.01 a	37.78±1.06 a	33.59±3.9 a	42.25±1.2 a
Citrus flower	26.08±2.62 a	40.17±.88 a	37.76±1.19 a	30.04±1.98 ab	33.44±1.46 b

Notes: (mean value ±standard deviation)

Values in the same column followed by the same letter are not significant different ($p < 0.05$) by the Tukey's multiple range test.

3.3 Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

After MIC the were subjected to MBC assay by subcultering 10 micro from well on Mueller Hinton agar for bacteria and Sabouraud Dextrose agar for fungi there showed honeys have a bacteriostatic effect.

No fungus or bacteria are killed the MBC, MFC could be >250.

Chapter Four

Discussion and conclusion

4.1 Discussion

In this investigation, almost all of the honey types showed positive inhibitory effect on *E. coil*, *S. aureus*, *S. typhimurium*, and *K.pneumoniae*, and these findings are consistent with previous research on the antibacterial activity of honeys from other countries and these results support a number of studies on the antibacterial action of honey from many countries (Nweze et al., 2016). Honey's antibacterial activity has been linked to osmolality, pH, the production of hydrogen peroxide (H2O2), flavonoids, phenolic compounds, and the presence of additional phytochemical components. These biological substances are transferred from nectar to honey and play an important role in honey's health-promoting properties. Honey contains a high concentration of phenolic chemicals, which may explain its antibacterial properties. The amount of phenolic acid in honey is affected by both geographical location and the plant from which the nectar was derived(Matzen et al., 2018).

Furthermore, this investigation also demonstrated that, similar to antibiotics, some organisms were susceptible to some honey at different degree. The MIC values of the honey types varying depending on the bacterial strains and the honeys with lower MIC values are more effective antibacterial agents.

The difference in antibacterial activity seen can be attributed to a variety of factors. One theory is that the sensitivity of each type of microbe to the antibacterial action of the honey utilized varies (Nzeako and Hamdi 2000; Ceyhan and Ugur 2001; Taormina et al. 2001). Furthermore, the varying findings reported amongst honeys might be owing to the diverse floral sources used by the bees as well as geographical characteristics such as temperature and humidity where the honey was generated. Another reason for these findings might be variations in the putative antibacterial agents present in different honeys. These agents may contain both hydrogen peroxide and non-peroxide antioxidants flavonoids, phenolic compounds.

This investigation show that the most sensitive strain was *S. aureus* to various samples of honey. These results appear to support the earlier findings (Wadi, 2022), on the Gram positive *S. aureus* bacteria. The Gram stain offers a significant categorization method for bacteria since the number of cell characteristics may be linked to cell. As their exterior layer, gram-positive bacteria have thick (20–80 nm) cell walls. In contrast, Gram negative bacteria have a cell wall that is just 10 nm thick, but they also have an extra outer membrane this membrane restrict the passages of many substances. The cell's reactions to external stimuli including heat, UV light, and antibiotics are affected by these variations in the cell membrane (Mai-Prochnow et al., 2016).

This study found that Multiflower honey significantly reduced the growth of the pathogens *K. pneumonia* and *S. aureus* when compared to other honey kinds, this might be explained by the claim that each type of Multiflower honey contains different nectar/phytochemical content. Honey's antibacterial activity is directly dependent on its botanical sources, which also strongly influence its physical and chemical characteristics, including its antimicrobial qualities (Cilia et al., 2020).

Multiflower honey, is made from the nectar of several flowers (*Chrysanthemum coronarium L, Anemone coronaria* and *Bellis perennis)* these are having alkaloids, flavonoids and phenolic acids which have antibacterial effects, the argument that each variety of honey labeled for wildflowers contains various nectar contents and phytochemicals may have inhibitory action against bacteria help to explain this (Masalha et al., 2018). The previous studies showed that the Citrus honey is distinguished by the presence of Phenol, 2-methoxy-4 (1-propanol), 1-Hydoxylinalool, and 2-Aminobenzoic acid methyl ester. Furthermore, these compounds are not present in any other honey samples this might be explained the high antibacterial activity of the citrus honey (Masalha et al., 2018), and this could be explained why citrus honey was have highly antibacterial activity against *E. coli* and *S. typhimurium*.

This study shows that Palestinian honey has no antifungal impact on *C. albicans.* According to earlier research using a variety of honeys evaluated, the growth of *C. albicans* was not inhibited by the honeys. This result is consistent with other reports (Omafuvbe & Akanbi, 2009). However, some of the test organisms may not have been susceptible to the honey samples because a resistant strain may have developed. The

antifungal properties of honey may be influenced by a variety of circumstances. These include its physicochemical characteristics, botanical origin, entomological origin, and symbiotic interactions with helpful microorganisms, among other things (Feás & Estevinho, 2011).

Four types of honeys have inhibitory effects. However, honey does not kill > 99.9% of these bacteria and fungi. So, honeys are called bacteriostatic which mean the agent prevent bacterial growth, it preserves them in the stationary phase of growth, and bactericidal" refers to the fact that it kills bacteria. There aren't actually two distinct groups of antibacterial agents (one that completely kills bacteria and another that only inhibits growth), Instead, most so-called bacteriostatic agent kills some bacteria within the first 18 to 24 hr following the test, but not enough (> 99.9%) to be considered bactericidal (Pankey & Sabath, 2004).

Last but not least, the agar disc diffusion technique is a major qualitative test for sensitivity by classifying microbes as susceptible, intermediate or resistant and its ineffective in determining the minimum inhibitory concentration (MIC).The disc diffusion technique cannot identify the antibacterial activity of honey components as the active material or components may have limited ability to diffuse into the agar, hence the diameters of the identified growth inhibition zones may be tiny and undetectable. Unlike broth micro-dilution techniques, which are among the most significant assays for the antibiotic susceptibility of microorganisms since it gives both the MIC and an interpretation of susceptible, intermediate or resistant. The bacteriostatic and bactericidal activity may be quantitatively assessed using the broth dilution technique (Hossain et al., 2022).

4.2 Conclusion

According to the results, the antibacterial activity of Palestinian honeys depends on their botanical origin, honey can be used as an antimicrobial agent against specific pathogenic bacteria, and the proliferation of bacterial strains is inhibited by these honeys. A comparison of the variations between honeys from different botanical origins, as well as the inhibitory effect of honey on human pathogenic bacteria and fungi, could be useful in gathering more comprehensive data.

4.3 Recommendations

Future medical practice will consider honey's in vivo therapeutic limits. To develop the active component into a pharmaceutical product, more study is required on the compounds that give natural bee honey its potent antibacterial activity. more investigation is required, further studies on natural chemicals that can be utilized to fight microbes with few side effects or consequences of overdose or heavy consumption.

List of Abbreviations

Abbreviation	Meanings
AMR	Antimicrobial resistance
ATCC	American Type Culture Collection
B1	Thiamin
B2	Riboflavin
B5	Niacin
B6	pyridoxine
°C	Celsius degrees
CFU/mL	clone-forming units per milliliter
ExPEC	extraintestinal pathogenic E. coli
g	Grams
H2O2	Hydrogen peroxide
HIV	Human immunodeficiency virus
InPEC	intestinal pathogenic E. coli
K	Phylloquinone
L	Liter
MBC	minimum bactericidal concentration
MFC	minimum fungicidal concentration
µL	Microliter
μm	Micrometers
mg	Milligram
MIC	minimum inhibitory concentration
mL	Milliliter
nm	nanometer
OD	optical density
PH	Potential of hydrogen
TV	Thymus vulgaris
WHO	World Health Organization

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جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

التأثير المضاد للميكروبات للعسل الفلسطيني من أصول نباتية مختلفة ضد البكتيريا والفطريات المسببة للأمراض البشرية

إعداد

حنين محمد كامل قلالوه

إشراف

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجه الماجستير في ماجستير العلوم الحياتية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

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الملخص

الخلفية: بسبب المقاومة المتزايدة لبعض الأنواع البكتيرية والفطرية للمضادات الحيوية المختلفة، أصبح استخدام المواد الكيميائية الأساسية الطبيعية مثل العسل والنباتات الطبية جذابة بشكل متزايد. العسل هو أحد أشهر المصادر الطبيعية المضادة للبكتيريا الموثقة في الطب القديم.

الأهداف: هدفت هذه الرسالة الى تقييم التأثيرات المضادة للبكتيريا ضد *Klebsiella pneumonia* و *Escherichia coli* و *Staphylococcus aureus* و *typhimurium* Salmonellae والتأثيرات المضادة للفطريات ضد *Candida albicans* لأربعة أنواع من العسل فلسطيني من مناطق فلسطينية مختلفة ذات أصول نباتيه مختلفة. وكانت هذه العينات الأربعة هي عسل السدر، عسل الزعتر، عسل الليمون وعسل الربيع المتعدد.

المنهجية: اتبعت في هذه الدراسة منهجية قياس التأثيرات المضادة للبكتيريا والفطريات من خلال تقييم تأثير العسل والنبات المضاد للبكتيريا، والفطريات عن طريق نشر القرص والتي تعتبر الاختبار النوعي الرئيسي للكشف عن قابلية البكتيريا والفطريات للمواد المضادة للميكروبات وطريقة التركيز المثبط الأدنى (MIC)الذي يعكس الكمية المطلوبة للتثبيط البكتيري والفطري والحد الأدنى من تركيز مبيد الجراثيم (MBC) والحد الأدنى لتركيز مبيد الفطريات (MFC) الذي يعكس الموت البكتيري والفطري على التوالي.

النتائج: تكشف الدراسة الحالية أن العسل الفلسطيني المحلي المختلف في الأصل النباتي مثل السدر، الزعتر، الأزهار المتعددة وعسل الموالح كان فعالاً في تثبيط النمو في المختبر لـ *Klebsiella pneumoniae*، *Escherichia coli*، *Staphylococcus aureus*، *Salmonellae typhimurium*. كان عسل الأزهار المتعددة أقوى من أنواع العسل الأخرى في تثبيط نمو بكتيريا *Klebsiella pneumoniae* و *Staphylococcus aureus* حيث كان mic50 هو 177.86 ± 9.75 و78.08 ± 23 على التوالي كما كان عسل الليمون أقوى من أنواع العسل الأخرى في تثبيط نمو *Salmonellae typhimurium* و*Escherichia coli* حيث كان mic50 هو 196.72±6.26 ، 2.85166±13.على التوالي. كانت عينات العسل بتركيزات مختلفة أكثر فاعلية ضد بكتيريا Staphylococcus aureus من البكتيريا الأخرى. ولم يكن هناك تأثير للعسل في *candida albican*

الخاتمة: وفقًا للنتائج الحالية ، يعتمد النشاط المضاد للبكتيريا للعسل الفلسطيني على أصله النباتي ، ويمكن استخدام العسل كعامل مضاد للميكروبات ضد البكتيريا المسببة للأمراض ، ويتم منع تكاثر السلالات البكتيرية بواسطة هذا العسل.

الكلمات المفتاحية: العسل ، البكتيريا ، الفطريات ،الاصل النباتي، فلسطين.