FERYAL TANOĞLU INVESTIGATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *Ficus sycomorus* FRUIT AND LEAF EXTRACTS NEU 2019

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A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By
FERYAL TANOĞLU

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

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Approval of Director of Graduate School of Applied Sciences

Prof. Dr. Nadire ÇAVUŞ

We certify this thesis is satisfactory for the award of the degree of Master of Science in Food Engineering

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I hereby declare that, all the information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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To my parents...

ABSTRACT

In this study, antioxidant and antimicrobial activity of *Ficus sycomorus* has been studied. The antimicrobial activity of the leaf and fruit extracts were calculated by using disk diffusion method against pathogenic microorganisms such as; *Escherichia coli, Enterobacter cloacae, Klebsiella spp*. *Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis* and *Candida albicans.* As a result of the antimicrobial test for leaf-acetone, leaf-methanol and leaf-ethanol extracts showed inhibition zone against *S. aureus* between 10-13 mm diameters. Leaf-acetone and leaf-ethanol extracts showed 10 mm and 12 mm inhibition zone against *C. albicans,* respectively. There was no inhibition zone against *E. coli, E. cloacae, Klebsiella spp., B. subtilis, E. faecalis, S. epidermidis*. Antimicrobial activity was not observed against all microorganisms used in fruit extracts but bacteriostatic activity against *E. faecalis* was observed in fruit-water extract. The minimum inhibition concentration (MIC) was recorded as the highest in leaf-ethanol extract against *S. aureus* at 25 mg/mL with 9 mm inhibition zone. The MIC value for *C. albicans* was recorded as the highest in leaf-ethanol extract at 50 mg/mL with 10 mm inhibition zone. In antioxidant studies, the highest antioxidant activity (DPPH) in the leaf was observed in methanol extract, the highest phenolic content was observed in chloroform extract and the highest flavonoid content was in acetone extract. The highest antioxidant activity (DPPH) in the fruit was observed in acetone, ethanol and methanol extract, the highest phenolic and flavonoid content observed in acetone extract. As a result of this study, leaf extracts can be used as a curative agent for the treatment of Gram-positive bacterial and fungal infections and may be effective against pathogenic microorganisms that are resistant to antibiotics. Antioxidant content of fruit and leaf extracts can be effective against the negative effects of free radicals.

Keywords: Antioxidant activity; antimicrobial activity; disc diffusion method; ethanol; *Ficus sycomorus*

ÖZET

Bu çalışmada, *Ficus sycomorus*'un antioksidan ve antimikrobiyal aktivitesi çalışılmıştır. Yaprak ve meyve özlerinin antimikrobiyal aktivitesi disk difüzyon yöntemi ile *Escherichia coli, Enterobacter cloacae, Klebsiella spp., Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis* ve *Candida albicans* patojen mikroorganizmalara karşı yapılmıştır. Yaprak özütleri için yapılan antimikrobiyal test sonucunda yaprak-aseton, yaprak-metanol ve yaprak-etanol özütleri *S. aureus*'a karşı 10-13 mm çapları arasında bir inhibisyon zonu ve *C. albicans*'a karşı yaprak-aseton özütünde 10 mm, yaprak-etanol özütünde 12 mm çapında inhibisyon zonu olduğu görülürken, *E. coli, E. cloacae, Klebsiella spp., B. subtilis, E. faecalis, S. epidermidis* suşlarına karşı bir inhibisyon zonu oluşmadığı görülmüştür. Meyve özütleri ile yapılan antimikrobiyal test sonucunda ise tüm mikroorganizmalara karşı antimikrobiyal aktivite görülmezken *E. faecalis'*e karşı 1.8 mm çapında bakteriyostatik aktivite görülmüştür. En yüksek minimum inhibisyon konsantrasyon (MIC) değeri 25 mg/mL'de, yaprak-etanol özütünde *S. aureus'*a karşı 9 mm çapında inhibisyon zonu olarak kaydedilmiştir. *C. albicans* için en fazla MIC değeri 50 mg/mL'de yaprak-etanol özütünde 10 mm çapında inhibisyon zonu olarak kaydedilmiştir. Antioksidan testinde, yaprak için en yüksek antioksidan aktivite (DPPH) metanol-özütte, en yüksek fenolik içerik kloroform-özütte ve en yüksek flavonoid içerik aseton-özütte görülmüştür. Meyve için en yüksek antioksidan aktivite (DPPH) aseton, etanol ve metanol özütte görülmüştür. En yüksek fenolik ve flavonoid içeriği ise aseton-özütte görülmüştür. Bu çalışmanın sonucunda, yaprak-özütte Gram-pozitif bakteriyel ve fungal enfeksiyonların tedavisi için iyileştirici bir ajan olarak kullanılabileceğini ve antibiyotiklere karşı direnç gösteren patojenik mikroorganizmalara karşı etkili olabileceği görülmüştür. Antioksidan çalışmaları sonucunda ise, meyve ve yaprak özütlerinin antioksidan içeriğinin serbest radikallerin olumsuz etkilerine karşı etkili olabileceği görülmüştür.

Anahtar kelimeler: Antioksidan aktivite; antimikrobiyal aktivite; disk difüzyon yöntemi; etanol; *Ficus sycomorus*

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LIST OF ABBREVIATIONS

UTI: Urinary Tract Infections

W.H.O: World Health Organization

DPPH: 2,2-diphenyl-1-picrylhydrazyl

MPT: Multipurpose trees

MIC: Minimum Inhibition Concentration

IC_{50}: Half maximal inhibitory concentration

NC: Negative control

PC: Positive control

TPC: Total Phenolic Content

TFC: Total flavonoid content

SD: Standard deviation

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

GAE: Gallic acid equivalent

RE: Routine equivalence

w/v: Percent Weight / Volume

rpm: Revolution per minute

^{o}C : Degree of Centigrade

%: The percent

µL: Microliter

mL: Milliliter

mg: Milligram

µg: Microgram

µm: Micrometer

mm: Millimeter

g: Gram

Ca^{2+}: Calcium ion

$C_7H_6O_5$: 3,4,5-trihydroxybenzoic acid

$NaHCO_3$: Sodium bicarbonate

$NaNO_2$: Sodium nitrite

$AlCl_3H_{12}O_6$: Aluminum chloride hexahydrate

NaOH: Sodium hydroxide

C_6H_{14} : Hexane

CHC_{l3} : Chloroform

$C_4H_8O_2$: Ethyl acetate

$C_4H_{10}O$: Butanol

H_2O: Water

CHAPTER 1
INTRODUCTION

Since ancient times, people have benefited from plants such as food supply, fragrance and flavoring, firewood, weapons, medicine and shelter construction. Especially with the extracts obtained from medicinal plants, many diseases have been tried to be treated and thus healing has emerged as a profession (Diken, 2009). In the traditional and modern medical applications, the plant used as herbal medicine is called the Medicinal Plant (Deveci et al., 2016). Properly to a report by the World Health Organization (WHO), over than 80% of the world's population is based on conventional drugs for the needs of first healthcare (Ghareeb et al., 2015). All drugs used for diseases are produced from two basic sources. First group is synthetic drugs, the second group is seconder metabolities named natural products. It is obtained from microorganism cultures or healing plants (Al-matani et al., 2015b). Secondary metabolites are isolated from different parts of plants and they are an important source for pharmaceutical medicaments (Jouda, 2013). Since 1800s, the pharmaceutical industry was born with the synthetic production of the active ingredients in the plants, and traditional methods were largely abandoned. However, in the last 25-30 years, there has been an interest in alternative medicine because synthetic drugs used in modern medicine; cannot achieve the desired success in treatment, have many negative side effects, have a single positive effect and similar reasons. Natural medicines derived from plants are often more attractive than synthetic drugs because they do not have a very important side effect and have more than one positive effect. For this reason, herbal medicine research, which has been a medical influence for many years, has become a very interested area of research. In the last twenty years great importance has been given to medicinal plants because the medicinal plants are rich in natural antioxidant content and therefore have been the focus of many studies (Diken, 2009). Another reason for the importance of natural plants is that they have antimicrobial properties. The antimicrobial properties of a plant can inhibit bacteria that gain antibiotic resistance. Therefore, it contributes to the treatment of resistant pathogenic microorganisms (Saleh et al., 2015).

1.1 Aim of the Thesis

The aim of this study is to investigate the antioxidant and the antimicrobial activity (antibacterial and antifungal) of fruit and leaf extracts of *Ficus sycomorus*.

1.2 Natural Antioxidants

Natural antioxidants are endogenous (synthesized by the organism) or exogenous (taken from outside food) structures. Natural antioxidant production of the organism decreases as the age increases. Therefore, experts consider herbal antioxidants to be a good alternative. The most important antioxidant sources are fruits and vegetables. Some of the most important antioxidants in plants, fruits and vegetables that cannot be synthesized in human body are; Karentoids, Lycopene, Lutein, Polyphenols, Phenolic acids, Flavanoids, Catechins (Flavonols), Gallic acid, Vitamin E (Tocopherols) and vitamin C (Ascorbic acid). The task of antioxidants is to prevent abnormal cell proliferation and to protect the cells from damage due to oxidation (Kasnak and Palamutoğlu, 2015; Kolaç et al., 2017). Antioxidants are substances that stop or destroy the formation and negative effects of free radicals in the human body and food. The free radical is the name given to single non-paired electron atomic or molecular structures. Single electron portions that are not matched in atomic or molecular structures are called free radicals. Also known as "oxidant molecules" or "reactive oxygen particles" (Ozturk, 2012). Free radicals cause cardiovascular diseases, cancer, cataracts, diabetes, liver damage and many other diseases. Antioxidants prevent the formation of these diseases and also delay aging (Kasnak and Palamutoğlu, 2015). Natural antioxidants are harmless compared to synthetic antioxidants when used as an additive. In the food industry, synthetic antioxidants are used to protect nutrients from oxidative degradation and increase shelf life. These synthetic antioxidants are very effective, stable and inexpensive, but have side effects. In addition, synthetic antioxidants are known to show carcinogenic and teratogenic effects in living organisms. Consumers prefer natural antioxidants for these

reasons. Consumer preferences have led the food industry to seek natural antioxidant resources (Deveci et al., 2016).

1.2.1 Phenolic compounds

Phenolic substances are the most important groups of natural antioxidants. The most common plant phenolic antioxidants are flavonoids, cinnamic acid derivatives, coumarins, tocopherols and phenolic acids (Deveci et al., 2016). Phenolic compounds are biologically active compounds which contain one or more aromatic rings and contain one or more hydroxyl groups. Phenolic acids are divided into two basic groups according to their chemical structure. The first group contains hydroxy benzoic acid in their structure, and gallic acid is an important member of this group. The members of the second group have hydroxy cinnamic acid groups in their structures. Kaffeic acid is one of the most important examples of this group (Onar, 2015). Under normal conditions, the damage caused by oxygen radicals is kept under control by the effective antioxidant systems of the organism. However, in pathological conditions, the oxidant and antioxidant balance change. Research has shown that certain phenolic antioxidants inhibit cell death as a result of oxidative stress. The antioxidant effects of plant phenolics are especially due to their redox properties. So reducing agents, hydrogen donors, single they act as oxygen inhibitors and metal chelating agents. Phenolic antioxidants have a preventive role in coronary heart failure due to their effects on Ca^{2+} homeostasis (Deveci et al., 2016).

1.2.2 Flavonoids

Flavonoids represent a broad group of phenolic compounds with their antioxidant activity. The basic structure of flavonoids consists of two aromatic phenyl benzo pirene rings. These aromatic rings are connected to each other by a chain containing 3 carbons (Onar, 2015).

1.2.3 Gallic acid

Gallic acid (3,4,5-trihydroxybenzoic acid; $C_7H_6O_5$) is of the class of hydroxybenzoic acids and can be obtained by acidic or basic hydrolysis of tannins. Gallic acid is a natural antioxidant that can be extracted from plants, especially green tea. It is used in foods, medicines and cosmetics to prevent spoilage caused by lipid peroxidation and decay. In addition, due to the antimicrobial properties of gallic acid, new food additives, the starting material of which are gallic acid, are being developed (Yavaşer, 2011).

1.2.4 DPPH (2,2-diphenyl-1-picrylhydrazyl) Determination of Radical Scavenging Activity

DPPH is one of the most widely used antioxidant methods for plant samples. DPPH is a stable free radical. It adopts an electron or hydrogen radical to form a stable diamagnetic molecule. The lower the absorbance read at 517 nm by the addition of DPPH on the standard antioxidant samples, the higher the free radical removal activity. The decrease in the amount of DPPH in the environment with the decrease in absorbance is proportional to a certain concentration of antioxidants. The reason for the decrease in absorbance is the removal of the radical by hydrogen bonding as a result of the reaction of radical and antioxidant molecules. Furthermore, the lower the calculated IC_{50} values (the amount of sample reducing the DPPH concentration by half), the higher the radical scavenging activity (Yavaşer, 2011).

1.3 Antimicrobial Activity

Antimicrobials are agents that destroy or prevent the development of microorganisms. Therefore, antimicrobial activity plays an important role against many diseases caused by microorganisms. The antibacterial activity of some plants has been associated with theirbioactive compounds such as saponins, tannins, steroids, flavonoids anthraquinone, glycosides and reducing sugars (Saleh et al., 2015).

1.4 The Significance of the Thesis

Many causes such as constantly developing technology, environmental pollution, contaminated waters, radiation, heavy metals, pesticides and oxygen metabolism in living cells cause the formation of free radicals in the human body (Kasnak and Palamutoğlu, 2015). Free radicals are known to cause many diseases, particularly cancer. Antioxidants protect our body against all damages caused by free radicals that threaten human health. The importance of foods containing antioxidants should be known and consumed in order to prevent the spread of cancer disease in Cyprus and all over the World. Another important problem is the resistance of bacteria to antibiotics. Nowadays, all around the world, exploratory work is going on to find effective solution against drug resistant bacteria (Braide et al., 2018). The discovery of new antimicrobials through plants provides new approaches and benefits for minimizing antibiotic resistance. In many studies, it has been mentioned that *Ficus* species have potential antibacterial activity (Saleh et al., 2015). *Ficus sycomorus* has been the subject of curiosity about antibiotic resistance that has become a problem in the world due to its properties. In addition, it is thought that *Ficus sycomorus* may be an effective solution against the diseases caused by pathogenic microorganisms thanks to its antimicrobial activity which is thought to be possible. Also, the fact that this important Cypriot plant has a value to the culture it belongs to, and that it is rare and very little known and that there is no study that has been conducted on it in North Cyprus or in Turkey makes this thesis worthwhile and valuable.

1.5 Overview of *Ficus sycomorus*

Ficus sycomorus belongs to the Moraceae, which is a family of flowering plants, containing about fourty genera and more than thousand species. This family is the best commonly found in tropical and subtropical areas and is often referred to as the mulberry family or the fig family (Al-matani et al., 2015b). The plant is indigenous to African countries and mostly grows well in tropical countries like Oman. It also grows well in the Arabian Peninsula and in Lebanon. It is also found in Cyprus, Madagascar, Israel and

Egypt. The plant grows to a height of about 10 to 20 m (In India, the plant can be longer than 30 m). The branches begin from the lower part of the body and form shapes like umbrellas. Leaves are dark green, yellow-veined, heart-shaped and about 10 to 14 cm long (Figure 1.1). The diameter of the fruits is about 2 to 3 cm and round. The fruits are green when it is raw, and it becomes yellow or red when it ripen (Figure 1.2) (Hossain, 2018). The most suitable area for *Ficus sycomorus* trees is near drainage lines, streams, rivers, springs or dams. This plant grows well in a deep and well-drained soil, with an annual average of 500-1800 (max. 2200 mm), in clay soils and in soils with ground water (Kassa et al., 2015). The fruits and leaves of the *Ficus sycomorus* are used as food. Fruits are eaten when they ripen or stored in stewed or dried and it can also be used to prepare an alcoholic beverage. Leaves are used in soup making and peanut dishes. In Ghana, the wood ash is usually used as a salt substitute. In Philistines, the leaves are dried and added to the cake, used as spice or consumed as raw or cooked as a soup (Dluya et al., 2015). There is a shortage of feed in Ethiopia, especially during dry seasons. *Ficus sycomorus* is preferred, which is an multipurpose trees (MPT) due to insufficient feeding or poor feed quality because *Ficus sycoromus* leaves have a high nutritional value (14- 17.95% crude protein) and 12 MJ/kg net energy on DM basis) for animals (cattle, goats and sheep) (Kassa et al., 2015). This plate is also used to obtain fuel, to provide shade and shelter, to prevent erosion (Orwa et al., 2009).

Figure 1.1: The leaves of *Ficus sycomorus* (Ahmad et al., 2016)

Figure 1.2: The fruits of *Ficus sycomorus*

1.5.1 Taxonomy of *Ficus sycomorus*

The classification of *Ficus sycomorus* is shown in Table 1.1

Table 1.1: Classification of *Ficus sycomorus* (CABI, 2019)

Domain	**Eukaryota**
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Magnoliopsida
Order	Urticales
Family	Moraceae
Genus	Ficus
Species	Sycomorus

1.5.2 *Ficus sycomorus* in Northern Cyprus

It is a fruit that is known as 'Cümbez' or 'Pharaoh fruit' among the people. The tree of cümbez is known to give fruit seven times a year. When the tree gives fruit, the fruit is scratched with a knife and the fruit is mature. Scratched fruits ripen after about 7-10 days and become ready to be consumed. The maturing fruit turns from green to pink-orange. It is said that the method of maturing the fruits with a knife was discovered by the Egyptians. In the past, the idea of splitting the fruits was intended to escape the flies in the fruit and later it was determined that the fruits were matured. The fruits ripen with the resulting ethylene gas. The most well-known *Ficus sycomorus* plant in Northern Cyprus is located in the courtyard of the Lala Mustafa Paşa Mosque in Famagusta (Figure 1.3). The height of the tree is 15 meters and the estimated age is 715. The body of the tree is surrounded by

smaller branches growing from the main body. The body is divided into 7 branches after 2.70 meters. Each branch around the main body is said to have coincided with a century. It is the oldest and most vivid tree in Cyprus. The fact that this tree is the oldest tree in the history of the island and witnessed many events from the past to the present makes the historical Cümbez tree in this region culturally important. It is estimated that the tree was erected in 1298 when the construction of the cathedral began. It is known for giving an impressive shadow to the front of the cathedral. Another characteristic of the tree is the fall of the leaves in February and the gives the impression that the tree is dead. However, the revival of leaves within a month makes a great impression on humans. The tree in Famagusta is under protection and is included in the national heritage list of the Ministry of Culture (Bulut, 2018).

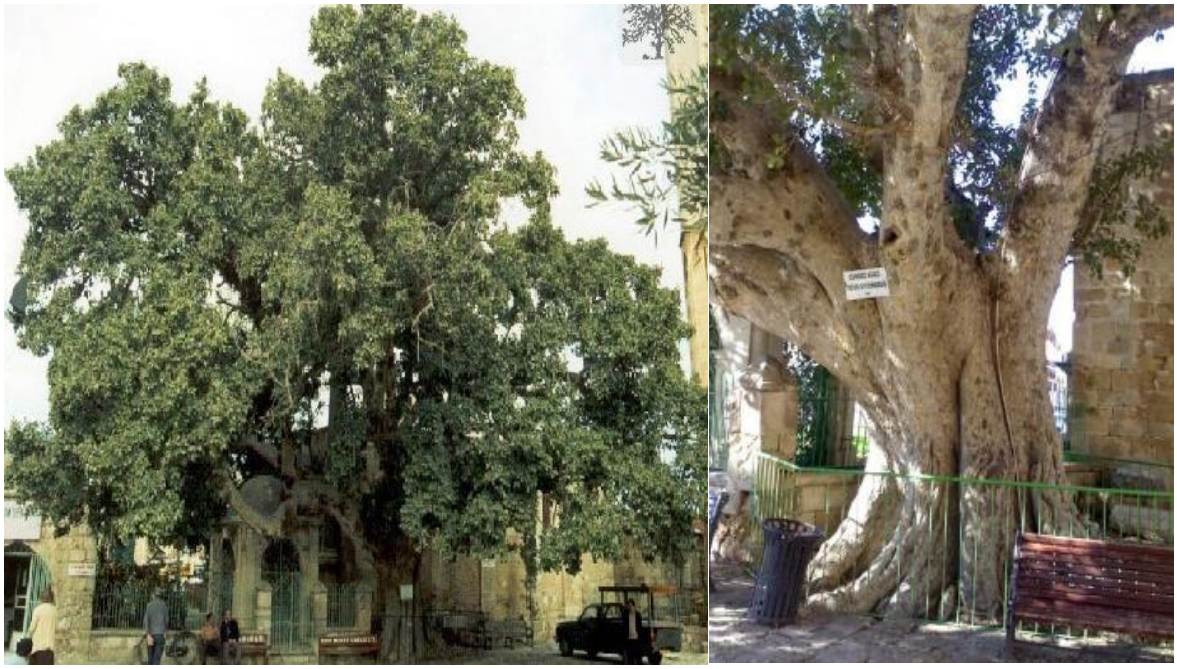

Figure 1.3: *Ficus sycomorus* in Lala Mustafa Paşa Cami avlusu, Gazimağusa (Anıt ve korunmaya değer ağaçlar, 2005)

1.5.3 Other names of *Ficus sycomorus*

Ficus sycomorus has local names by country such as baure in Hausa, opoto in Yaruba, ba'are in Fulbe, subula in Arabic, gular in Hindi, figuier sycomore or sykomore in French, sicomoro in Spanish, mukuyuchivuzi in Swahili, in English is known as wild fig, strangler fig, Sycamore, sycamore fig, bush fig, common cluster fig (Ahmad et al., 2016) and in Cyprus it is known as Cümbez tree.

1.5.4 The place in public medicine and its benefits to health

In Tanzania, particularly in the rural areas, the leaves of the plant are used in the treatment of jaundice, snake bites and at the same time they are used as latex to impact for chest diseases, cold and dysentery (Ahmad et al., 2016). In Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast extract of fruits, leaf, root and stem bark of *Ficus sycomorus* are used to treat various ailments such as cough, diarrhea, skin infections, stomach disorders, liver disease, epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility, sterility and diabetes mellitus (Dluya et al., 2015). The leaves of*Ficus sycomorus* have been informed to have antidiabetic and antioxidant properties (70% methanol extract). It also displays antitumor activity and antibacterial activity, but no antifungal activity (Abubakar et al., 2015). The organic root extracts of *F. sycomorus* have been reported to have more antifungal activity than the aqueous extracts (Jouda et al., 2015). *Ficus sycomorus* is also known for its antimicrobial activity in the treatment of fungal infections. The dry leaf of *Ficus sycomorus* contains high amounts of protein and raw fiber. In addition, the ash, lipid and carbohydrate content are in the desired proportions for dry leafy vegetables. It is used as spice in Philistines. The leaves are dried and added to the cake, consumed as raw or cooked as a soup (Dluya et al., 2015). The sedative and anticonvulsant properties of *Ficus sycomorus* has been reported and suspected to has antidiarrhoeal activity (Jouda et al., 2015).

1.6 The Microorganisms

In this study, seven pathogenic bacterial species and one type of fungus were used.

1.6.1 *Escherichia coli*

Escherichia coli is classified as part of the Enterobacteriaceae family of gamma-proteobacteria. It is a gram-negative and rod-shaped bacteria that is widely found in the lower intestine of warm-blooded organisms. The harmless strains of *E.coli* are a member of the normal flora of the intestines and produce vitamin K2 to their hosts, thus inhibiting the formation of pathogenic bacteria in the gut and providing benefit. *E. coli* is also known to cause urinary tract infections. Antibiotics that can be used to treat *E. coli* infection include; amoxicillin, semisynthetic penicillins, cephalosporin, carbapenem, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and aminoglycosides (Wikipedia contributors, 2019).

1.6.2 *Bacillus subtilis*

Bacillus subtilis is a gram-positive, catalase-positive bacteria, found in the soil and in the gastrointestinal tract of ruminants and humans. *B. subtilis* cells are rod-shaped, and are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter. *Bacillus subtilis* is a facultative anaerobe and spore-forming bacterium. It was recognized by the FDA that non-toxic and non-pathogenic strains of *B.subtilis* are widely available and are safely used in various food applications (Wikipedia contributors, 2019). *B. subtilis* is found in dust, soil, fertilizer, water, plants and animals. It causes spoilage in milk drinks, bread, vegetables and fruits. Suspected of causing food poisoning. *Bacillus subtilis* can cause eye inflammations such as panophtalmia and iridoxilide as a result of entering into the eye (Kalaylı and Beyatlı, 2003).

1.6.3 *Staphylococcus aureus*

S. aureus is a gram-positive, round-shaped, facultative anaerobe, non-softening and non-spore-forming bacteria. It is a member of the microbiota of the body and located on the upper respiratory tract and on the skin (Wikipedia, 2019). *S. aureus* causes superficial skin lesions (boils, shallots), localized abscesses, deep-seated infections such as osteomyelitis and endocarditis, more severe skin infections (furunculosis), infection of hospital-acquired (nosocomial) surgical wounds, intoxication of food by releasing enterotoxins to food and release of superantigens into the bloodstream causes toxic shock syndrome. *S. aureus* multiple antibiotic resistance is gradually increasing. The resistance to methicillin causes outbreaks in hospitals (Baron, 1996). The treatment for *S.aureus* infection is penicillin, β-lactam antibiotic, vancomycin (Wikipedia, 2019).

1.6.4 *Staphylococcus epidermidis*

Staphylococcus epidermidis is a gram-positive and facultative anaerobic bacterium. It is found in normal skin flora, human flora and mucosal flora. *S. epidermidis* is generally not pathogenic but patients with weakened immune systems are at risk of developing infection. The most common sources of infections of these bacteria are hospitals. The ability of biofilm formation in plastic devices is a basic virulence factor for *S. epidermidis.* It allows binding of other bacteria to existing biofilms and forms a multilayer biofilm. Such biofilms reduce the metabolic activity of bacteria in it. This decreasing metabolism, along with disrupted antibiotic spread, make it harder for antibiotics to destroy such infections. *S. epidermidis* strains are usually resistant to antibiotics such as methicillin, fluoroquinolones, gentamicin, rifamycin, clindamycin, sulfonamides and tetracycline (Wikipedia, 2019).

1.6.5 *Klebsiella spp.*

Klebsiella species are a Gram-negative and rod shaped bacteria belong to the Enterobacteriaceae family. They are mostly found in the environment and in the human

intestinal tract. *Klebsiella* species may cause various infections such as pneumonia, bloodstream infections, surgical site infections orwound, and meningitis. It is endogenously derived from the patient's own intestinal flora or exogenously from the health environment. Many patients with poor immune system carry the risk of infection. Infections can be associated with patient-to-patient spread, contaminated hands of healthcare workers, environmental contamination, the use of invasive devices, or medical procedures. *Klebsiella spp.* can become resistant to a broad range of antibiotics through a various of mechanisms for example, production of extended-Spectrum, Beta-lactamases or carbapenemase (Public Health England, 2017).

1.6.6 *Enterobacter cloacae*

Enterobacter cloacae is a gram negative, facultatively anaerobic, rod shaped bacterium. *E. cloacae* is oxidase-negative and catalase-positive. *E. cloacae* is found in the normal intestinal flora of most people and is generally not a primary pathogen. Some strains cause urinary and respiratory tract infections in humans with weakened immune systems. The treatment of these infections is possible with cefepime and gentamicin (Wikipedia., 2019).

1.6.7 *Candida albicans*

C. albicans is a member of our natural flora or the microorganisms living in or on our bodies. It is to exist in the gastrointestinal tract, vagina and mouth. *Candida albicans* is the most common cause of fungal infections in humans. *Candida species* cause fungal urinary tract infections (UTI), genital fungal infections, fungal skin infection, oral thrush. *Candida* types are a piece of the natural microflora of the gastrointestinal tract, skin, and vagina, and don't reason illness. Some conditions, such as using long-term antibiotics or having a poor immune system, may cause *Candida* infection. The best known *Candida* infections are

skin and vaginal infections that can be cure with antifungal drugs (MedicalNewsToday, 2018).

1.6.8 *Enterecoccus faecalis*

Enterecoccus faecalis is a gram-positive, commensal bacterium. These bacteria live in the our gastrointestinal tract, mouth and vagina. *E. faecalis* normally lives harmlessly in our guts. However, if it is dispersed to other region of the body, it may cause a more important infection. They are very resistant, so they can keep alive in hot, salty, or acidic environments. *E. faecalis* bacteria generally do not reason problems in healthy people. However, people with certain health conditions or a weak immune system are more likely to get sick. These bacteria are found in feces, so they can be transmitted through contaminated hands or from sources of contact with the infected hand. Especially in hospitals, it is transmitted from the dirty hands of health workers or medical devices that cannot be cleaned properly. *E. faecalis* causes several different types of infections in humans these are bacteremia, endocarditis, meningitis, periodontitis, urinary tract infections, wound infections. *E. faecalis* infections are treated with antibiotics. However, these bacteria are resistant to many antibiotics. Antibiotic used to treat *E. faecalis* infections include ampicillin, daptomycin, gentamicin, linezolid, nitrofurantoin, streptomycin, tigecycline, vancomycin. *E. faecalis* bacteria are sometimes also resistant to vancomycin (Healthline, 2017).

CHAPTER 2

RELATED RESEARCH

In this section, information was given about other studies on antimicrobial and antioxidant activities of *Ficus sycomorus*.

In study by Ghareeb et al. (2015), The leaves of *F. sycomorus* were dried and powdered then was kept in a dark room in a closed container until extraction. 200 g of the ground leaves soaking it in 2000 mL, then extracted separately with 85% methanol. Then extract was filtered and evaporatored (40 ± 2ºC). The 85% methanol crude extracts (20-30 g) were washed with petroleum ether at 60-80°C. 20 g extracted methanol extracts were fractionation with Chloroform, Ethyl acetateand n-Butanol (4x150 mL solvent). *F. sycomorus* leaves were tested for their In vitro antimicrobial activities. The antimicrobial test was calculated by disc diffusion method towards *E. coli, S. aureus, C. albicans* and *A. niger. F. sycomorus* extracts (leaf-methanol, leaf-methylene chloride, leaf-nBuOH, leaf-ethyl acetate, leaf-petroleum ether) exhibited antimicrobial spectrum towards *E. coli, C. albicans, S. aureus* with inhibition zones between 13-27 mm, but no activity against *A. niger.*

In study by Al-Matani et al. (2015a), The ground leaves which were extracted with MeOH using the maceration method were evaporated. The obtained extract was suspended in H_2O and extracted in C_6H_{14}, CHC_{l3}, $C_4H_8O_2$ and $C_4H_{10}O$ solvents. Total flavonoid content was evaluated by aluminum chloride method. The maximum flavonoid content was recorded as CHC_{l3}, C_6H_{14}, $C_4H_{10}O$, $C_4H_8O_2$ and H_2O extracts, respectively. Antimicrobial activity of the leaf extracts was evaluated by a slightly modified disc diffusion method towards various pathogenic microorganisms. The leaf extracts of *F. sycomorus* created inhibition zone between 0-12 mm towards *Proteus spp*., *H. İnfluenza* and *S. aureus, E. coli.*

In study Jouda et al. (2015), The antibacterial effect of *F. sycomorus* leaf and stem bark extracts and their synergistic antibiotics towards *P. Aeruginosa, E. coli* and *S. aureus* were investigated. The fresh leaves and stems of *F. sycomorus* were dried in the shade for one

week and it was ground with an electric mill. 20 g ground leaf and stem barks were extracted with 150 mL methanol and ethanol by a soxhlet extractor. Aqueous extraction was done by boiled on slow heat for 2 hours. Then the extracts were filtered and evaporated in oven at 45 ºC. The dried extract was dissolved in Dimethyl sulfoxide. Antibacterial activity of the leaf and stem-bark methanol, ethanol and water extracts of *F. sycomorus* against *S. aureus, E. coli* and *P. aeruginosa* was performed by paper disk diffusion assay. According to these results leaf-methanol extract of *F. sycomorus* exhibited inhibition zone towards *S. aureus* (11 mm), but no antibacterial activity *E. coli* and *P. aeruginosa*. Leaf-ethanol extract showed inhibition zone against *S. aureus* (12 mm), *E.coli* (8 mm), *P. aeruginosa* (7 mm). There is no inhibition zone against *P. Aeruginosa*, *S.aureus* and *E.coli* in leaf-water extract. The leaf-methanol and leaf-water extract of *F. sycomorus* were importantly active displaying the highest potency with MIC from 6.25-3.125 mg/mL towards *S. aureus*. The strongest effect against *S. aureus* was recorded when water extracts of *F. sycomorus* leaf and bark were mixed with Ceftriaxone. And the strongest effect on *E. coli* was observed when *F. sycomorus* leaves and bark were mixed with Ofloxacin. The strongest effect against *P. areuginosa* was observed when Ceftazidime was combined with *F. sycomorus* leaves and bark.

In study of Saleh et al (2015), 500 g shade-dried ground stem-barks and leaf of *F. sycomorus* were extracted with methanol and acetone solvent. All samples were evaporated. The concentration of extracts was 100 mg/mL. The antimicrobial test was performed by disc diffusion method towards sensitive and resistant species of *S. aureus* and *A. baumannii* microorganisms. Diameter of inhibition zone was 15–23.5 mm for methanol and 16–27 mm for acetone extracts. The value was calculated to be 26 mm for acetone leaf and 27 mm stem bark extracts and 23 mm for methanol leaf and, 23.5 mm for stem bark extracts. The MIC values for methanol leaf and stem bark extracts was 3.7–17.3 mg/mL and 2.5–13.5 mg/mL for acetone leaf and stem bark extract.

The most antibacterial activity was observed in sensitive *A. baumannii* 2.5 mg/mL for acetone-leaf extract and 4.9 mg/mL for acetone-stem bark extracts. It was found 3.7 mg/mL for methanol-leaf extract and 6.7 mg/mL for stem bark-methanol extract.

In study of Saleh and Al-Mariri (2017), fresh leaves and stem-bark were shade dried and extracted with etheric and acetonic solvents. The antimicrobial test was performed by disc diffusion method towards *L. monocytogeneses*, *S. aureus*, *B. cereus*, *E. coli* O:157, *S. typhimurium*, *B. melitensis*, *P. mirabilis* , *Y. enterocolitica* O:9, *P. aeruginosa* and *K. pneumonia*. Diameter of inhibition zone was 12-23 mm for stem bark-acetone and 16–27 mm for leaf-acetone extract. In the Saleh and Al-Mariri study, ether extract was found to have no inhibitory effect on all bacterial pathogens tested. MIC was determined by Microdilution broth assay. The MIC value calculated between 32.5-130.3 mg/mL and 52–182.3 mg/mL for stem bark-acetone and leaf-acetone extract respectively. It was observed that terpenoids were alkaloids as a result of phytochemical analysis, coumarins and fatty acids either in leaf and Stem bark. As for acetone extract, it was viewed that phenol content presented in the same trend with ether extract, in an reverse tendency to flavonoids. Whereas, alkaloids, saponins, terpenoids and tannins were not detected either in leaf or stem bark acetonic extracts.

In study Atiku et al (2016), phytochemical and antioxidant activity properties of leaf-ethanol extract of *F. sycomorus* were researched. The plant material was air dried under shade. 2.5 kg of plant material was exposed to cold maceration with 75% ethanol for 24 hours. The extract was filtered and evaporated. The remaining crude ethanol extract from evaporation was one after another fractionated using n-hexane, chloroform, ethylacetate and n-butanol. In this study, crude ethanol extract, n-hexane fraction and ethylacetate fraction were used. The crude ethanol extract, ethyl acetate and n-Hexane fractions of the leaves of the plant were subjected to preliminary phytochemical screening using standard procedure with qualitative and quantitative antioxidant activity using DPPH method. The conclusion of phytochemical screening displayed that the crude ethanol extract contains flavonoids, alkaloids, tannins, saponins, terpenoids and anthraquinones, the n-hexane fraction contains, terpenoids, alkaloids and anthraquinones while the ethylacetate fraction contains alkaloids, tannins, saponins, flavonoids, terpenoids, anthraquinones and cardiac glycosides coumarins were found to be unavailable in the leaves. The results of the antioxidant test the leaf extracts have IC_{50} of 44.83 µg/mL, 58.46 µg/mL and 42.00 µg/mL

for crude n-hexane, ethanol and ethylacetae respectively. Vitamin C was found IC_{50} of 25.00 µg/mL.

In study of El-Sayed et al (2009), total phenolic contents and antioxidant activity were investigated. Dried powdered 100 g of leaf were extracted with MeOH, MeOH-water mixtures and distilled water then filtered and concentrated by rotary evaporator. The obtained crude MeOH (70%) extract was defatted with petroleum ether and fractionated by; $CHCl_3$, $C_4H_8O_2$ and n-Butanol. The antioxidant activity of leaf extracts was appraised by using DPPH method and total antioxidant content using phosphomolybdenum technique. The extract of methanol (70%) containing the most value of phenolic compounds showed the most antioxidant activity in all analyzes. Thus, the extract of methanol (70%) showed the highest effective solvent for the extraction of antioxidant compounds from the leaf of *F. sycomorus*. The activity of the extracts varied according to different different temperatures, pH values and storage.

In study of Ramde-Tiendrebeogo et al (2012), the antioxidant and antibacterial activities of phenolic compounds from *F. sur* and *F. sycomorus* types were investigated. 25 grams of ground leaves extracted in a Soxhlet system with chloroform, ethanol 90%, distilled water. The results of *F. sycomorus* extracts (336 mg TAE/g and 203 mg TAE/g) were higher than the results of *Ficus sur* extracts (247 mg TAE/g of extract and 120.8 mg TAE/g). As a result of the DPPH test, that extracts of *F. sycomorus* present the most antiradical activity with IC_{50} value of 9.60 µg/mL against 31.83 µg/mL for *Ficus sur*. The IC_{50} value of quercetin, was of 4.6 µg/mL. The latex of *F. sycomorus* showed the MIC value towards *S. aureus* (0.13 mg/mL) and *E. coli* (0.25 mg/mL).

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials and Equipment Used

Glass materials: Graduated cylinders, Sterile bottles, Round bottomed flasks, Conical flasks, Beaker, Glass funnel, Volumetric flasks, Graduated glass pipettes.

Used kits, solvents, broths and solutions: Mueller-Hinton Agar, Blank antimicrobial dicks (BioAnalyse Limited), Susceptibility antibiotic discs (Bioanalyse Limited (Ciprofloxacin) 5 µg, (Tetracycline) 30 µg, (Teicoplanin) 30 µg and (Nystatin) Oxoid 100 units), Phoenix ID Broth, Methanol, Ethanol, Pure water, Acetone, Chloroform, Folin-Ciocalteu reagent, DPHH (2,2-diphenyl-1-picrylhydrazyl), Sodium bicarbonate ($NaHCO_3$), Sodium nitrite ($NaNO_2$), Aluminum chloride hexahydrate ($AlCl_3H_{12}O_6$), Sodium hydroxide (NaOH), Agar plates, Sterile wooden cotton applicator stick, Whatman quantitative filter papers, Autoclave band, Pippettes, Cotton wool, Foil, Bunsen burner.

Equipment: Fume hood, Autoclave (OT 40L), Excalibur parallax food dehydrator, Densitometer (McFarland Phoenix Spec), Incubator (Heraeus thermo scientific), Vortex Mixer (VELP SCIENTIFICA), Washing machine (LANCER), Weighing balances (SHIMADZU ELB 300 and METTLER TOLEDO), Rotary evaporator (Buchi Rotavapor R-210, Switzerland and Heidolph Laborota 4001), Bandelin Sonerex (Digital 10 P ultrasonic baths), IKA Shakers (KS 260 Basic), VITEK 2 Compact (Automated ID/AST Instrument), Belimed (Infection Control Steam Sterilizer), Belimed (Infection Control Medical Heat Sealer), Class 6 Steam Emulating Indicator, Spectrophotometry (Biochrom Libra S60 B,England).

NOTE: All the laboratory materials were mechanically washed using a washing machine (LANCER) and then the materials were packaged in the Medical Heat Sealer device (Belimed Infection Control) and sterilized in the steam sterilization machine (Belimed Infection Control) with Class 6 Steam Emulating Indicator (Used for routine monitoring of steam sterilization cycles).

3.2 Collection and Preparation of Plant Material

Fruits and leaves of *Ficus sycomorus* were collected from the Kyrenia region of Northern Cyprus in July. The collected fruit and leaves were washed to remove dust and soil and then dried. The washed fruits were cut into thin slices with a knife (Figure 3.1) and dried in a food dehydrator machine (Figure 3.2) and the washed leaves were dried at room temperature (Figure 3.4) The dried leaves and fruits were ground with an electric mixer (Figure 3.3) and stored in a +4°C refrigerator until the day of use in the laboratory.

Figure 3.1: Sliced fruits

Figure 3.2: Slices of fruit placed in a food dehydrator machine

Figure 3.3: Grinding of dried fruits with electric mixer

Figure 3.4: Leaves left to dry at room temperature

3.3 Preparation of Leaf and Fruit Extracts

The ground leaves are weighed into sterile empty glass bottles at 10 grams and the ground fruits are weighed into glass bottles at 20 grams for five different solvents. 100 mL of 5 different solvents (Methanol, Ethanol, Acetone, Distilled water, Chloroform) were added into the bottles with ground leaf samples (1:10 [w/v]) and 200 mL of 5 different solvents are added into the bottles with fruit samples (1:10 [w/v]). Then the bottles were closed and the leaf and fruit samples were extracted with the solvents in the shaker (IKA, KS 260 Basic) for 72 hours at room temperature (Figure 3.5). At the end of 72 hours, the shaken samples were filtered into a sterile glass bottle with filter paper (Figure 3.6).

Table 3.1: Properties of organic solvents used (Kimyaevi, 2018)

Solvent	**Formula**	**Polarity Index**	**Boiling point (°C)**
Methanol	CH_4O	6.6	65.0
Ethanol	C_2H_6O	5.2	78.5
Chloroform	$CHCl_3$	4.4	61.7
Acetone	C_3H_6O	5.4	56.2
Water	H_2O	9.0	100.0

Figure 3.5: Extracting grated fruits and leaves with solvents in a shaker

Figure 3.6: Extracted samples filtered into sterile bottles with filter paper

3.4 Extraction of Fruit and Leaf Extracts

Each of the fruit and leaf extracts in a sterile bottle was transferred to the round bottomed flask with the funnel for extraction. Then, the round bottomed flask was fitted to the rotary evaporator and the machine was operated by adjusting the appropriate pressure and temperature for each solvent (Table 3.2). After this process, the solvents of each sample were evaporated (Figure 3.7) and the extracts were obtained. The obtained extracts was allowed to cool and after that, the methanol is added in round bottomed flask with the accordance the total extraction yield at a concentration of 100 mg/mL and placed in the ultrasonic bath to dissolve dry extracts adhering to the round bottomed flask (Figure 3.8). All samples were transferred to sterile bottles with pipette after dissolving in ultrasonic bath and stored in +4°C refrigerator until the time they were used in laboratory.

Figure 3.7: Evaporation of leaf and fruit extracts with a rotary evaporator

Figure 3.8: Thawing process in ultrasonic bath

Table 3.2: The amount of pressure (mbar) required for solvents to evaporate at 40°C

Solvent Vacuum in mbar for boiling point at 40°C	
Methanol	337
Ethanol	175
Chloroform	474
Acetone	556

3.4.1 Calculation of total extraction yield in percentage

1. The tare of the empty bottle is taken.
2. Add the sample into the vial and put it on the rotary evaporator.
3. After evaporation, we weigh the flask again and we find the amount of extract we obtained by removing the bottle weight.
4. How many grams of ground leaves or fruits are present in the sample is calculated in percentages with the obtained extract.

% Total Extraction yield (g/g): 100 × (Weight of round bottom flask after evaporation – Empty round bottom flask) / Amount of ground samples

The amount of ground sample is 20 g for fruits and 10 g for leaves.

3.5 Preparation of the Mueller-Hinton Agar

Mueller hinton agar is used to test the sensitivity of clinically important pathogens. 1 L of water is added into the conical flask. Dissolve 34.0 g mueller hinton agar in 1 L of water. The conical flask is heated and shaken in boiling water to ensure better dissolution. The conical flask is sealed and sterilized in an autoclave at 121°C for 15 minutes. After the

autoclave, is cooled to 50-45 °C and poured into the sterile petri dishes under the fume hood. After than, cooled at room temperature and stored at +2-8 °C.

Table 3.3: Composition of mueller hinton agar

Ingredients/Composition	**g/L**
Beef Extract	2.0 g
Acid Hydrolysate of Casein	17.5 g
Starch	1.5 g
Agar	17.0 g

3.6 Antimicrobial Test

NOTE: This test was done under the fume hood with bunsen burner.

The antimicrobial activity of the leaf and fruit extracts were evaluated by using the Kirby-Bauer Disk Diffusion Method (Bauer et al., 1966). 10 samples in sterile bottles (fruit-water, fruit-chloroform, fruit-methanol, fruit-ethanol, fruit-acetone, leaf-water, leaf-chloroform, leaf-methanol, leaf-ethanol, leaf-acetone) are removed from the refrigerator.

Each sample is placed on the shaker before using and 20 µL sample taken with the pipette and released into blank discs (Figure 3.9). The process of released the sample into blank discswas carried out in a sterile petri dishes (Plates). It is then left to dry.

The microorganism names and sample names are written on a mueller hinton plates with a pen. On the agar plate, the YA represented the leaf-acetone, YM represented the leaf-methanol, YE represented the leaf-ethanol, YK represented the leaf-chloroform, YS represented the leaf-water, MA represented the fruit-acetone, MM represented the fruit-methanol, ME represented the fruit-ethanol, MK represented the fruit-chloroform and the MS represented the fruit-water samples.

A total of 1 fungal and 7 bacteria species were used for the antimicrobial test. They include three gram-negative bacterial specie; *Escherichia coli* ATCC 29922, *Enterobacter cloacae* and *Klebsiella spp*. while the gram-positive bacteria included; *Bacillus subtilis* B-354, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis*. The fungus specie used was *Candida albicans* ATCC 90028.

Enterobacter cloacae, Klebsiella spp. and *Staphylococcus epidermidis* were suspended in glycerol and identified by VITEK 2 Compact, Automated ID/AST instrument. *E. coli, E. faecalis, C. albicans, B. subtilis* and *S. aureus* were grown in stock culture. Stock culture of *C. albicans* was suspended in Muller-Hinton broth and then incubated for a day before use. The fungal and bacterial species were grown in cultures. Then a small amount was taken bacteria and fungus type with the cotton applicator stick and transferred into Phoenıx ID broth (4.5 mL). The bottle containing the phoenix ID broth was placed on a vortex (Velp Scientifica) and agitated on 30 hertz. Then, the bottle placed in a densitometer (McFarland Phoenix Spec) to prepare microbial suspension from pure colony at 0.45-0.55 (standard McFarland number and for antibiogram). A densitometeris used to measure the turbidity of the cell suspension. 10 µL of the microbial suspension is taken with a pipette and transferred to the center of mueller hinton agar and then spreads homogeneously to the surface with a wooden cotton applicator stick (Figure 3.10).

Then, the discs absorbed by the samples (YA, YM, YS, YE, YK, MA, MM, MS, ME, MK) are placed on the mueller hinton agar surface at regular intervals with PC (positive control) and NC (negative control). Pure methanol was used as the negative control for all samples. 20 µL methanol taken with the pipette and released into blank discs and after placed in the agar.

As the positive control, Tetracycline (Bioanalyse Limited, 30 µg) was used for *Bacillus subtilis* B-354, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis.* Ciprofloxacin (Bioanalyse Limited, 5 µg) was used for *Escherichia coli* ATCC 29922, *Enterobacter cloacae* and *Klebsiella spp.* Nystatin (Oxoid, 100 units) was used for *Candida albicans* ATCC 90028. Teicoplanin (Bioanalyse Limited, 30 µg) was used as positive control for *Enterococcus faecalis* ATCC 29212.

The plates are kept at room temperature for 20-30 minutes. Then the plates were placed in the incubator for 18-24 hours at 37°C. Following the incubation, the clear zones around the discs were evaluated and their diameters were measured.

Figure 3.9: Sample taken with the pipette and released into blank discs

Figure 3.10: Spreading the bacterial suspension onto the mueller hinton agar surface

3.7 Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentrations (MIC) of the *Ficus sycomorus* leaf and fruit extracts that showed antimicrobial activity against test microorganisms were determined. This analysis was performed based on fact that the lowest inhibitory concentration determines to effective on test micoorganisms. In this test, the 12.5, 25, 50, 75 and 100 mg/mL concentrations of the acetone leaf extracts, ethanol leaf extracts, methanol leaf extract and pure water fruit extract were investigated for their inhibitory effects against *C. albicans*, *S. aureus* and *E. faecalis*. The minimum inhibition concentration was done using the disc diffusion method. The samples (acetone-leaf extracts,ethanol leaf extracts, methanol leaf extract and pure water fruit extract) were released into blank discs and left to dry. Then a small amount was taken bacteria and fungus type with the cotton applicator stick andtransferred into Phoenıx ID broth (4.5 mL). The bottle containing the phoenix ID broth was placed on a vortex (Velp Scientifica) and agitated on 30 hertz. Then, the bottle placed in a densitometer (McFarland Phoenix Spec) to prepare bacteria suspension from pure colony at 0.45-0.55 (standard McFarland number and for antibiogram). A densitometeris used to measure the turbidity of the cell suspension. 10 µL of the bacterial suspension is taken with a pipette and transferred to the center of mueller hinton agar and then spreads homogeneously to the surface with a sterile swab. The microorganism namesand sample names are written on a mueller hinton plates with a pen. Then, the discs absorbed by the samples (Acetone-leaf extracts (YA), Ethanol-leaf extracts (YE), methanol leaf extract (YM) and pure water fruit extract (MS)) are placed on the mueller hinton agar surface at regular intervals with PC (positive control) and NC (negative control). Pure methanol was used as the negative control for all samples. 20 µL methanol taken with the pipette and released into blank discs and after placed in the agar. As the positive control, Tetracycline (Bioanalyse Limited, 30 µg) was used for *Staphylococcus aureus* ATCC 25923. Nystatin (Oxoid, 100 units) was used for *Candida albicans* ATCC 90028. Teicoplanin (Bioanalyse Limited, 30 µg) was used as positive control for *Enterococcus faecalis* ATCC 29212. The plates are kept at room temperature for 20-30 minutes. Then the plates were placed in the incubator for 18-24 hours at 37 °C. Following the incubation, the clear zones around the discs were evaluated and their diameters were measured.

3.8 Total Antioxidant Test

The antioxidant activity of extracts were determined by DPPH Radical Scavenging Method. This method is based on the reduction of DPPH, a dark violet color compound and the absorbance reduction is measured by UV-GB spectrophotometer (Büyüktuncel, 2013). The antioxidant activities of the extracts, which are expressed as the activity of capturing free radicals, were determined by the use of DPPH (2,2-diphenyl 1-picrylhydrazyl) radicals as according to the method of Yılmaz (2011); Uçan Türkmen et al. (2016). DPPH radical (0.025 g/L) prepared in 3.9 mL of methanol was added to 100 µL of the extracts. The mixture was incubated at room temperature and in the dark for 30 minutes. In this analysis based on the opening of purple color of the DPPH solution, the residual amount of DPPH was measured at 515 nm by using spectrophotometer. Inhibition of DPPH was calculated as percent by following formula. All analyzes were repeated 3 times.

For the control value: Methanol + DPPH, For Blank: Methanol,

Against Blank (methanol): Methanol + DPPH (control), Against Blank: Plant sample + DPPH were used.

% Inhibition = [(Control Absorbance – Sample Absorbance / Control Absorbance)] × 100

3.9 Total Flavonoid Content

According to the method reported by Sharma and Vig (2013), 1 mL of extracts were diluted with 5 mL of distilled water. To the samples 0.3 mL $NaNO_2$ (5%) was added and incubated for 5 min at room temperature. Then 0.6 mL of $AlCl_3.6H_2O$ (10%) was added to the mixture and after incubation under the same conditions, 2 mL of 1M NaOH was added and the final volume of reaction mixture was completed to 10 mL with distilled water. The absorbance of the prepared mixtures was determined spectrophotometrically at 510 nm. Total flavonoid content was expressed as mg routine equivalents (mg RE/g) per gram

(Figure 3.11). All analyzes were repeated 3 times. There is no blank and control value. The total flavonoid content was calculeted by calibration curve. Calculated according to the slope value (y = 10,954x).

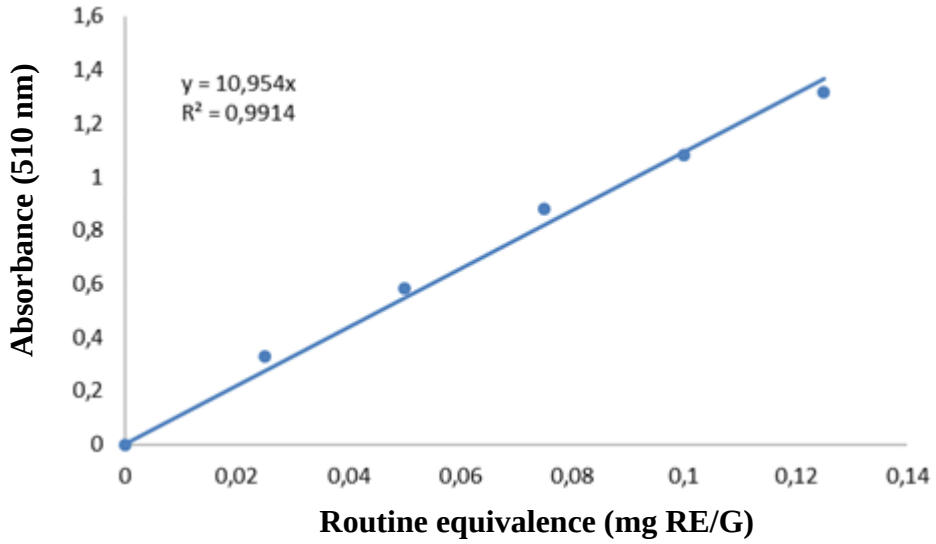

Figure 3.11: Total flavanoid calibration curve

3.10 Total Phenolic Content

Soluble phenolic content of fruit and leaf extracts were determined using Folin-Ciocalteu reagent. 0.5 mL of extracts were incubated in a water bath at 45°C for 45 minutes with the addition of 2.5 mL of Folin-Ciocalteu reagent (10%) and 2.5 mL of $NaHCO_3$ (7.5%). The absorbance of the mixtures was measured spectrophotometrically at 765 nm. According to the calibration graph using gallic acid as standard, the total phenolic content is expressed as mg gallic acid equivalents (mg GAE/g) per gram (Stankovic 2011). All analyzes were repeated 3 times (Figure 3.12). There is no blank and control value. The total phenolic concent was calculated by calibration curve. Calculated according to the slope value (y = 8,8286x).

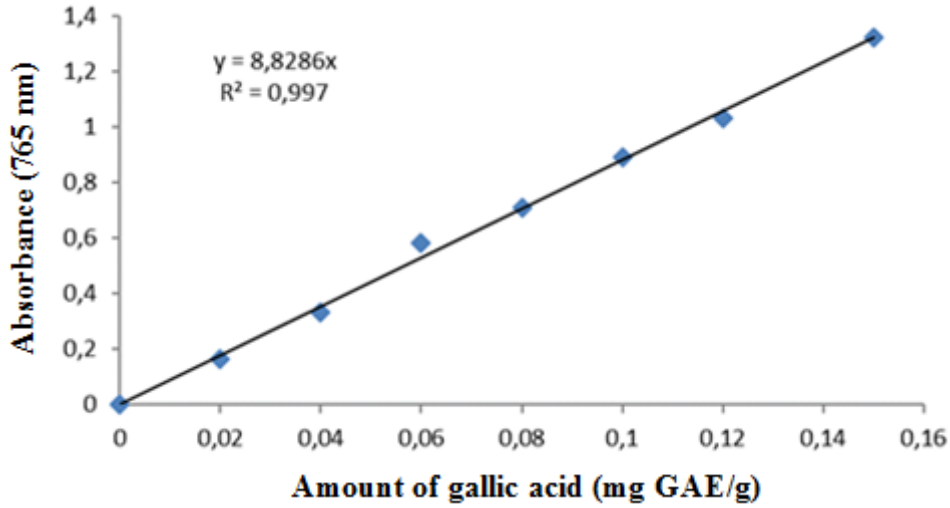

Figure 3.12: Total phenolic calibration curve

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Percentage Yield of Extraction

Table 4.1: Percentage extraction yield for leaf extracts

Name of Extract	Amount of leaf extracts after evaporation (g)	Percent yield of leaf extracts (%)
Leaf-Water	0.85	8.5
Leaf-Methanol	1.14	11.4
Leaf-Ethanol	0.53	5.3
Leaf-Chloroform	0.53	5.3
Leaf-Acetone	0.48	4.8

The percentage extraction yield of the leaf extracts showed in Table 4.1. According to these results, the maximum extraction yield saved in the leaf-methanol extract as 11.4%. The distilled water, ethanol, choloroform extracts had an extraction yield of 8.5%, 5.3% an 5.3% respectively. The minimum extraction yield was saved in the leaf-acetone extract as 4.8%. In study of Ahmad et al. (2016), percentage yield of the leaf-ethanol fraction was 40.07%, leaf-chloroform was 6.54%, leaf-methanol was 14.72%, leaf-distilled water was 4.01 %. The results of the extraction yields of Ahmad et al were higher than the results of the extraction efficiency of this study. The extraction yield for the leaf-chloroform extract (6.54%) from Ahmad et al.'s study was found to be close to the leaf-chloroform extraction yield in this study (5.3%). Another study of Ghareeb et al. (2015) percentage yield extraction of the leaf-methanol extract was found 14.0%. The extraction yield for the leaf-

methanol extract (14.0%) from Ghareeb et al study was found to be close to the leaf-methanol extraction yield in this study (11.4%).

Table 4.2: Percentage extraction yield for fruit extracts

Name of Extract	Amount of fruit extracts after evaporation (g)	Percent yield of fruit extracts (%)
Fruit-Water	6.49	32.45
Fruit-Methanol	10.33	51.65
Fruit-Ethanol	5.48	27.4
Fruit-Chloroform	0.68	3.4
Fruit-Acetone	0.97	4.85

The percentage extraction yield of the fruit extracts showed in Table 4.2. According to these results, the maximum extraction yield was saved in the fruit-methanol extract as 51.65%. The water, ethanol and acetone extracts had an extraction yield of 32.45%, 27.4% and 4.85%, respectively. The minimum extraction yield was saved in the fruit-chloroform extract as 3.4%. In study of Al-matani et al. (2015b), percentage yield extraction of the fruit-methanol extract was found 9.6%, fruit-chloroform was 17.9%, fruit-water extract 15%. In this study, the extraction yield of fruit-methanol (51.65%) and fruit-water extract (32.45%) was higher than the fruit-methanol (9.6%) and fruit-water extraction (15%) yield of Al-matani et al' s study. Fruit-chloroform extraction (17.9%) yield of Al-matanin et al study was found to be higher than the fruit-chloroform yield in this study (3.4%).

4.2 Antimicrobial Activity of Leaf Extracts

Table 4.3: Diameter of the inhibition zone (mm) of the methanol-leaf extracts (100 mg/mL concentration, 20 µL) against *B. subtilis, S. aureus and S. epidermidis*

Microorganisms tested	Leaf-Acetone	Leaf-Chloroform	Leaf-Methanol	Leaf-Ethanol	Leaf-Water	Methanol (NC)	Tetracycline (PC)
B. subtilis	-	-	-	-	-	-	25
S. aureus	11	-	10	13	-	-	23
S. epidermidis	-	-	-	-	-	-	14

(-) represents a no inhibition zone against microorganisms.
PC: Positive control, NC: Negative control

Table 4.4: Diameter of the inhibition zone (mm) of the methanol leaf extracts (100 mg/mL concentration, 20 µL) against *E. coli, Klebsiella spp.* and *E. cloacae*

Microorganisms tested	Leaf-Acetone	Leaf-Chloroform	Leaf-Methanol	Leaf-Ethanol	Leaf-Water	Methanol (NC)	Ciprofloxacin (PC)
E. coli	-	-	-	-	-	-	42
Klebsiella spp.	-	-	-	-	-	-	34
E. cloacae	-	-	-	-	-	-	26

(-) represents a no inhibition zone against microorganisms.
PC: Positive control, NC: Negative control

Table 4.5: Diameter of the inhibition zone (mm) of the methanol-leaf extracts (100 mg/mL concentration, 20 µL) against *E. faecalis*

Microorganisms tested	Leaf-Acetone	Leaf-Chloroform	Leaf-Methanol	Leaf-Ethanol	Leaf-Water	Methanol (NC)	Teicoplanin (PC)
E. faecalis	-	-	-	-	-	-	19

(-) represents a no inhibition zone against microorganisms.
PC: Positive control, NC: Negative control

Table 4.6: Diameter of the inhibition zone (mm) of the methanol-leaf extracts (100 mg/mL concentration, 20 µL) against *C. albicans*

Microorganisms tested	Leaf-Acetone	Leaf-Chloroform	Leaf-Methanol	Leaf-Ethanol	Leaf-Water	Methanol (NC)	Nystatin (PC)
C. albicans	10	-	-	12	-	-	15

(-) represents a no inhibition zone against microorganisms.
PC: Positive control, NC: Negative control

As seen from Table 4.3, antimicrobial activity was only leaf-acetone (11 mm), leaf-methanol (10 mm) and leaf-ethanol (13 mm) against *S.aureus*. No antibacterial activity against *B. subtilis* and *S. epidermidis*. In Table 4.4 and 4.5, all the leaf samples displayed no antibacterial activity against *E. coli, Klebsiella spp., E. cloacae and E. faecalis*. In Table 4.6, antifungal activity was only leaf-acetone (10 mm) and leaf-ethanol (12 mm) against *C. albicans.* These antifungal activities against *C. albicans* were found to be significant when compared with positive control (15 mm). According to these results, the maximum zone diameter found in the leaf-ethanol extract against *S. aureus* as 13 mm. Subsequently, the leaf-ethanol extract was recorded as 12 mm against *C. albicans* and the leaf-acetone extract was recorded as 11 mm against *S. aureus*. The minimum zone diameter was recorded in the leaf-acetone extract as 10 mm against *C. albicans* and in the leaf-methanol extract as 10 mm against *S. aureus*.

According to other studies conducted on *Ficus sycomorus* leaves; Ghareeb et al. (2015) reported leaf-methanol extract of *Ficus sycomorus* showed antimicrobial activity against *E.coli* (14 mm), *S. aureus* (27 mm), *C. albicans* (16 mm) but no antifungal activity against *A. niger*. In addition this study, antimicrobial activity against *E. coli* and *C. albicans* was not seen in leaf-methanol extract, but it was seen in the study of Ghareeb et al. In this study, anti-fungal activity against *C. albicans* was not observed in the leaf-methanol extract but it was seen in leaf-acetone and leaf-ethanol. In addition, in Ghareeb et al. study, the inhibition zone diameter of the leaf-methanol extract against *S. aureus* (10 mm) was greater than the result of this study.

According to the study of Jouda et al. (2015), The antibacterial effect of *F. sycomorus* leaf and stem bark extracts and their synergistic antibiotics against *E. coli*, *S. aureus* and *P. aeruginosa* were investigated. Leaf-methanol extract of *Ficus sycomorus* showed antibacterial activity against *S. aureus* (11 mm), but no antibacterial activity *E. coli* and *P. aeruginosa*. Leaf-ethanol extract was observed inhibition zone towards *S. aureus* (12 mm), *E. coli* (8 mm), *P. aeruginosa* (7 mm). There is no inhibition zone towards *E. coli*, *S. aureus* and *P. aeruginosa*in leaf-water extract. In study of Jouda et al., the zone diameter of leaf-methanol extract against *S. aureus* (11 mm) found little difference to this study result (10 mm). Also, in this study inhibition zone diameter of leaf-ethanol extract (13 mm) against *S. aureus* found a little bit more than the result in Jouda et al. study (12 mm).

Another study by Saleh and Al- Mariri (2017), antibacterial effects were observed against different bacteria. Leaf-acetone extract showed antibacterial activity against *L. Monocytogeneses* (10 mm), *S. aureus* (9 mm), *B. cereus* (10 mm), *E. coli* O:157 (17 mm), *S. typhimurium* (19 mm), *B. melitensis* (15 mm), *P. mirabilis* (18 mm), *Y. enterocolitica* O:9 (17 mm), *P. aeruginosa* (11 mm) and *K. pneumonia* (13 mm). In this study, the zone diameter of leaf-acetone extract against *S. aureus* (11 mm) was higher than the result obtained in Saleh and Al- Mariri (2017) study (9 mm).

In the study of Braide et al (2018), no inhibition zone towards *E.coli, Klebsiella spp.*, *S. aureus*, *P. aeruginosa* observed in the leaf-methanol extract. In the study of Braide et al., there was no antibacterial activity in the leaf-methanol extract against *S. aureus* but it was seen in this study (10 mm).

There is no previous research has been conducted on *Ficus sycomorus* leaf extracts towards the *E. faecalis*, *E. cloacae*, *S. epidermidis* and *B. subtilis.*

In general, the extracts from this study have been shown to have more effective antibacterial activity towards *S. aureus* than previous studies (Braide et al., Saleh and Al-Mariri, Jouda et al.). In this study, inhibition against *C. albicans* and *S. aureus* at 100

mg/mL was observed. If the amount of concentration is increased, the inhibition zone against these microorganisms may increase. *S. aureus* causes superficial skin lesions (boils, shallots), localized abscesses, deep-seated infections, severe skin infections (furunculosis), infection of hospital-acquired surgical wounds and food poisoning. *C. albicans* cause fungal urinary tract infections (UTI), genital fungal infections, fungal skin infection, oral thrush. The susceptibility of *C. albicans* and *S. aureus* to leaf extracts of *Ficus sycomorus* has shown that it can be effective against diseases caused by these organisms and can be used as a healing agent.

4.3 Antimicrobial Activity of Fruit Extracts

Table 4.7: Diameter of the inhibition zone (mm) of the methanol-fruit extracts (100 mg/mL concentration, 20 µL) towards *B. subtilis, S. aureus and S. epidermidis*

Microorganisms tested	Fruit-Acetone	Fruit-Chloroform	Fruit-Methanol	Fruit-Ethanol	Fruit-Water	Methanol (NC)	Tetracycline (PC)
B. subtilis	-	-	-	-	-	-	25
S. aureus	-	-	-	-	-	-	23
S. epidermidis	-	-	-	-	-	-	14

(-) represents a no inhibition zone against microorganisms.
PC: Positive control, NC: Negative control

Table 4.8: Diameter of the inhibition zone (mm) of the methanol-fruit extracts (100 mg/mL concentration, 20 µL) towards *E. coli, Klebsiella spp.and E. cloacae*

Microorganisms tested	Fruit-Acetone	Fruit-Chloroform	Fruit-Methanol	Fruit-Ethanol	Fruit-Water	Methanol (NC)	Ciprofloxacin (PC)
E. coli	-	-	-	-	-	-	42
Klebsiella spp.	-	-	-	-	-	-	34
E. cloacae	-	-	-	-	-	-	26

(-) represents a no inhibition zone against microorganisms.
PC: Positive control, NC: Negative control

Table 4.9: Diameter of the inhibition zone (mm) of the methanol-fruit extracts (100 mg/mL concentration, 20 µL) towards *E. faecalis*

Microorganisms tested	Fruit-Acetone	Fruit-Chloroform	Fruit-Methanol	Fruit-Ethanol	Fruit-Water	Methanol (NC)	Teicoplanin (PC)
E. faecalis	-	-	-	-	1.8	-	19

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

Table 4.10: Diameter of the inhibition zone (mm) of the methanol-fruit extracts (100 mg/mL concentration, 20 µL) towards *C. albicans*

Microorganisms tested	Fruit-Acetone	Fruit-Chloroform	Fruit-Methanol	Fruit-Ethanol	Fruit-Water	Methanol (NC)	Nystatin (PC)
C. albicans	-	-	-	-	-	-	15

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

In Table 4.7, 4.8 and 4.10, all the fruit extracts displayed no inhibition zone towards all the bacterial and fungus species. In Table 4.9, bacteriostatic activity was observed in fruit-water (1.8 mm) against *E. faecalis*. In study by Braide et al (2018) the fruit-methanol extract showed antibacterial activity against *E.coli* (18 mm), *Klebsiella spp.* (7 mm), *P. aeruginosa* (10 mm) and *S. aureus* (17 mm). Another study by El-Beltagi et al (2019), fruit-ethanol extract showed antimicrobial activity against *E.coli* (18 mm), *Klebsiella spp.* (7 mm), *S. aureus* (17mm) and *P. aeruginosa* (10 mm).

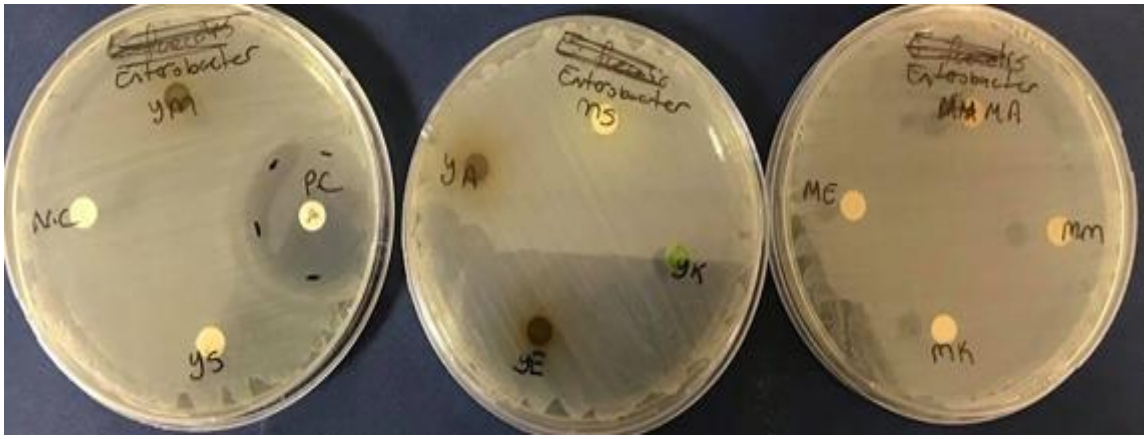

Figure 4.1: No inhibition zone of all fruit and leaf extracts towards *Enterobacter cloacae*

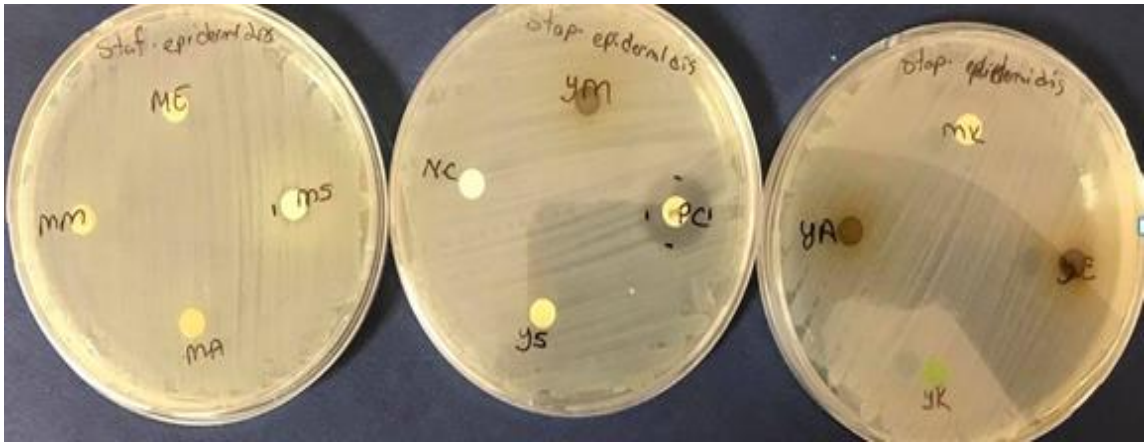

Figure 4.2: No inhibition zone of all fruit and leaf extracts towards *Staphylococcus epidermidis*

Figure 4.3: No inhibition zone of all fruit and leaf extracts towards *Klebsiella spp.*

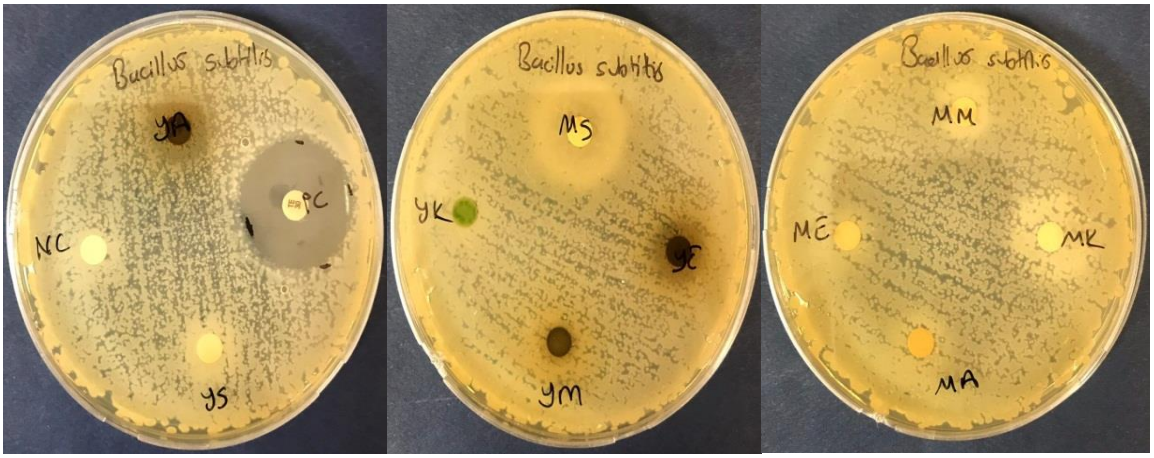

Figure 4.4: No inhibition zone of all fruit and leaf extracts towards *Bacillus subtilis*

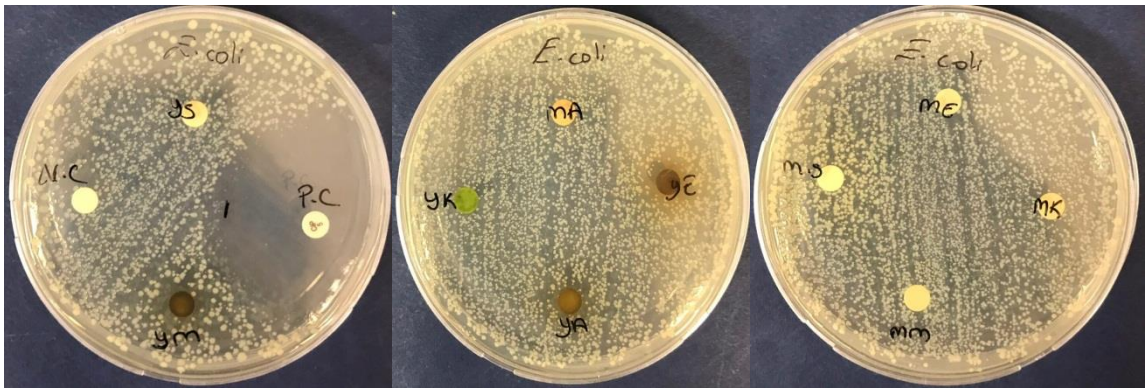

Figure 4.5: No inhibition zone of all fruit and leaf extracts towards *Escherichia coli*

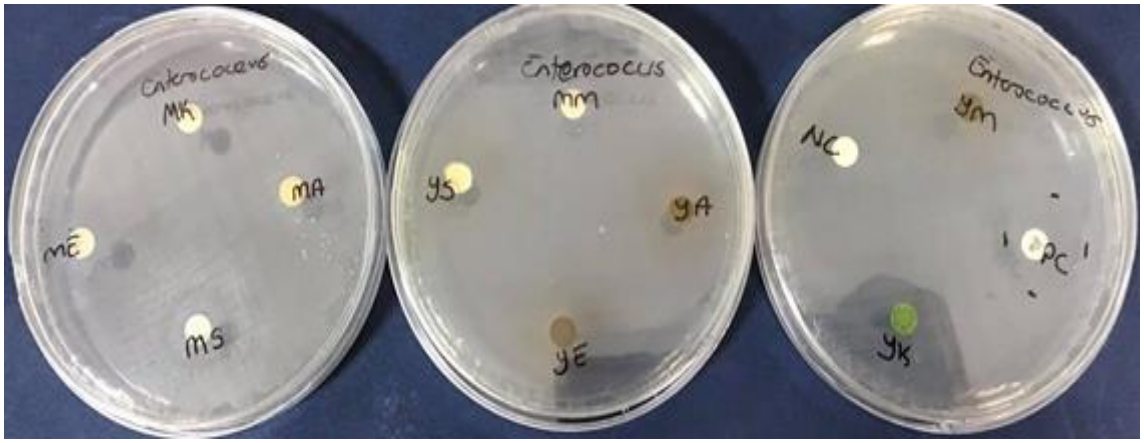

Figure 4.6: Bacteriostatic activity of fruit-pure water (MS) extract towards *Enterococcus faecalis*, no inhibition zone of other fruit and leaf extracts

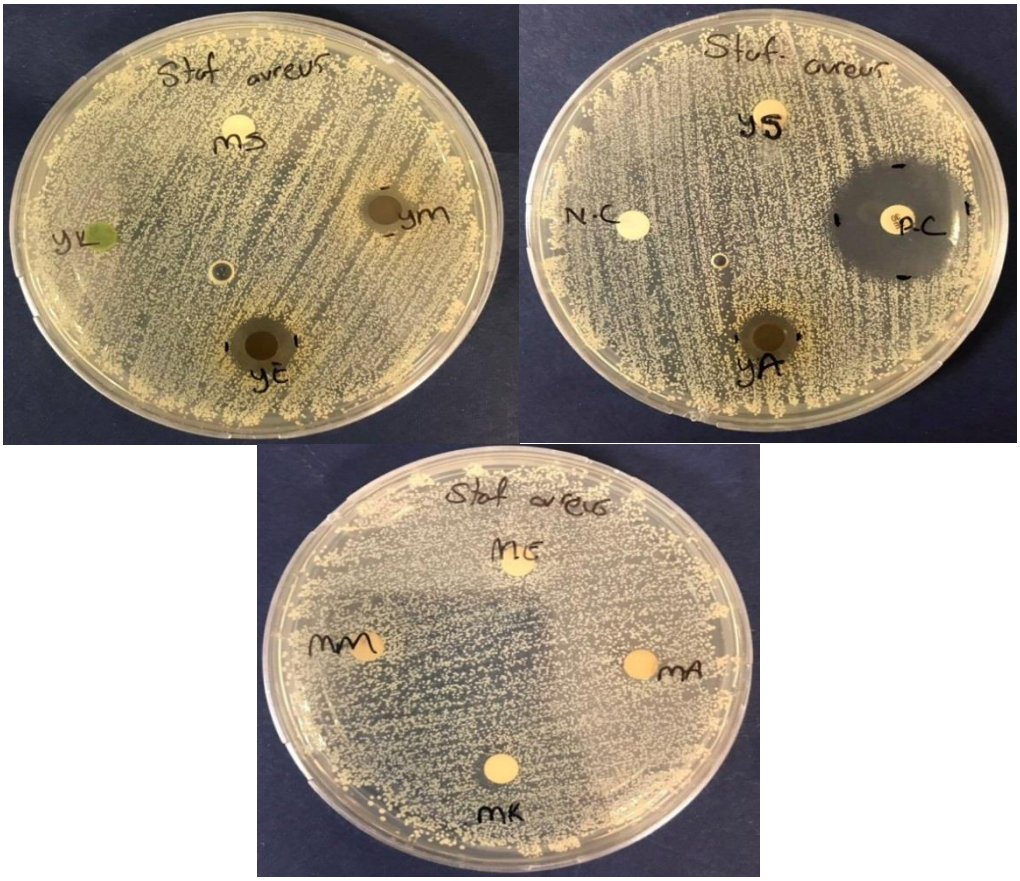

Figure 4.7: Inhibition zone of leaf-acetone (YA), leaf-methanol (YM) and leaf-ethanol (YE) extracts against *Staphylococcus aureus*, no antibacterial activity of other fruit and leaf extracts

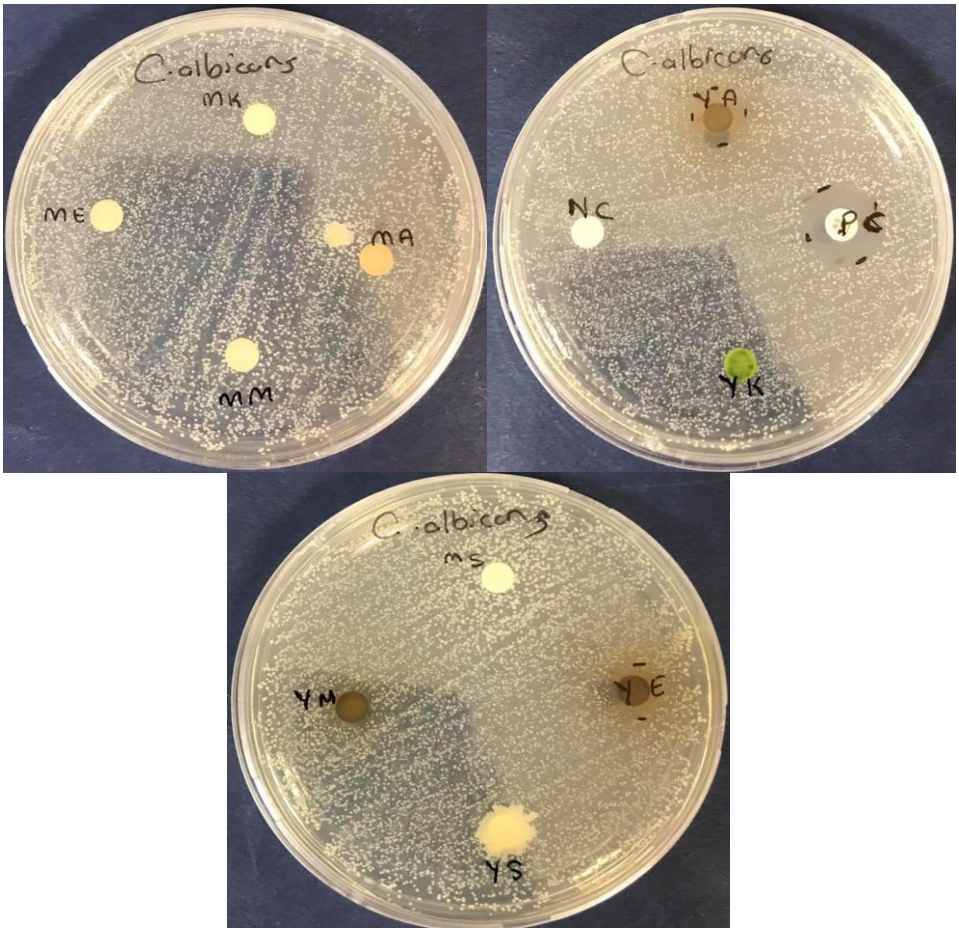

Figure 4.8: Inhibition zone of leaf-acetone (YA) and leaf-ethanol (YE) extracts towards *Candida albicans*, no antifungal activity of other fruit and leaf extracts

4.4 Minimum Inhibition Concentration

Table 4.11: MIC values of leaf-acetone extracts against the *S. aureus*

Microorganisms tested	Leaf-Acetone (12.5 mg/mL)	Leaf-Acetone (25 mg/mL)	Leaf-Acetone (50 mg/mL)	Leaf-Acetone (75 mg/mL)	Leaf-Acetone (100 mg/mL)	Methanol (NC)	Tetracycline (PC)
S. aureus	-	-	-	9	11	-	24

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

As shown in Table 4.11 above, *S. aureus* bacterium at 75 and 100 mg/mL showed susceptibility to leaf-acetone extract and inhibition occurred. However, at 12.5, 25, 50 mg/mL, the bacterium showed resistance and no inhibition occurred. Accordingly, the MIC value is 75 mg/mL for leaf-aceton extract against *S. aureus*. In study of Saleh et al. (2015), the minimum inhibitory concentrations was evaluated by microdilution broth method to establish the pathogens susceptibility to the acetone-leaf extract. These values were found 7.3 mg/mL for resistance *S.aureus* and 6.6 mg/mL for sensitive *S. aureus*. Another study of Saleh et al. (2017), the MIC value of the leaf extract was evaluated by using Microdilution Broth Method. Accordingly, the minimum inhibition concentration of the leaf-acetone extract towards *S. aureus* was 130.2 mg/mL. The study of Saleh et al exhibited that the *E. coli* bacterium was also susceptible to leaf-acetone extract. This value was found 52 mg/mL.

Table 4.12: MIC values of leaf-ethanolextracts against the *S. aureus*

Microorganisms tested	Leaf-Ethanol (12.5 mg/mL)	Leaf-Ethanol (25 mg/mL)	Leaf-Ethanol (50 mg/mL)	Leaf-Ethanol (75 mg/mL)	Leaf-Ethanol (100 mg/mL)	Methanol (NC)	Tetracycline (PC)
S. aureus	-	9	11	13	13	-	26

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

In Table 4.12 above, *S. aureus* bacterium at 25, 50, 75 and 100 mg/mL showed susceptibility to leaf-ethanol extract and inhibition occurred. However, at 12.5 mg/mL, the bacterium showed resistance and no inhibition occurred. Accordingly, the MIC value is 25 mg/mL for leaf-ethanol extract against *S. aureus*. In study by Jouda et al. (2015) the MIC value of the leaf extract was evaluated by the Microdilution Method. Accordingly, the minimum inhibition concentration of the leaf-ethanol extract towards *S. aureus* was found to be the same as in this study 25 mg/mL. The study of Jouda et al showed that the *E. coli* bacterium was also susceptible to leaf-ethanol extract. This value was found 12.5 mg/mL.

Table 4.13: MIC values of leaf-methanolextracts against the *S. aureus*

Microorganisms tested	Leaf-Methanol (12.5 mg/mL)	Leaf-Methanol (25 mg/mL)	Leaf-Methanol (50 mg/mL)	Leaf-Methanol (75 mg/mL)	Leaf-Methanol (100 mg/mL)	Methanol (NC)	Tetracycline (PC)
S. aureus	-	-	-	8	10	-	24

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

In Table 4.13, *S. aureus* bacterium at 75 and 100 mg/mL showed susceptibility to leaf-methanol extract and inhibition occurred. However, at 12.5, 25, 50 mg/mL, the bacterium showed resistance and no inhibition occurred. Accordingly, the MIC value is 75 mg/mL for leaf-methanol extract against *S. aureus*. In study of Saleh et al (2015), the minimum inhibitory concentrations was evaluated by microdilution broth method to establish the

pathogens susceptibility to the methanol-leaf extract. These values were found 9.2 mg/mL for resistance *S.aureus* and 8.7 mg/mL for sensitive *S. aureus*. In study by Jouda et al. (2015) the MIC value of the leaf extract was evaluated by using Microdilution Method. Accordingly, the minimum inhibition zone of the leaf-methanol extract towards *S. aureus* was 6.25-3.125 mg/mL. The study of Jouda et al (2015) showed that the *E. coli* bacterium was also susceptible to leaf-methanol extract. This value was found 12.5-6.25 mg/mL.

Table 4.14: MIC values of leaf-acetoneextracts against the *C. albicans*

Microorganisms tested	**Leaf-Acetone (12.5 mg/mL)**	**Leaf-Acetone (25 mg/mL)**	**Leaf-Acetone (50 mg/mL)**	**Leaf-Acetone (75 mg/mL)**	**Leaf-Acetone (100 mg/mL)**	**Methanol (NC)**	**Nystatin (PC)**
C. albicans	-	-	-	9	10	-	13

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

In Table 4.14, *C. albicans* fungus at 75 and 100 mg/mL showed susceptibility to leaf-acetone extract and inhibition occurred. However, at 12.5, 25, 50 mg/mL, the fungus showed resistance and no inhibition occurred. Accordingly, the MIC value is 75 mg/mL for leaf-acetone extract against *C. albicans*. In order to compare the result of the minimum inhibition concentration, no previous research has been conducted on *Ficus sycomorus* leaf-acetone extract against *C. albicans.*

Table 4.15: MIC values of leaf-ethanolextracts against the *C. albicans*

Microorganisms tested	**Leaf-Ethanol (12.5 mg/mL)**	**Leaf-Ethanol (25 mg/mL)**	**Leaf-Ethanol (50 mg/mL)**	**Leaf-Ethanol (75 mg/mL)**	**Leaf-Ethanol (100 mg/mL)**	**Methanol (NC)**	**Nystatin (PC)**
C. albicans	-	-	10	11	12	-	13

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

In Table 4.15, *C. albicans* fungus at 50, 75 and 100 mg/mL showed susceptibility to leaf-ethanol extract and inhibition occurred. However, at 12.5 mg/mL, 25 mg/mL the fungus showed resistance and no inhibition occurred. Accordingly, the MIC value is 50 mg/mL for leaf-ethanol extract against *C. albicans*. In order to compare the result of the minimum inhibition concentration, no previous research has been conducted on *Ficus sycomorus* leaf-ethanol extract against *C. albicans.*

Table 4.16: MIC values of fruit-waterextracts against the *E. faecalis*

Microorganisms tested	**Fruit-Water (12.5 mg/mL)**	**Fruit-Water (25 mg/mL)**	**Fruit-Water (50 mg/mL)**	**Fruit-Water (75 mg/mL)**	**Fruit-Water (100 mg/mL)**	**Methanol (NC)**	**Teicoplanin (PC)**
E. faecalis	-	-	-	-	1.8	-	22

(-) represents a no inhibition zone against microorganism.
PC: Positive control, NC: Negative control

In Table 4.16, *E. faecalis* bacterium showed resistance at 12.5, 25 , 50 , 75 mg/mL to fruit-water extractand no inhibition occurred. In order to compare the result of the minimum inhibition concentration, no previous research has been conducted on *Ficus sycomorus* fruit-water extract against *E. faecalis*.

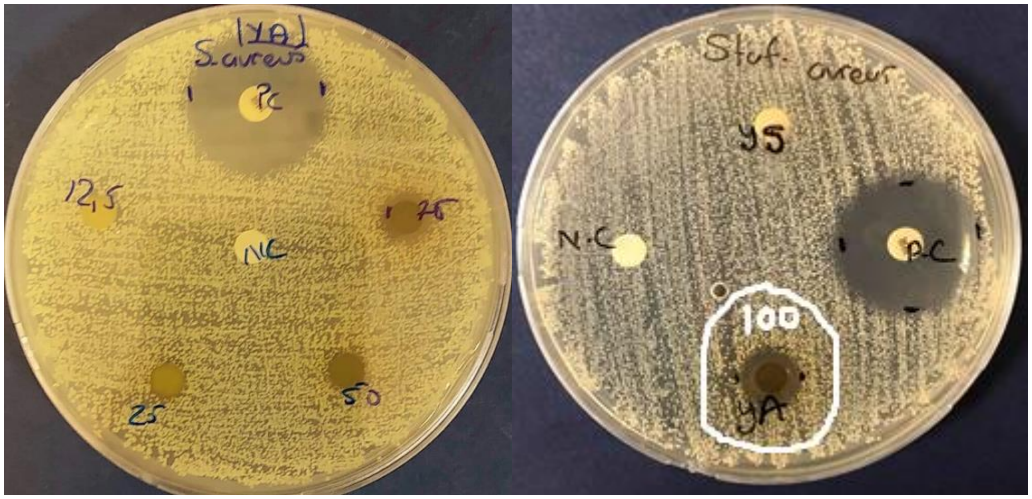

Figure 4.9: The inhibition zone towards *S. aureus* at different concentration of the leaf-acetone extracts (YA)

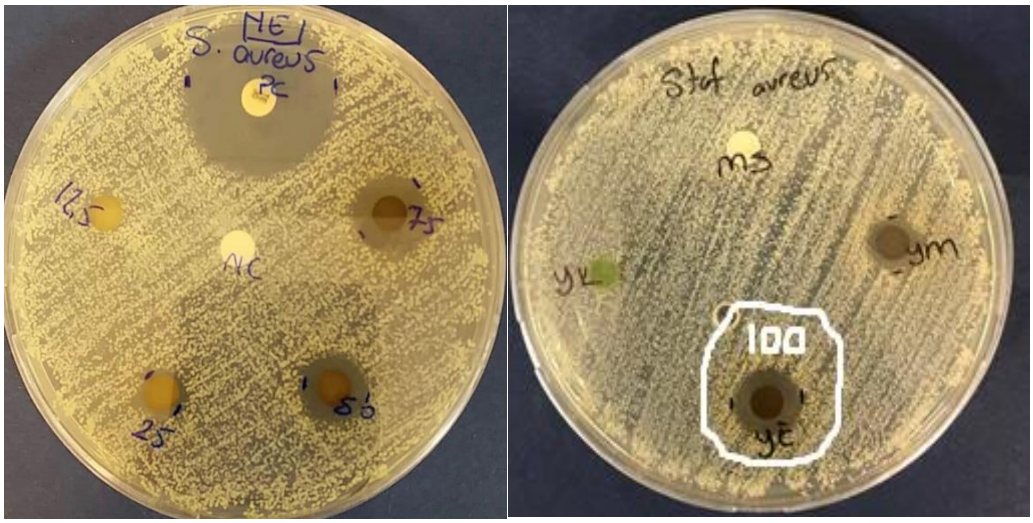

Figure 4.10: The inhibition zone towards *S. aureus* at different concentration of the leaf-ethanol extracts (YE)

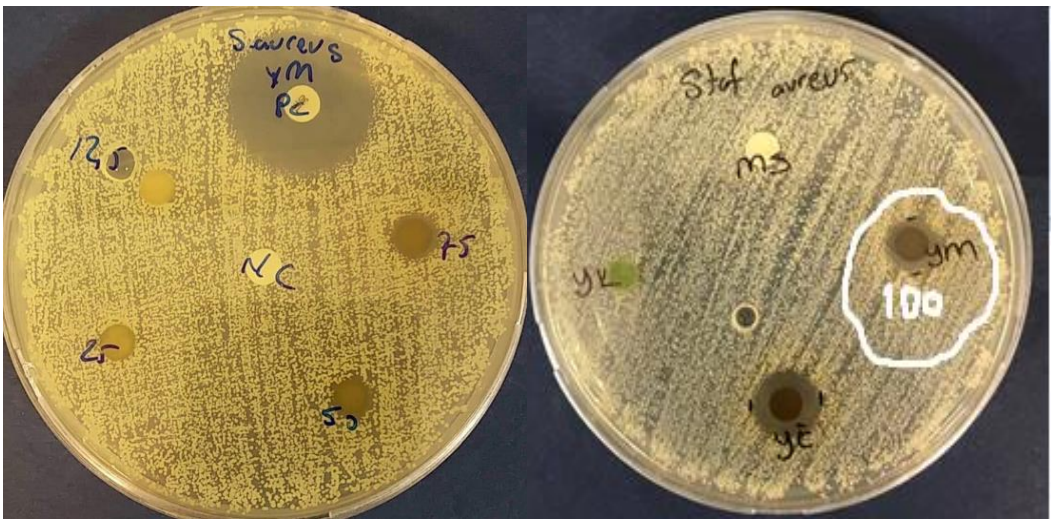

Figure 4.11: The inhibition zone towards *S. aureus* at different concentration of the leaf-methanol extracts (YM)

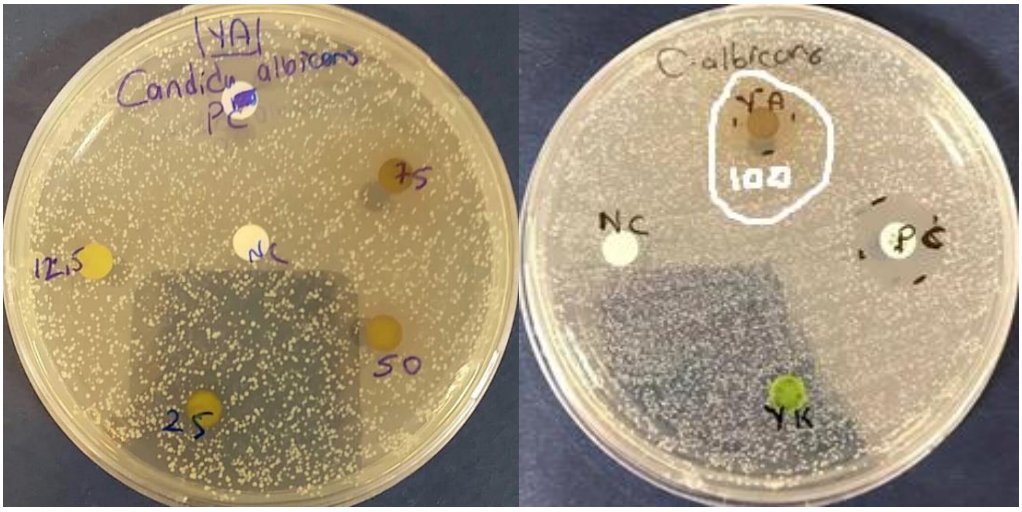

Figure 4.12: The inhibition zone towards *C. albicans* at different concentration of the leaf-acetone extracts (YA)

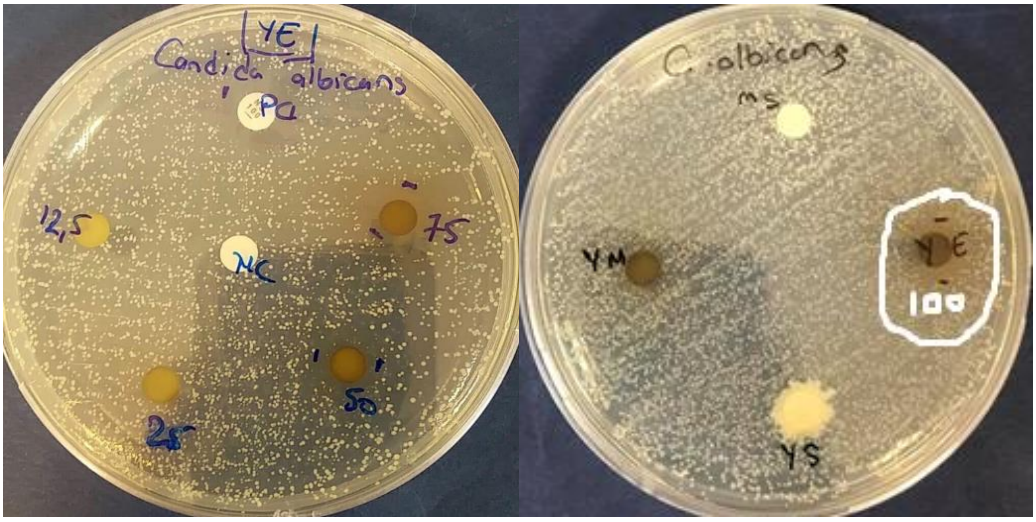

Figure 4.13: The inhibition zone towards *C. albicans* at different concentration of the leaf-ethanol extracts (YE)

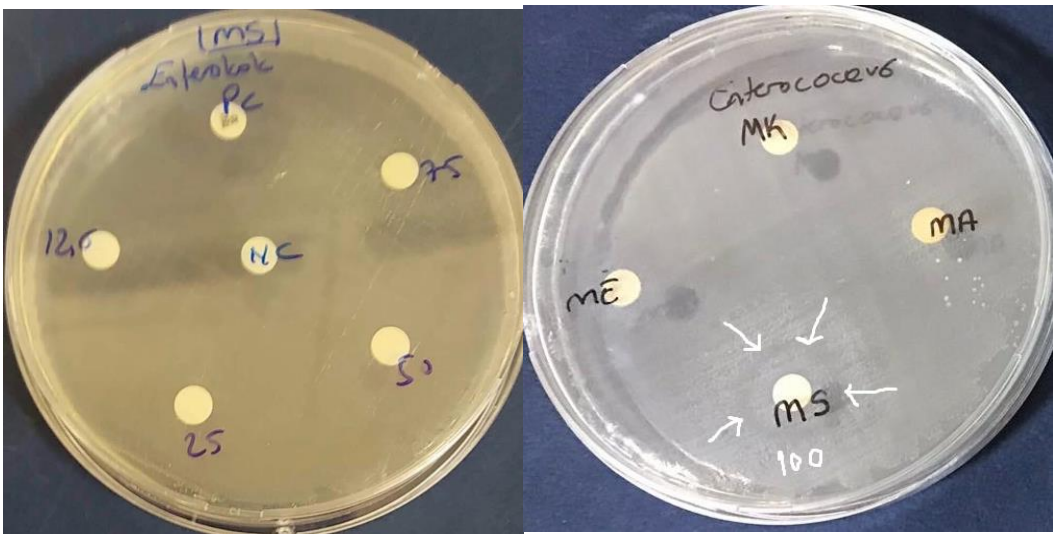

Figure 4.14: The inhibition zone towards *E. faecalis* at different concentration of the fruit-water extracts (MS)

4.5 Total Antioxidant Activity, Total Phenolic and Total Flavonoid Content of Leaf and Fruit Extracts of *Ficus sycomorus*

Table 4.17: Total phenolic content (TPC), Total flavonoid content (TFC) and Antioxidant activity (DPPH scavenging) of the different leaf extracts of *Ficus sycomorus*

Sample	TPC (mg GAE/g)	TFC (mg RE/g)	DPPH (%)
Leaf-water	3.72±0.08	0.19±0.015	1±2.55
Leaf-acetone	2.55±0.38	1.38±0.306	33±3.38
Leaf-chloroform	7.09±0.23	1.24±0.064	42±0.13
Leaf –ethanol	2.23±0.00	1.37±0.246	18±0.13
Leaf –methanol	2.38±0.09	0.84±0.107	47±2.17

Values are mean ± Standard deviation (SD) of three replicate analysis. 100 mg/mL concentration was used for the tests

The highest antioxidant activity (DPPH) in the leaf was observed in methanol. Although the phenolic content of methanol was lower than acetone, DPPH activity was highest in methanol. Each phenolic substance dissolved in the plant may not have DPPH effect. In such cases specific tests such as Fe chelating activity assay, superoxide anion radical scavenging activity assay, ABTS radical scavenging activity assay, Trolox equivalent activity assay should be tried because acetone exhibited high flavonoid and phenolics agent solubility, and DPPH activity of acetone may be high in specific tests. Although phenolic substance is dissolved in water, there is low DPPH activity but other specific antioxidant tests should be performed. The highest phenolic content found in chloroform and the highest flavonoid dissolved in acetone. The chloroform has the least polarity index compared to the other solvents, but has solved the highest phenolic substance. This may be due to the high hydrophobicity of the compounds. The results of flavonoids for all leaf samples were lower than the results of phenolic substances. This is because flavonoids are the subgroup of phenolics. According to the study by El-Sayed et al (2009), the total

phenolic content of *Ficus sycomorus* leaf-methanol extract was 124.00±4.96 (mg GAE/g ext.), and the free radical scavenging potential (DPPH SC_{50}) was 20.93±0.21 [mg/mL] and the total phenolic content of leaf-water extract was 26.31±3.76 (mg GAE/g ext.), and the free radical scavenging potential (DPPH) was 66.58±0.75 [mg/mL]. The total phenolic content of *Ficus sycomorus* leaf-choloroform extract was 180.79±1.88 (mg GAE/g ext.), Free radical scavenging potential (DPPH SC_{50}) was 132.41±1.17 [mg/mL], Total flavonoids was 4.66±0.66 (mg RE/g ext.). In the study conducted by El-Beltagi et al (2019), DPPH % in leaf-ethanol extracts values with concentrations of 40, 80, 120 and 150 (µg/mL) found as 58.426, 63.541, 67.426, 75.249 µg/mL respectively. Half maximal inhibitory concentration (IC_{50}) found 18.443 µg/mL. The reducing power activity (µg Gallic acid/100g) found 22.53±0.37 in DPPH % in ethanolic extract. Another study by Samuel et al (2017) % inhibition (DPPH scavenging effects) of the ethanol leaf extracts values with concentrations of 50, 100, 200, 400, 600, 800 and 1000 (µg/mL) found a 13.10, 19.31, 23.86, 36.18, 48.17, 65.82 and 79.02 µg/mL respectively. The IC_{50} values of DPPH scavenging effects of ascorbic acid in leaf ethanol extracts found 585.54 µg/mL.

Table 4.18: Total phenolic content (TPC), Total flavonoid content (TFC) and Antioxidant activity (DPPH scavenging) of the different fruit extracts of *Ficus sycomorus*

Sample	**TPC (mg GAE/g)**	**TFC (mg RE/g)**	**DPPH (%)**
Fruit-water	5.62±0.05	0.07±0.004	76±2.23
Fruit-acetone	11.29±0.39	1.38±0.021	86±0.06
Fruit-chloroform	7.78±0.33	1.08±0.058	69±1.21
Fruit-ethanol	1.93±0.27	0.32±0.009	86±4.78
Fruit –methanol	1.91±0.33	0.12±0.013	86±0.19

Values are mean ± Standard deviation (SD) of three replicate analysis. 100 mg/mL concentration was used for the tests.

Antioxidant activity (DPPH) in fruit samples was high in high polarity solvents. Methanol, ethanol, acetone, water and chloroform respectively. The highest phenolic and flavonoid substance dissolved in acetone. Although the polarity index of acetone was lower than methanol, it solved more phenolics. This may be due to the high hydrophobicity of the compounds. The results of flavonoids for all fruit samples were lower than the results of phenolic substances. This is because flavonoids are the subgroup of phenolics. Although the phenolic compounds dissolved in fruit-ethanol were less than fruit-water and fruit-chloroform and fruit-ethanol extract showed more antioxidant activity (DPPH). This may be because more soluble compounds have less antioxidant effects. In the study conducted by El-Beltagi et al. (2019), DPPH % in fruit-ethanol extracts values with concentrations of 40, 80, 120 and 150 (µg/mL) were found as 55.003, 59.232 65.763, 72.471 respectively. IC_{50} found 20.312 µg/mL. The reducing power activity (µg Gallic acid/100g) found 15.58±0.44 in DPPH % in ethanolic extract.

Compared to all these results, the antioxidant activity (DPPH) in the fruit is higher than the leaves for all solvents. The amount of phenolic compounds in the fruit-water sample was high and phenolic compounds showed antioxidant activity. However, specific antioxidant tests should be performed because there may be no activity in the total antioxidant test (DPPH). However, it should be noted that not every soluble phenolic substance may have an antioxidant effect. In the leaf-water sample phenolic substance is dissolved but less than fruit. Also, showed low antioxidant activity (DPPH). The amount of flavonoid compound of leaf-water is higher than the fruit-water. Therefore, specific antioxidant tests should be tried. The amount of phenolic substance in fruit-acetone was high and phenolics showed antioxidant activity. Leaf-acetone has DPPH activity and phenolic substance but less than fruit. The antioxidant activity (DPPH) in the fruit-water is higher than the leaf-water. The amount of phenolic substance in fruit-chloroform was high and phenolics showed antioxidant activity. The amount of phenolic substance found to be high in leaf-cloroform but less than fruit-cloroform. The antioxidant activity (DPPH) in the fruit-chloroform is higher than the leaf-chloroform and the amount of flavonoid compound of leaf-chloroform is higher than the fruit-chloroform. Although the amount of phenolic and flavonoid in leaf-ethanol is more than fruit-ethanol, the fruit-ethanol is more than the antioxidant activity

(DPPH). In such cases, other specific antioxidant tests such as Fe chelating assay should be attempted because not every phenolic substance dissolved may have DPPH activity. We can say the same thing here again when we look at methanol. Because the amount of phenolic and flavonoids in leaf-methanol was higher than that of fruit-methanol, but the DPPH activity of leaf-methanol was less than fruit-methanol. Therefore, other specific antioxidant tests should be attempted.

CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, *Ficus sycomorus* has no antimicrobial activity against *B. subtilis, S. epidermidis, E. coli, Klebsiella spp., E.cloacae and E. faecalis* bacteria in all leaf extracts (1:10 w/v). In leaf-acetone, leaf-methanol and leaf-ethanol extracts (1:10 w/v) diameter of inhibition zone showed 10-13 mm against *S. aureus* and leaf-acetone and leaf-ethanol extracts (1:10 w/v) showed 10 mm and 12 mm inhibition zone against *C. albicans*, respectively. The maximum inhibition zone was recorded against *S. aures* in the leaf-ethanol extract (13 mm). The inhibition zone observed in leaf extracts against *C. albicans* showed a significant activity when compared to the inhibition zone of Nystatin (15 mm) which was used as a positive control. The minimum inhibition concentration (MIC) was recorded as the highest in leaf-ethanol extract against *S. aureus* at 25 mg/mL with 9 mm inhibition zone. The MIC value for *C. albicans* was recorded as the highest in leaf-ethanol extract at 50 mg/mL with 10 mm inhibition zone.

Antimicrobial activity was not observed against all pathogenic microorganisms used in fruit extracts (1:10 w/v) but bacteriostatic activity against *E. faecalis* was observed in fruit-water extract.

The results of this study showed that leaf extracts can be used as a curative agent for the treatment of Gram-positive bacterial and fungal infections and may be effective against pathogenic microorganisms that are resistant to antibiotics.

In antioxidant studies, the highest antioxidant activity (DPPH) in the leaf was observed in methanol, the highest phenolic content was observed in chloroform and the highest flavonoid content was in acetone. The highest antioxidant activity (DPPH) in the fruit was observed in acetone, ethanol and methanol, the highest phenolic and flavonoid content observed in acetone.

According to these results, the presence of antioxidant compounds of fruit and leaf extracts could be effective against the negative effects of free radicals. It also indicates that the presence of phenolic substances may be effective in antimicrobial as well as in antioxidant effect.

5.2 Recommendation

This study may be used as an alternative to antibiotics that are resistant to pathogenic microorganisms. In this study, leaf extracts which were found to be effective against gram-positive *Staphylococcus aureus* bacteria may also be effective against other gram- positive bacteria such as *Streptococcus* species (cough, diarrhea, skin infections and food poisoning). It can also be used as a medicine or food against infections caused by various bacteria and fungi. Fruits and leaves of the plant can be consumed as antioxidants against free radicals formed in the human body. With this study, *in-vivo* and *in-vitro* antioxidant mutagenic toxicity tests can guide the future studies on the effect on eukaryotic cells.

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