Anatomical, Chemical and Antimicrobial Activity of *Pistacia lentiscus* L.

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ABSTRACT

The genus *Pistacia* L. is a member of the family Anacardiaceae and consists of 10 accepted species. According to monograph of Zohary published on 1952, the genus is divided into 4 sections and 11 species included. In Northern Cyprus 4 *Pistacia* species occur, wild ones: *P. atlantica* Desf. , *P. lentiscus* L., *P. terebinthus* L., and cultivated one *P. vera* L.. The male trees use for to extract resin and female trees produce red drupe fruits which is use to make traditional hard biscuits at patisseries.

Pistacia lentiscus is an evergreen shrub or small dioecious tree. Leaves are paripinnate with strong resin odour. Leaflets 6 or 8 pairs are elliptical, apex and base acute, margin entire. At spring-summer seasons it gives white flowers. It is multi-trunked plant with many branches and generally resists to breakage.

In this study leaf anatomical characteristics and antimicrobial effect of its fruit have been check with methanol (CH3OH), chloroform (CHCl3), dichloromethane (CH2Cl2), acetone (CH3COCH3), hexane (C6H14), ethanol (C2H5OH) and distilled water (H2O) extracts against gram positive and gram negative bacteria such as; *E. coli* ,*K. pneumoniae* *S aureus* and *E. faecalis*, was investigated. All examined specimens collected from wild population in Karpaz region.

The cross section of the leaflets made by hand, it is observed that on both surface q single layer of thin walled epidermal cells, covered by thick layer cuticula. Leaves are dorsiventral (bifacial) adaxial palisade consists of one layer, and the abaxial the abaxial spongy parnchima consists of several layers and the cell wall are very similar in appearance. Stoma only occurs on upper surface, no hairs.

MIC of all chemicals extracts is investigated to be 128 mg/L.

Keywords: *Pistacia lentiscus,* anatomical structure of seeds, antibacterial activity

ÖZ

Pistacia L. cinsi, Anacardiaceae familyasının bir üyesidir ve son literatüre göre 10 kabul edilen türden oluşmaktadir. 1952'de yayınlanan Zohary'nin monografisine göre, cins olarak 4 seksiyona ayrılmıştır ve 11 türe vardır.. Kuzey Kibris'ta 4 *Pistacia* türü bulunmaktadır, yabani olanlar *P.atlantica* Desf., P. lentiscus L., *P. terebinthus* L., ve yetiştirilen tek tür P. vera L. Erkek ağaçlardan recine elde edilmekte ve dişi ağaçlar kırmızı sert meyveleri Kıbrıs'ta pastanelerde geleneksel olarak peksemet (sert bisküvi) yapımında kullanılmaktadır. *Pistacia lentiscus,* yaprak dökmeyen bir çalı veya küçük bir ağaçtır. Yapraklar paripennattır ve güçlü reçine kokusuna sahiptir. Yaprakçıklar 6 veya 8 eliptik, akut, kenarları tam olayaprakçıktan oluşur. İlkbahar-yaz mevsiminde beyaz çiçekler açmaktadır. Çok gövdeli ve çok dallı bir bitkidir, genellikle kırılmaya karşı dayanıklıdır. *Pistacia* türlerinde az sayıda anatomik çalışma yayınlanmıştır. Bu çalışmada, Karpaz yöresinden doğal ortamından toplanan örneklerin yapraklarının anatomik özellikleri ve meyvelerinin antibakteriyel aktivitesi metanol, kloroform, diklorometan, aseton, heksan, ethanol ve su ekstrelerinin gram positive ve gram negative microrganismalarinin ki bunlar; S. *aureus, E. faecalis, E. coli* ve *K. pneumonia*e üzerindeki etkisi incelendi. Bitkinin yaprak kesitleri el ile alınmıştır, microskope incelemesi sonucunda yaprağın her iki yüzeyinde de ince tek tabaka halinde epiderma hücreleri, kutiküla kalın, yapraklar dorsiventral tiptedir, üstte palizat parenkiması, alt yüzde ise sünger parenkiması yer alır. Palizat parenkiması tek sıra hücreden oluşmaktadır. Sünger parenkiması ise birbirine benzeyen çok sıralı hücreden meydana gelmiştir. Stomalar sadece üst yüzeyde bulunur ve yapraklarda tüy gözlenmemiştir.

Meyvenin minimum inhibitör konsantrasyonunun 128 mg/L'den olduğu metanol, kloroform,diklorometan, aseton, heksan, ethanol ve su ekstrelerini kullanarak yapilan deneylerde belirlenmiştir.

Anahtar Kelimeler: *Pistacia lentiscus,* meyvesinin anatomikal yapısı, antibakteriyal aktivitesi

DEDICATION

I dedicate my thesis to my family, my husband and daughter, my mother and father for all their support, patience and love.

ACKNOWLEDGMENT

I would like to express my gratefulness to my advisor Assoc. Prof. Dr. Mustafa Gazi from faculty of Arts and Sciences and co-advisor Prof. Dr. F. Neriman Özhatay from faculty of Pharmacy; for their continuous supports of my study and related research, for their patience, motivation, and immense knowledge.

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LIST OF SYMBOLS AND ABBREVIATIONS

^{o}C	Celsius
ATCC	American Type Culture Collection
C	Carbon atom
cfu	Colony forming unit
Cl	Chloride atom
cm	Centimeter
conc	Concentration
Desf	René Louiche Desfontaines
DMSO	Dimethyl sulfoxide
E. faecalis	*Enterococcus*
E. coli	*Escherichia coli*
gr	Gram
H	Hydrogen atom
K. pneumoniae	*Klebsiella pneumoniae*
l	Liter
L	Carl Linnaeus
s	Second
m	Meter
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minute
ml	Milliliter
mm	Millimeter

O	Oxygen atom
P	*Pistacia*
S	*Staphylococcus aureus*
µl	Microliter

Chapter 1

INTRODUCTION

1.1 *Pistacia lentiscus* L.

1.1.1 Cultivation History

Pistacia lentiscus L. is always evergreen, native plant and covers the soil, with separate female and male plants. By the covering of soil it can be also prevents erosion. It has the ability to regenerate itself in a short time even under bad conditions such as forest fire.

It is and largely distributed in extreme ecosystems of the Mediterranean basin, from Morocco, France, Canary Islands, Iraq, Turkey, East of Iran etc. It is cultivated especially in western Aegean (Çeşme-Karaburun) and mostly found on the island of Chios at Greece. In Cyprus mostly grown at Larnaca, Paphos and Karpaz region.

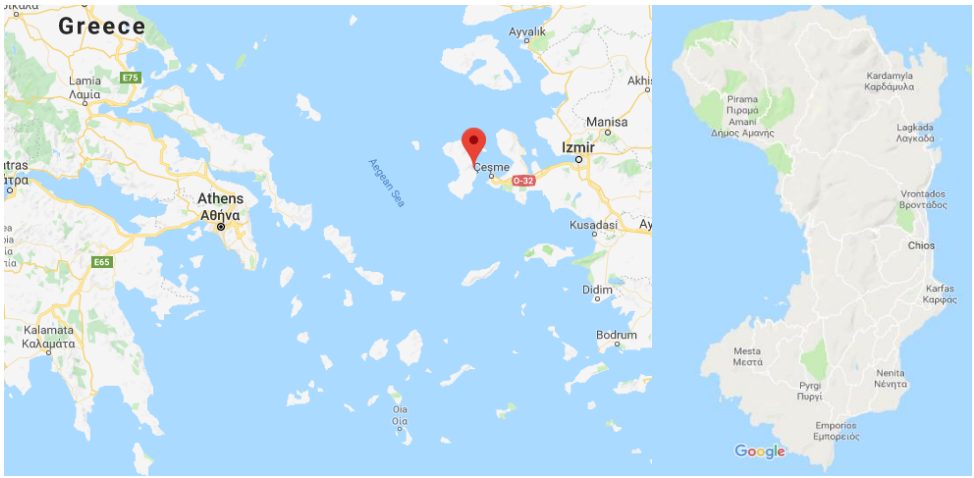

Figure 1.1: Map of Greece, the Island of Chios

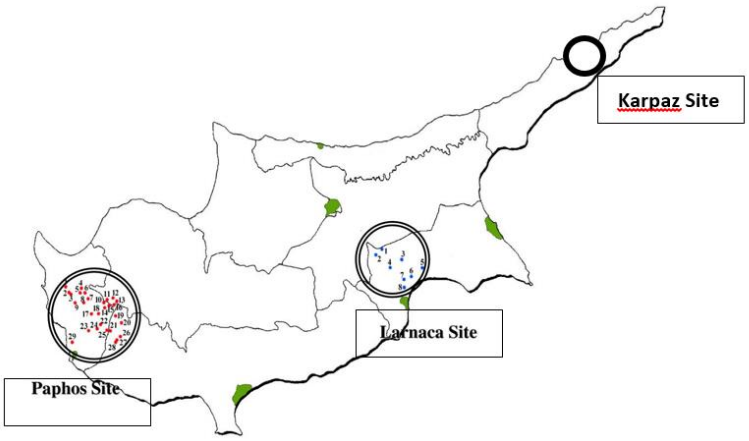

Figure 1.2: Map of Cyprus, *Pistacia lentiscus* L. Distribution

This plant has strong smell of resin. Only male trees of plant can start to produce resin after 6 years, between 12-15 years period the plant rich high quality of production approximately 320 gr per tree. Then the tree can reach actual size between 40-50 years, it often becomes a tree of up to 7 m. The resin harvesting period take place between June to September during summer session. This resin is called as mastic (Zitouni Amel, 2016).

1.1.2 Taxonomy

Anacardiaceae family plants are trees, shrubs, or lianas which produce a milky or watery sap. The compound leaves of the family are alternate arrangement, shape with pinnate venation. The flowers of family are small, often in red colour and hermaphrodite, five-merous, actinomorphic and may have either pollen-bearing or ovule-bearing parts, or both. The ovary of the flower has 3 styles and 5 stamens inserted. The fruits are drupe and small seeds surrounded by a dry or fleshy covering. The genus *Pistacia*, is an evergreen tree, without falling it leaves in winter (Mohannad G. AL-Saghir, 2012).

Table 1.1: Anacardiaceae Family Flower Characteristics

Anacardiaceae Family Flowers	
Calyx and corolla	3-5
Stamens	5 or 10
Ovary	3 Styles

Figure 1.3: Flowers of *Pistacia lentiscus* L Plant

Table 1.2: Taxonomy of *Pistacia lentiscus* L.

Taxonomy of *Pistacia lentiscus* L.	
Domain:	Eukaryota
Kingdom:	Plantae
Phylum:	Spermatophyta
Subphylum:	Angiospermae
Class:	Dicotyledonae
Order:	Sapindales
Family:	Anacardiaceae
Genus:	Pistacia
Species:	Pistacia lentiscus

1.1.3 Characteristics of Plant and Identity

Table 1.3: Identification of *Pistacia lentiscus L.*

Identity of plant	
Scientific Name	*Pistacia lentiscus* L.
Common Name	Mastic
Local Names	**English:** Mastic tree
	Spanish: Lentisco
	French: Arbre au mastic; Lentisque
	Portuguese: aroeira
	Germany: Mastix- Pistazienstrauch; Mastixstrauch
	Italy: Lentischio; Lentisco
	Netherlands: Mastikboom

The fruit looks like a grape bunch, which is green first then turns to red and at the end of the summer it turns to black colour when ripe. Fruits are drupe and edible, about 4 mm in diameter. Fruits are ripening until late October to mid-December (YALÇIN, 2011).

The flowers are very small with red colour the female plants flower divided into 3 lobes and male plants flowers have 5 stamens. Perianth-free flowers develop on leaf seats of plant with 1 year shoots. The flowering period takes place between March to April. Male flowers are 1-2.5 cm long with compound bunches and female flowers are in the form of sparsely branched bunches with 1-3 cm in length (INAL, 2005).

The stems of the plant get grey colour while becoming older, when it is young it has reddish colour (Ömer Faruk AKDEMİR, 2013).

Leaves are compound with 6 or 8 pairs of leaflets and never carry terminal leaf, not have any hairs on their surface. The male and female plants show some difference in their leaf shapes (Neriman ÖZHATAY, 1999).

Figure 1.4**:** Leaves of *Pistacia lentiscus* L. (6 or 8 leaflets)

Table 1.4: Leaf Description of the Plant

Shape	Alternate, Eliptical
Venation: Veins	Pinnate
Margin: Edge of leaf.	Entire
Apex: Tip of leaf	Acute
Base: Region that connects the blade to the petiole.	Acute

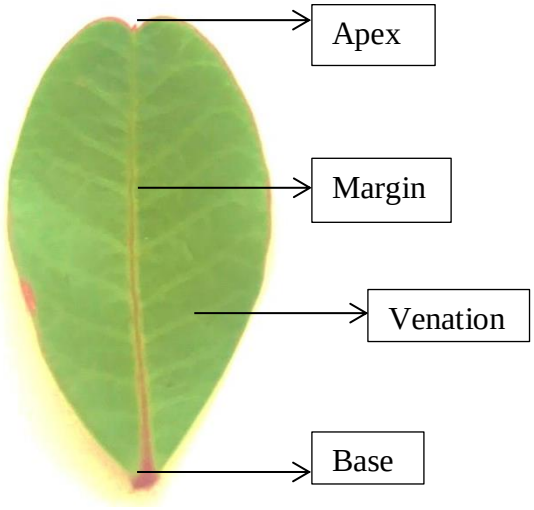

Figure 1.5: A Leaflet Identification of *Pistacia lentiscus* L.

1.2 Anatomy, Chemical Extraction and Antimicrobial Effect of *Pistacia lentiscus* L.

1.2.1 Anatomy

Leaf is the main organ of plant which makes photosynthesis, transpiration and respiration; stems, roots, flowers, fruits, and seeds are other organs of plant. Protective outer layer of leaf is called epidermis, which is a single cell layer covering the both lower (abaxial) and upper (adaxial) surfaces of the leaf. The cuticle; which is a thin waxy layer covers the epidermis and protect the leaf from water retain, its minimizing the loss of water from the leaf surface. Guard cells are the special cells that regulate gas exchange between leaf and environment. These pores that make exchange are called stomata; pores let the carbon dioxide, oxygen and water vapours exchange (Tattini M1, 2006).

Mesophyll is located between lower (spongy mesophyll) and upper (palisade mesophyll) epidermis, the mesophyll leaf layer is composed of a palisade mesophyll

region and a spongy mesophyll region. The inner layer of the leaf is rich in chlorophyll which is called palisade mesophyll (University, 1995).

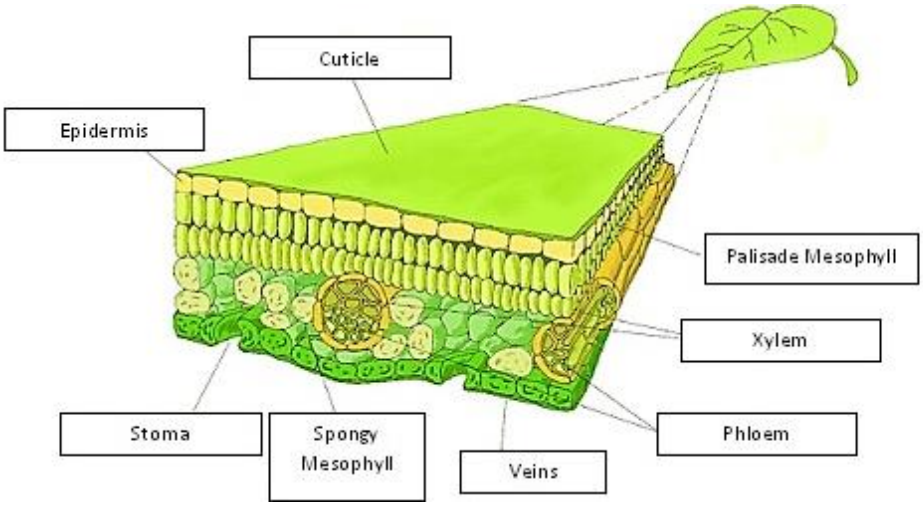

Figure 1.6: Cross-section of Leaf

Plant anatomy is the general term that studies the shape, structure, and size of plants. It is a field in botany that focuses for internal structure, body parts and systems that make plant. Plant anatomy investigated the cellular level of plant, which is involves the tissues and microscopy. The pant body can be vegetative organs which are roots, stem and leaf and for reproductive part it includes flowers, fruits and sees (University, 1995)

1.2.2 Chemical Extraction

Extraction is a very important separation technique in chemistry, which is used to separate the components of a mixture by their solubility. Different phases separate according to their solubility. It is a basic method used for isolating compounds from plant materials. Extraction is done between two phases. In chemicals extraction there is 2 main extraction types which including; liquid to liquid extraction and solid to phase extraction.

Liquid to liquid extraction is an important separation technique used for a wide range of applications in the chemical process industries. In liquid to liquid extraction, dissolved solute is transferred from one liquid phase to another (NN, 2015).

In this study, Solid to liquid extraction take place, which use solvents to allow soluble components to be removed from solid samples, in this technique solute is transferred from a solid phase to a liquid phase. By using this technique oil can be obtained from oil seeds (NN, 2015).

1.2.2.1 Soxhlet Extraction

Franz von Soxhlet invented soxhlet extractor in 1879, which is use for extractions of solid to liquid samples. It setup from three main parts; a round botom flask, an extraction chamber, and a condenser. Often used to remove weakly soluble solvents from solutes. Soxhlet extraction allows a small amount of solvent to be used with a large solid sample (Deiner, 2019).

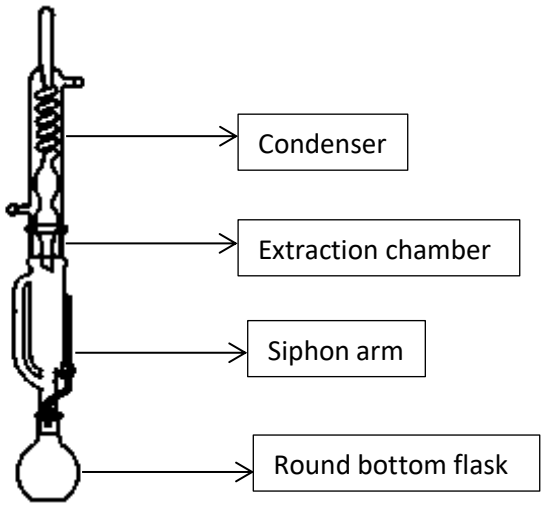

Figure 1.7: Soxhlet Apparatus

1.2.2.1 Rotary Evaporator

A simple rotary evaporator system was invented by Lyman C. Craig in 1950 and in 1957 the Swiss company Büchi was first commercialized it (Deiner, 2019). The rotary evaporator can be used for; chemical, pharmaceutical and biological laboratories. It is designed to separate solutions such as; distillation, drying, recovery, extraction, etc.

Rotary evaporators used for, removes solvents and low boiling organic chemicals from compound mixture of samples by evaporation. Low boiling organic chemicals, usually solvents are removed by a simple distillation.

Figure 1.8: Lab Tech EV311 Rotary Evaporator

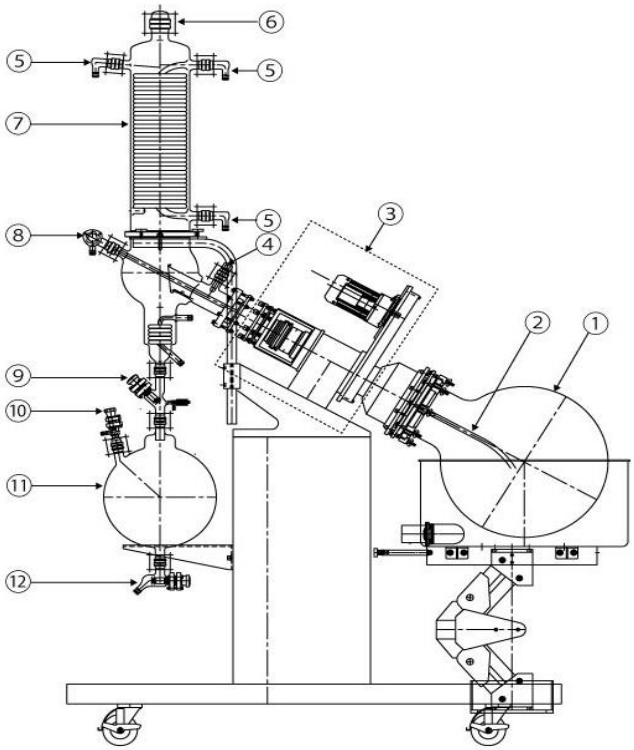

Figure 1.9: Rotary Evaporator

Table 1.5: Parts of Rotary Evaporator

Parts of Rotary Evaporator			
1	Rotation Flask	**7**	Condenser, Reflux Divider
2	Feed Tube, PTFE	**8**	Liquid Feed Valve
3	Mechanical Seal	**9**	Straight valve
4	Thermometer Pocket	**10**	Vacuum vent valve
5	Hose connector, L-shape	**11**	Receiver
6	Plug	**12**	Glass drain port

1.2.3 Antimicrobial Effect

Antimicrobial means; anti is against and microbial means microorganisms, so antimicrobial are against the microorganisms. It's an agent that destroying or inhibiting the growth of microorganisms or inhibits the growth pathogens (Nabila Benhammou, 2008).

Substances such as disinfectants, antibiotics and antimicrobial additives are an example for antimicrobial. In pharmaceutical industries, the antimicrobial activity of medicinal plants has a key role. For the production of new drugs active compounds in the plants synthesized. Medicinal plants are may contain compounds that have anti-inflammatory, healing, antidiabetic, anticancer, antioxidant as well as antimicrobial activities (AYESHA SIDDIQUI1, 2017).

In 1910; the first antimicrobial agent in the world was discovered by Ehrlich, which is use for syphilis treatment and named as a salvarsan.

In 1928 Alexander Fleming discovered penicillin, it is the first natural antimicrobial fungus from the Penicillium genus. 1940s The antibiotic successfully used to treat a *Streptococcus* infection for clinical use (Tomoo SAGA, 2009).

A range of laboratory methods used to evaluate in vitro, antimicrobial activity of a sample. The basic methods are disk-diffusion and broth or agar dilution methods (Mounyr Balouiri, 2016). The methods are used to determine antimicrobial activity of a sample, check Minimum Inhibitory Concentration (MIC) which is lowest concentration of an antimicrobial, usually a drug, which inhibits visible growth of bacterium (Nickson, 2017)

Chapter 2

USE AREAS OF *Pistacia lentiscus* L.

2.1 Medical Industry

Some species of Anacardiaceae family used for medical industry because of their medicinal properties. Thee *Pistacia lentiscus* used in the treatment of many diseases because of its metabolites in the content (Onay, 2016).

Table 2.1: Medicinal Use of *Pistacia lentiscus* L.

Treatment of some diseases
• Antifungal • Antibacterial • Antimicrobial • Anti-inflammatory • Anti-Helicobacter Pylori Activity • Anti-tumor Activity • Wound-healing Activity • Liver Protective Activity • Blood Pressure-lowering • Anti-cancer Activity

2.2 Food Industry

Mastic Oil of *Pistacia lentiscus* which is obtained from resin of plant, use for some desserts making, chewing gum, in ice cream production, vegetable preserves and smoked foods.

In Cyprus the fruits of pictesia lentiscus used at bakeries to make traditional hard biscuit, the mature fruits collect from wild environment, wash and then live for dry before use. The traditional sugar-free biscuits serve with tea.

Figure 2.1: Cyprus Traditional Biscuits with *Pistecisa lentiscus* L.

Chapter 3

EXPERIMENTAL

3.1 Samples Collection

The leaves and fruits of *Pistacia lentiscus* L. were harvested in February 2018 from spontaneous native plants at Karpaz region, which is located at north side of Cyprus. The Leaves and fruits of *Pistacia lentiscus* L. collect from spontaneous trees from same region. Same of the samples used as a fresh and some dry. Harvested plants fruits were washed with distilled water and leave for 10 days dry at dark room.

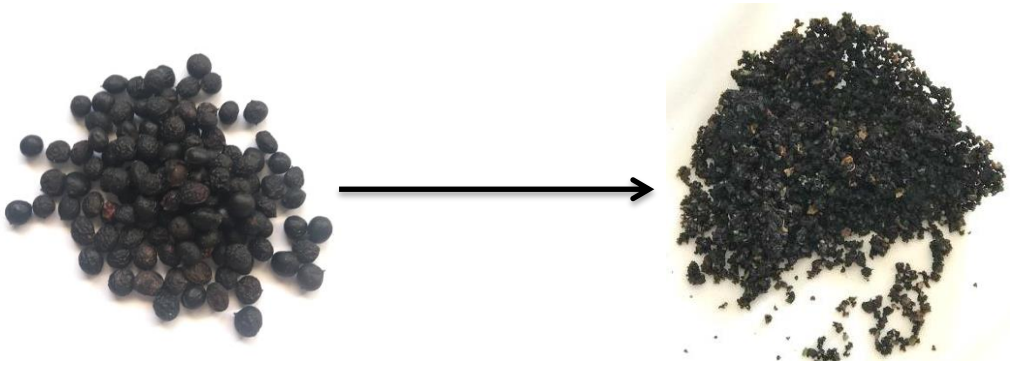

Figure 3.1: Crushed Dried Fruits

Figure 3.2: *Pistacia lentiscus* L. Tree

Figure 3.3: *Pistacia lentiscus* L. Tree and Immature Fruit

3.2 Anatomical Structure

3.2.1 Materials

For materials; Microscope slide, cover slip, tweezer, razor blade, leaf, and SARTUR reagent used. The leaves of *Pistacia lentiscus* L. were harvested in February 2018 from spontaneous wild plants at Karpaz region and used as a fresh.

The SARTUR reagent; is a composite indicator. It is prepared by Prof. Dr. Sarim Çelebioğlu and Prof. Dr. Turhan Baytop in 1949 to identify many elements in the same preparation and it shows its reagent effect after heating. It does not have any affect on Calcium Oxalate Crystals (biyologlar, 2008).

Table 3.1: Ingredients of SARTUR Reagent

Lactic acid	60 ml
Cold saturated lactic acid with Sudan III	45 ml
Pure aniline	2.0 gr
Iodine	0.2 gr
Potassium iodide	1.0 gr
Ethanol 95	10 ml
Distilled Water	80 ml

3.2.2 Instruments

2 main instruments were used which are, Olympus cx21 microscope and velp scientifica heater.

3.2.3 Methods

Green leaf of *Pictecia lentiscus* taken from plant sample and small piece of cross section of a leaf upper face and lower face taken by using specialized razor blade. Then by using tweezer cross sectıon of leaf transfer to microscope slide. The 1-2 drops of special reagent which is called SARTUR is added to cross section of leaf. Heat for 10 seconds by using heater. Placed under Olympus cx21 microscope. Start with low power and increase gradually and record observation. Pictures of samples taken under microscope.

3.3 Chemical Extraction

3.3.1 Materials

As a material; Ground fruit of Pistacia lentiscus, soxhlet apparatus, distilled water, 6 different solvents (ethanol, methanol, chloroform, hexane, acetone, and dichloromethane), sensitive electronic balance, stop watch, filter paper, stands and clamps to support the extraction apparatus used.

3.3.2 Instruments

The main instruments, Lab tech EV311 Rotary evaporator, grinder, sensitive electronic balance and electro-mag heater used.

3.3.3 Methods

3.3.3.1 Soxhlet Method

Harvested mature plant fruits were dried and ground into small particles by using grinder to provide a greater surface area and 10 gr of plant sample placed into a filter paper cone. The filter paper cone is placed in an extraction chamber. The round

bottom flask filed with 500 ml of solvent and attached to a Soxhlet extractor and condenser. The heater fix to 80 ^{o}C, solvent start to evaporates and moves up into the condenser part of soxhlet. The condensate then drips into the reservoir containing the filter paper. Cycle begins when the level of solvent reaches the siphon and pours back into the flask. The process continues up to 16 hours.

Figure 3.4: Soxhlet Extraction of *Pistacia lentiscus L.*

3.3.3.2 Rotary Evaporator

After soxhlet extraction the collected sample taken to Lab tech EV311 Rotary evaporator to evaporated excess solvent, only a small yield is enough to leave for glass bottom flask (James Redfern1, 2014).

Figure 3.5: Evaporation of Extracts of *Pistacia lentiscus L.* by Lab Tech EV311

3.4 Antimicrobial Effect

3.4.1 Materials

Test Organism (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922) Mueller Hinton Broth Media, test tubes, heater, Dimethyl sulfoxide (DMSO), sterile disposable inoculating loop, sensitive electronic balance, volumetric flask, plate, glass rod for determining the antimicrobial activity of *Pistecis lentiscus L*, in total four different test organisms, two for Gram positive cocci and two for Gram negative bacilli were used.

Table 3.2: Test Organisms

Gram positive cocci	**Gram negative bacilli**
Staphylococcus aureus ATCC 29213	*Klebsiella pneumoniae* ATCC 700603
Enterococcus faecalis ATCC 29212	*Escherichia coli* ATCC 25922

All organisms that used for a test were providing by Eastern Mediterranean University, Famagusta, North Cyprus. Department of Microbiology and Virology, Faculty of Pharmacy.

3.4.2 Instruments

Selecta presoclave-II Autoclave,

3.4.3 Methods

3.4.3.1 Mueller Hinton Broth Media

21 grams of the medium was dissolved in 1 liter (1000 ml) of distilled water and then it is stirred till a homogenous solution was acquired. Then, the ph of the medium was adjusted to 7 and the medium was autoclaved 15 min at 121°C under 1 atmosphere pressure. After autoclaving, 5 ml of the medium was distributed into individual test tubes.

For next section, plants exracts that was obtained from extraction of rotary evaporater were weighted by using sensitive electronic balance. In total, 7 different 1.024gr(1024 mg) plant extract was transferred into the volumetric flasks. Each of the 7 different extracts of *Pistacia lentiscus L* fruit was dissolved in 25 ml Dimethyl sulfoxide (DMSO) to obtain 4096 mg/l of stock solution.

3.4.3.2 Macro Dilution Method

Antimicrobial activities of 7 different extracts prepared by chloroform, Methanol, Ethanol, Dichloromethane, Hexane Acetone and distilled water solvents using the macrodilution method were examined against *S. aureus*, *E. faecalis*, *E. coli* and *K. pneumoniae*. All 4 test microrganisms were adjusted to 0.5 Mac Farland ($1.5x10^8$ cfu/ml) in Mueller Hinton broth. Then diluted 1:100 using Mueller Hinton broth in order to obtained a conc. of $1,5x10^6$ cfu/ml for each 4 different test microrganisims (Donna M. Hacek, Dana C. Dressel, Lance R. Peterson, 2014).

Stock solutions (4096 mg/l) concentration of were diluted by 1:16 using mueller hinton broth so that the concentrations of 7 different plant extracts were reduced to 256 mg/l.

Later, 5 ml of each *S. aureus*, *E. faecalis*, *E. coli* and *K. pneumoniae* microorganisms, at the concentration of $1,5x10^6$ cfu/ml were mixed with 5 ml of 7 different extracts at 256 mg/l. So the final concentration of extracts became 128 mg/l and the final concentrations of test microorganisms became 10^5 cfu/ml. For obtaining the final concentration of the extracts as 64 mg/l, the stock solution at 4096 mg/l was diluted 32 fold and the diluted extract was mixed with the equal volme (5 ml) of test organisms at the concentrations of $1,5x10^6$ cfu/ml. For the extracts at 128 mg/l final Dimethyl sulfoxide conc were 3% and for extracts at 64 mg/l conc was 1.5%.

3.4.3.3 Agar Well Diffusion Method

All four test miccrorganisms; *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922 at the conc of 1,5x108 cfu/ml (0,5 Mac Farland) were transferred to all the entire surface of individual mueller hinton agar plates. Then for next section, plates leave for dried at the room temperature and by using sterile glass rod 6 mm diameter wells bored on plates. Then into each of wells, 100 µl of different extract at 128 mg/l, were pipeted and plates were left incubation for 24 hours at 37ºC under aerobic atmosphere. After incubation period, by using ruler inhibition zone diameter was measured in millimeters (Mounyr Balouiri, 2016).

The plates then dried at the room temperature and by using sterile glass rod 6 mm diameter wells bored on plates. Then into each of 6 mm wells, 100 µl of each

different extract at 128 mg/l, were pipeted. The plates were left incubation for 24 hours at 37ºC under aerobic atmosphere. After incubation period, inhibition zone diameter was measured in millimeters using ruler.

Chapter 4

RESULTS AND DISCUSSION

4.1 Anatomical and Morphological Structure of *Pistacia lentiscus* L.

Plant samples collected spontaneously from the same region of Karpaz region north Cyprus in February 2018. For the anatomical structure fresh leaves used. The cross section of leaf, inner and outer layer examine with sartur reagent. As result of microscopic investigation leaves are dorsi-ventral (bifacial), adaxial palisade consists of one layer and the abaxial spongy parenchyma consists of several layers. Both surface of leaf has single thin walled epidermis and thick layer cuticula. The cell walls of both side are very similar in appearance. Stomata only occurs upper surface of leaf and not an hairs present at surface. While cheking the reserch to compare that, the cross section of leaflet has same thin epidermal cells on both inner and outer of the leaf and also cove with cruticula.

Figure 4.1: Cross Section of *Pistacia lentiscus L*. Leaf

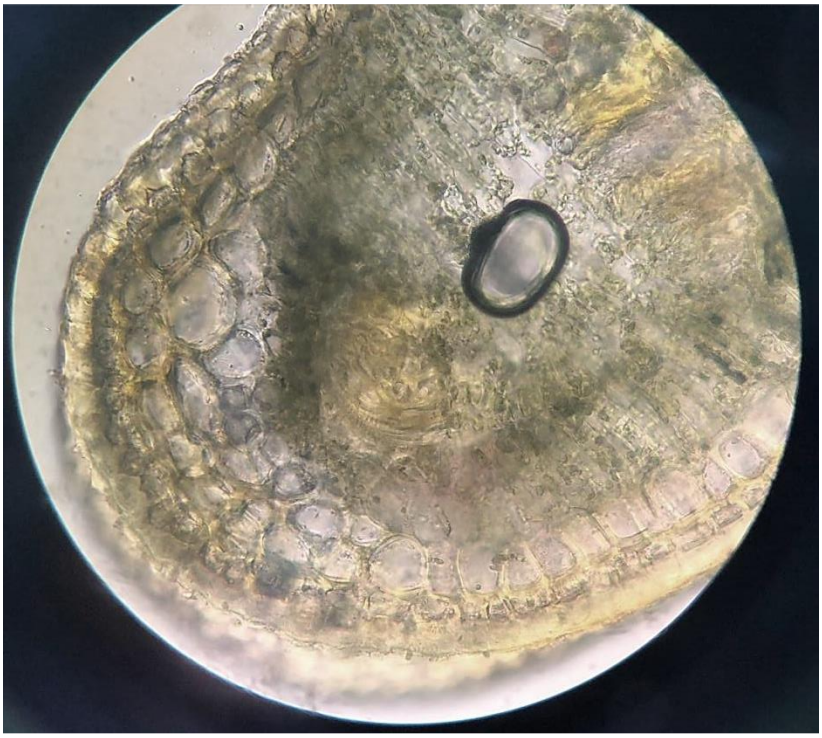

Figure 4.2: Spongy Parenchyma of *Pistacia lentiscus* L.

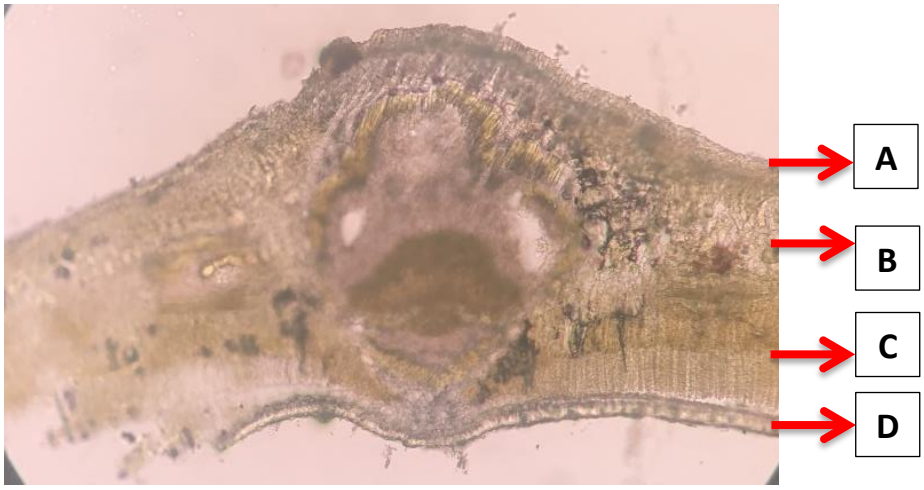

Figure 4.3: Cross Section of *Pistacia lentiscus* L. from Midrib.
A) Upper Epidermis, B)Spongy Parenchyma, C)Palisade Parenchyma D)Lower Epidermis

4.2 Anti-Microbial Activity of the *Pistacia lentiscus L.*

4.2.1 Macro Dilution Method

Table 4.1 shows the results of, Minimum Inhibitory Concentration of all extracts against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922. MICs of; methanol, ethanol, chloroform, dichloromethane, hexane, acetone and distilled water extracts were obtained to be 128 mg/l against *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. For *K. pneumoniae* and *E. coli*, MICs of dichloromethane, methanol, chloroform, distilled water and hexane extracts were determined to be 128 mg/l. Ethanol and acetone extracts inhibit the growth of *E. coli* at 128 mg/l but not at 64 mg/l. Therefore, the MIC of these extracts were regarded as 128 mg/l. *K. pneumoniae* did not show any growth at the conc. of 128 mg/l and 64 mg/l ethanol and acetone extracts which requires the further investigation of lower concentrations.

Table 4.1: Minimum Inhibitory Concentrations of Methanol (M), Ethanol (E), Chloroform (C), Dichloromethane (D), Hexane (H), Acetone (A), and Distilled Water (dH_2O) Extracts of *Pistecia lentiscus* L. against *S.aureus, E. faecalis, K.pneumania* and *E. coli*

Bacterial Isolates	**MIC (mg/l)**						
	M	**E**	**C**	**D**	**H**	**A**	**dH_2O**
S. aureus	128	128	128	128	128	128	128
E. faecalis	128	128	128	128	128	128	128
K. pneumonia	128	*	128	128	128	*	128
E. coli	128	128	128	128	128	128	128

** Will be further investigated*

4.2.2 Agar Well Diffusion Method

In this method, Methanol, Chloroform, Acetone, Distilled Water, Hexane, and Dichloromethane of the plant extract against all strains that tested gives any zone (0 mm). Only Ethanol showed 12mm zone against *S. aureus* at 256mg/l concentration on Mueller Hinton Agar.

Chapter 5

CONCLUSION

As a result of the study, anatomical structure of *Pistacia lentiscus* L. leaves are bifacial, both inner and outer surface of leaf has thin walled epidermis and thick layer cuticula with simillar apperance of cell wall. Leaf has no hairs on surface and stomata cells occurs only outer surface of leaf.

In terms of the antimicrobial activity of *P. lentiscus*, the minimum inhibitory concentrations of all extracts, except ethanol and acetone, against two Gram negative and two Gram positive bacteria were found to be 128 mg/l. Ethanol and acetone extract shows shows promising antibacterial activity against *K. pneumoniae* which needs further investigation at lower concentrations. Moreover, the chemical ingredients of *Pistacia lentiscus* that lead to the antibacterial activity should further be investigated.

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