Anatomical, Chemical and Antimicrobial Activity of *Corchorus olitorius* L. (Molokhia)

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ABSTRACT

The genus *Corchorus* L. which is a member of the family Tiliaceae, is native to tropical and subtropical regions. According to the plant list, it contains about 75 accepted taxa. *Corchorus olitorius* L. is an annual plant growing to 3.5 m high. Its leaves are simple, shine green, elliptic-ovate, acute and serratulate with short petiole. The flowers are yellow and small. The flowering time is from August to October and the seeds ripen in October.

In this study, anatomical characters and antibacterial activity of leaves have been investigated. The examined specimens have been collected in the early summer season from fields in Çayönü village (Famagusta, North Cyprus). The cross-sections, surface sections and powder of leaves have been examined microscopically and antibacterial activity of methanol, chloroform, dichloromethane, acetone, hexane, ethyl acetate and water extracts against *S. aureus*, *E. faecalis*, *E. coli*and *K. pneumoniae* was investigated by Muller Hinton broth media for Macrodilution and agar well diffusion method.

The results obtained in this research, anatomically leaves are dorsiventral (bifacial), on both sides there is one layered epidermal. Outer wall of epidermis cells are thick and the cuticula thin. Midrib is very prominent with 4 big secretion cell. Palisade parenchyma is 1-2 layered, spongy parenchyma is 2-3 layered. Prismatic crystals are abundant and mainly runs as one row along the veins. Stomata are on the lower surface. There are no hairs but some small scabrous cavities are present.

Microbiologically, the Minimum Inhibitory Concentrations of all/some extracts were found to be 128 mg/l for all of the Gram positive and Gram negative strains tested. The results obtained in this study appear to confirm the antibacterial potential of *Corchorus olitorius* leaves, as well as its beneficial in the treatment of related diseases.

Keywords: *Corchorus olitorius*, leaf anatomical structure, antimicrobial activity

ÖZ

Corchorus L., tropikal ve subtropikal bölgelere özgü olan bir bitki olup Tiliaceae familyasının bir üyesidir. Bitki listesine göre, yaklaşık 75 kabul edilen türden oluşmaktadır. *Corchorus olitorius* L., 3.5 m yüksekliğe ulaşan yıllık bir bitkidir. Yaprakları kısa saplı, basit, parlak yeşil, eliptik-oval, akut ve serratülattır. Çiçekler sarı ve küçüktür. Çiçeklenme zamanı ağustos ayından ekim ayına kadardır ve tohumlar ekim ayında olgunlaşır.

Bu çalışmada yaprağın anatomik karakterleri ve antibakteriyel aktivitesi araştırılmıştır. İncelenen örnekler, yaz mevsiminde Çayönü köyünden (Gazimağusa, Kuzey Kıbrıs) toplanmıştır. Yaprakların çapraz-kesiti, yüzey kısımları ve tozları mikroskopta incelenmiş ve metanol, kloroform,diklorometan, aseton, heksan, etil asetat ve su ekstrelerinin S. *aureus, E. faecalis, E. coli* ve *K. pneumonia*e suşlarına karşı antibakteriyel aktivitesi Mueller Hinton sıvı besiyeri ile makrodilüsyon ve agar kuyu difüzyon yöntemiyle araştırılmıştır.

Anatomik olarak bu araştırmada elde edilen sonuçlar, yapraklar dorsiventral (bifasiyal) özellikte olup, her iki tarafta bir katmanlı epiderma vardır. Epiderma hücrelerinin dış duvarı kalın ve kutikül ise incedir. Midrib 4 büyük salgı hücresi ile çok belirgindir. Palisade parankim bir-iki katmanlı, süngerimsi parankim 2-3 katmanlıdır. Prizmatik kristaller bol ve esas olarak damarlar boyunca bir sıra olarak bulunur. Stomata alt yüzeydedir. Yaprağın yapısında tüy bulunmamaktadır, ancak bazı küçük çürük boşluklar mevcuttur.

Mikrobiyolojik olarak, test edilen tüm Gram pozitif ve Gram negatif türlerinin tümü / bazı ekstraktların Minimum İnhibitör Konsantrasyonları 128 mg/l olarak bulundu. Bu çalışmada elde edilen sonuçlar, *Corchorus olitorius* yapraklarının antibakteriyel potansiyelini ve bununla ilgili hastalıkların tedavisinde faydalı olduğunu doğrulamaktadır.

Anahtar Kelimeler: *Corchorus olitorius*, yaprağın anatomic yapısı, antimikrobiyal aktivite

In memory of
my father

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LIST OF SYMBOLS AND ABBREVATIONS

ºC	Degree Celsius
%	Percent
ATCC	American Type Culture Collection
Ca	Calcium
$CHCl_3$	Chloroform
CH_2Cl	Dichloromethane
CH_3OH	Methanol
C_3H_6O	Acetone
$C_4H_8O_2$	Ethyl acetate
C_6H_{14}	Hexane
cfu/ml	Colony-forming units per milliliter
cm	Centimeter
Cu	Copper
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
Fe	Iron
g	Gram
H_2O	Water
IU	International Unit
K	Potassium
kcal	Calorie
kJ	Kilojoule

K. pneumoniae	*Klebsiella pneumoniae*
L	Carl Linnaeus
M	Meter
mg	Milligram
mg/L	Milligram/Liter
mg	Magnesium
MIC	Minimum Inhibitory Concentration
ml	Milliliter
mm	Millimeter
Mn	Manganese
Na	Sodium
P	Phosphorus
Se	Selenium
S. aureus	*Staphylococcus aureus*
TRNC	Turkish Republic Northern Cyprus
µg	Microgram
WHO	World Health Organization
Zn	Zinc

Chapter 1

INTRODUCTION

1.1 General Information About *Corchorus olitorius* L.

The genus *Corchorus* L. which is a member of the family Tiliaceae, is native to tropical and subtropical regions. According to the plant list, it contains about 75 accepted taxa. *Corchorus* species are generally grown in warm regions, tropical and sub-tropical climates, but they are mostly found in Eastern and South Africa. *Corchorus olitorius* L. is known to have originated in North Australia and Africa, and is thought to emigrate to India and China via Egypt and Syria (International Jute Study Group, 2018).

Corchorus olitorius L. is an annual plant growing to 3.5 m high. Its leaves are simple, shine green, elliptic-ovate, acute and serratulate with short petiole. The flowers are light yellow and hermaphrodite and the pollensare transferred by insects. It needs a moist soil and a sunny climate to grow. The flowering time is from August to October and the seeds ripen in October. The highly branched *Corchorus olitorius* L. is generally harvested when it reaches 90-120 cm. Leaves are 6-10 cm long and 3-5 cm wide.In general, the leaves are consumed fresh or dried. Fresh leaves can be used in salads and dried leaves used in soups and traditional dish in Northern Cyprus (SOYKUT, 2018).

The body of the plant contains large amounts of cellulose and lignin. The fiber products of the *Corchorus olitorius* are separated from the body by various chemical

methods and they are frequently used in textile sector. *Corchorus olitorius* is the most useful plant that is used as a fiber products after cotton. The yarn that is obtained from fiber products of the plant are used in to produce natural dresses and to produce nature shopping bags that can disappear in nature. Especially in India and Bangladesh this plant is an important commercial product and plays a major role in the exportation of biologically degradable nature (Food and Agriculture Organization of the United Nations, 2018).

1.1.1 *Corchorus olitorius* L. Plant Taxonomy

Table 1: Taxonomy of *Corchorus olitorius* L.(Islam, 2013).

Rank	**Scientific Name**	**Common Name**
Kingdom	Plantae	Plants
Subkingdom	Tracheobionta	Vascular Plants
Superdivision	Spermatophyta	Seed Plants
Division	Magnoliophyta	Flowering Plants
Class	Magnoliopsida	Dicotyledonae
Subclass	Dilleniidae	
Order	Malvales	
Family	Tiliaceae	Linden Family
Genus	*Corchorus* L.	*Corchorus*
Species	*Corchorus olitorius* L.	Nalta Jute
English Name	Jute	

1.1.2 *Corchorus olitorius* L. Plant and Nutrient Content

Table 2: Nutrient Content of *Corchorus olitorius* L.(United States Department of Agriculture , 2019).

Nutrient	Unit	Value(100g)
Proximates		
Water	g	87.72
Energy	kcal	34
Energy	kJ	142
Protein	g	4.65
Total lipid (fat)	g	0.25
Ash	g	1.58
Carbohydrate, by difference	g	5.8

Table 3: Mineral Contents of *Corchorus olitorius* L.(Islam, 2013).

Nutrient	Unit	Value(100g)
Minerals		
Calcium, Ca	mg	208
Iron, Fe	mg	4.76
Magnesium, Mg	mg	64
Phosphorus, P	mg	83
Potassium, K	mg	559
Sodium, Na	mg	8
Zinc, Zn	mg	0.79
Copper, Cu	mg	0.255
Manganese, Mn	mg	0.123
Selenium, Se	µg	0.9

Table 4: Vitamin Contents of *Corchorus olitorius* L.(United States Department of Agriculture , 2019).

Nutrient	Unit	Value(100g)
Vitamins		
Vitamin C, total ascorbic acid	mg	37
Thiamin	mg	0.133
Riboflavin	mg	0.546
Niacin	mg	1.26
Pantothenic acid	mg	0.072
Vitamin B-6	mg	0.6
Folate, total	µg	123
Folic acid	µg	0
Folate, food	µg	123
Folate, DFE	µg	123
Vitamin B-12	µg	0
Vitamin A, RAE	µg	278
Retinol	µg	0
Vitamin A, IU	IU	5559
Vitamin D (D2 + D3)	µg	0
Vitamin D	IU	0

Table 5: Lipid Contents of *Corchorus olitorius* L.(United States Department of Agriculture , 2019).

Nutrient	Unit	Value(100g)
Lipids		
Fatty acids, total saturated	g	0.038
16:00	g	0.03
18:00	g	0.006
Fatty acids, total monounsaturated	g	0.017
16:1 undifferentiated	g	0.003
18:1 undifferentiated	g	0.014
Fatty acids, total polyunsaturated	g	0.12
18:2 undifferentiated	g	0.117
18:3 undifferentiated	g	0.002
Fatty acids, total trans	g	0
Cholesterol	mg	0

Table 6: Amino Acid Contents of *Corchorus olitorius* L.(United States Department of Agriculture , 2019).

Nutrient	Unit	Value(100g)
Amino Acids		
Tryptophan	g	0.03
Threonine	g	0.164
Isoleucine	g	0.221
Leucine	g	0.388
Lysine	g	0.219
Methionine	g	0.065
Cystine	g	0.04
Phenylalanine	g	0.212
Tyrosine	g	0.147
Valine	g	0.248
Arginine	g	0.248
Histidine	g	0.11
Alanine	g	0.256
Aspartic acid	g	0.567
Glutamic acid	g	0.493
Glycine	g	0.214
Proline	g	0.246
Serine	g	0.182

1.2 Anatomical Structure of Plants

Plant anatomy is a botanical branch that examines the outer and inner structures of multicellular plants. The term of anatomy is derived from the word of thomus. The term of morphology is derived from morphosor shape and logos in science. Initial research on plant anatomy were begins 3rd century with Theophrastus. This researcher differentiated between root, stem, leaf, flower and fruit and also used another anatomical terms such as bark, essence and wood. After the presence of the microscope in the Renaissance and 17th century, researches on plant histology rapidly increased. Marcello Malpighi and Nehemiah Grew set the basic rules of histology and anatomy. In recent years, with the studies done with electron microscopy, the anatomical parts of the plants are fully illuminated (Giovanna Ginestra, 2009).

Plant anatomy, is required all the oxygen for our life which is produced by plants. If there was not even one of the photosynthesis enzymes that plants carried out, there would be no living things on earth. The most important benefit of plants to living things is not just photosynthesis. In addition, the plant has been a source of food and a nest for insects, mammals, birds and almost all creatures (ENCYCLOPEDIA.COM, 2018).

Plant morphology is a botanical branch that defines the physical form and external structures of a plant. Also known as a study of tissue organization. Morphology of the plant is concerned with the examination of all structures such as histology and cytology of a plant. It is known as a phytomorphology. Plant morphology also plays a role in the identification of hereditary, environmental determinants and their relationships by the controlled-environment method and vaccination experiments on embryos. Plant anatomy and morphology are related to the research of structural and functional of plants in kingdom Plantae.There are four basic parts of the plant. These parts are defined as roots, stem leaves and flowers (Silverio, 2016).

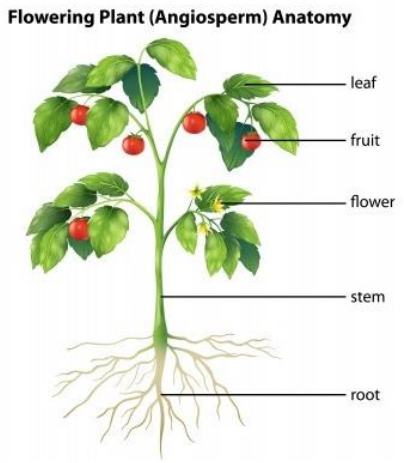

Figure 1: The Basic Four Parts of the Plant

Plant roots are usually found under the ground. The plant binds to the soil by using their roots. It provides absorption of water and water soluble mineral substances from soil. It stores food and water when is needed (ENCYCLOPEDIA.COM, 2018).

Plant stem are usually found above the soil. The body of the plant is carries the organs which are branches, leaves, bud and fruit. It keeps the plant upright and helps leaves to get the light from the sunlight. It transmits the water and mineral substances to the leaves which are absorbed from the roots. They are also involved in the storage of food and support leaves and reproductive structure (ENCYCLOPEDIA.COM, 2018).

The leaves of the plant play the important role in photosynthesis. They are also included in food storage and helps respiration of the plants. They are also known as site of gas exchange (ENCYCLOPEDIA.COM, 2018).

Flowers of the plants are plays a role in producing fruits and helps insects for pollination. Also it contains the sexual organs of the plants (ENCYCLOPEDIA.COM, 2018).

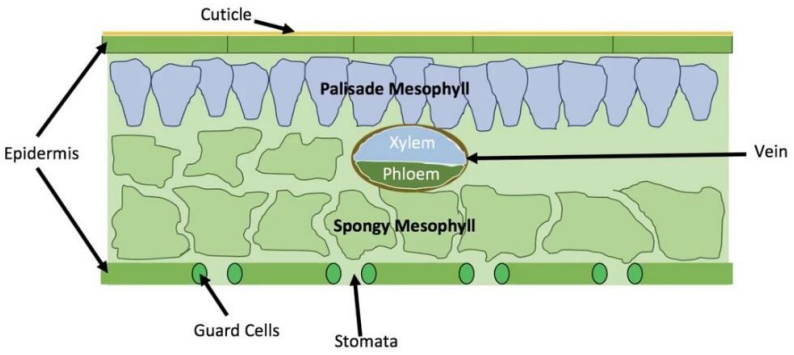

Figure 2: The Cross Section of the Leaf.

Anatomical structure of the leaf is investigated under the microscope. The important parts that seen under the microscope from the cross section of the leaf is epidermis, cuticle, palisade mesophyll, spongy mesophyll, guard cells, stomata and vein (xylem, phloem) as it shown in Figure 2 (Giovanna Ginestra, 2009).

In the cross-section of the leaf the cuticle layer at the top waxy, non-cellular part of the leaf which prevents the water loss of the plant. The cuticle layer of the plants is thick in arid zone and is thin in moist zone. Cuticle secretes the epidermis. Epidermis is playing the important role in protecting the leaf. They are similar to skin like layer of the cells which are found on both sides. Above the epidermis; the leaf contains cells called stomata to allow air inside to enter the photosynthesis. Stoma cells are the holes which is present in the lower epidermis to provide gas exchange when they are open and close. The inner layer of the leaf is called the mesophyll layer. Mesophyll layer consists of two parts. These sections are palisade mesophyll which is rich in chloroplasts and spongy mesophyll which is poor in chloroplast. Palisade mesophyll are present below the upper epidermis and plays important role in photosynthesis. Spongy mesophyll are present just under the palisade mesophyll and plays important role in storing the products of photosynthesis. Guard cells are located around of the

stomata and they are responsible from the opening and closing of the stomata (Boundless Biology - Lumen, 2018).

1.3 Extraction Methods of Plants

Extraction is the process for purposes of purification organic solvents vegetable, animal or synthetic raw materials by using alcohol, water at a given temperature and pressure. The extraction process is applied in different ways according to the final product desired from the raw material (ABBASOGLU, 1996). In general extraction as applied in medicinal and aromatic plants, is a process of removing soluble substances in the vegetable material through this solvent by passing a solvent through the material to be extracted. The products of the plant that obtained are impure liquids, semisolids or powders intended only for oral or external use (Mariam Gökçebağ, 2017).

1.3.1 Extraction Methods of Medicinal Plants

Extraction is an important step for the separation, identification, and use of valuable compounds from different plants (Jibrin Mohammed Danlami, 2014). The selection of suitable technique to obtain maximum efficiency and highest purity depends on the nature of the target compound. The extraction of the compounds from the plants are used numerous chemical and mechanical processes such as solvent extraction and steam distillation (Handa, 2008). Existing techniques for extracting plant leaves and essential oils of the leaves include Soxhlet, hydro distillation and maceration with alcohol. The mass transfer resistances due to the inclusion of two or more phases in the system continuously limiting the usage of techniques of traditional Soxhlet extraction. Extraction method requires a long time according on the diffusion rates of the solvents (Singh, 2008).In addition, standard extraction techniques are energy intensive. These techniques are manual processes, and reproducibility is a major challenge (Rane, 2008).

1.3.2 Traditional Soxhlet Extraction

The basic techniques for the plants extraction are based firstly on the selection of solvent, including the use of heat and agitation. Soxhlet extraction which is known as an oldest extraction method, is the most widely used technique to evaluate the performance of solid-liquid extraction methods (Rane, 2008).

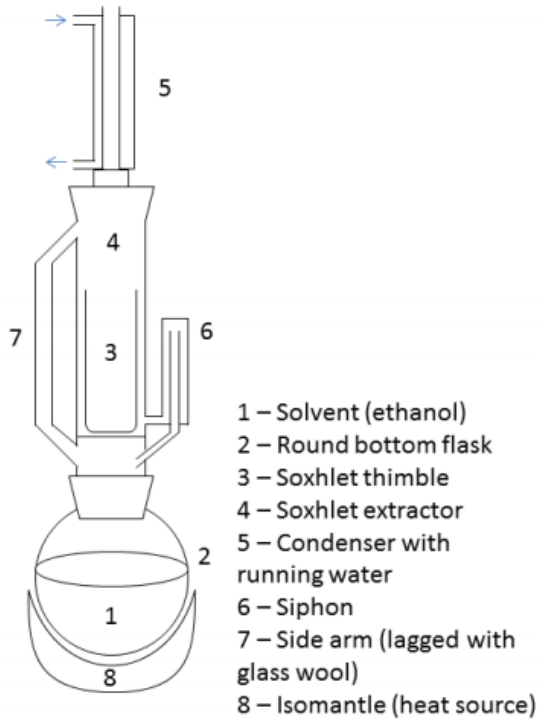

Figure 3: Diagram of Soxhlet Extraction Equipment (James Redfern, May 2014).

Figure 3 shows the standard Soxhlet system. The system should start by constructing the extraction apparatus by supporting the stands and used clamps. Subsequently, the chemical solvent is added to a round bottom flask connected to a Soxhlet extractor and condenser (Figure 3) on an isomantle. The powdered plant material is wrapped with the filter paper and transfer into the Soxhlet thimble placed in the Soxhlet extractor. The side arm is covered with glass wool. The solvent is heated by using heating mantle and will begin to changing from liquid to vapor by moving from the apparatus towards

the condenser. The condensation then started to drop down into the chamber containing the thimble. When the solvent level reaches the siphon, it is poured back into the round bottom flask and the cycle starts again. The process of the extraction using the Soxhlet apparatus should run for a total of 16 hours (James Redfern, May 2014).

1.3.3 The Advantages and Disadvantages of Using the Soxhlet Extraction Techniques

Table 7: Benefits and Drawbacks of Soxhlet Extraction Techniques (Rane, 2008).

Using the Soxhlet extraction techniques	
Advantages	Disadvantages
To expose constantly the solid matrix to fresh solvent keep the system far from equilibrium	Time is long for the extraction
To maintain the temperatureto be able to recover the compounds of interest	Alarge amount of solvent is consumed
Not essential for filtration	To speed up the process stirring the material cannot be possible in during the using of this method
Straight forward method and a low-cost technique	The large amount of solvent used in the an evaporation procedure

1.4 Biological Activities of Plants

1.4.1 Anti-Microbial Activities

In the treatment of infections, there is a continuous increase in the number and structure of the drugs used(M.N. Somchit, 2003).Researchers are working on new substances with the smallest dose effective against a wide range of microorganisms that will affect human beings with minimal damage. Physical, chemical, biological, pharmacological and technological studies are being carried out to determine the specific uses of new substances that are synthesized or isolated from plants. One of the priority studies that they investigate is *in vitro* biological activity (M.N. Somchit, 2003). In order to determine the antimicrobial activity of the substances, antiviral, antifungal and antibacterial activity studies are carried out. Further studies are carried out after determining the concentrations of substances that stop or inactivate the growth of viruses, fungi and bacteria (Zeid, 2002).

On the other hand; searching for the antimicrobial activity on clinical isolates to achieve the most effective results in the use of antimicrobial drugs considering the occurrence of resistant microorganisms or factors related to the patient determining the effectiveness of drugs, has become routine (Mounyr Balouiri, 2016).

Various *in vitro* methods are used to determine the antimicrobial activity of the substances regardless of the purpose. These methods, to determine whether the substance has antimicrobial activity uses the Minimal Inhibition Concentration (MIC) and certain microorganism spectrum. In the choice of the method; the number, quantity, solubility of the substances, the type of microorganisms to be used, the property and the density play an important role (Mounyr Balouiri, 2016).

1.5 Medicinal Uses of Plants

1.5.1 Medicinal Plants in North Cyprus

Drug plants are the plants that used for treatment of disease or to avoid the people from the diseases are called medicinal plants. The World Health Organization (WHO) defined medicinal plants in 1980 as a kind of plant that can be therapeutic or disease-inhibiting with one or more organs, or which can be a precursor of any chemical pharmaceutical synthesis. The method of treatment with plants has been transferred from generation to generation throughout history and treatment methods constitute the accumulations in this field (İ.Ümit YAPICI, 2009).

Cyprus is an Island which is one of the Mediterranean regions that has a rich flora. Mediterranean region is known as that is among the richest areas in the world for wild and cultivated plant species (Gianniantonio Domina, 2012).Considering the flora of Cyprus, there are 1,610 species or 1,738 taxa and 108 species or 143 taxa are endemic. Endemic plants of the Northern Cyprus are only 19 out of the 108 species. Considering the rich flora of The Turkish Republic of Northern Cyprus (T.R.N.C.), has a number of woody and herbaceous plants in parallel with this richness (YILDIRIM, 2010) (Ciftcioglu, 2015).

Some examples for medicinal plants that found in Northern Cyprus;
Narcissus tazetta L., *Pistacia terebinthus* L. and *Lagoecia cuminoides* L.. The uses of these plants as medicinal; if flowers of Narcissus tazetta L. used as like as tea, it is helping for treatment papilloma. Making tea from the leaves of Pistacia terebinthus L., is getting good for stomach pains. *Lagoecia cuminoides* L. is getting good for toothache when it is chewed (YILDIRIM, 2010).

1.5.2 Medicinal Use of *Corchorus olitorius* L.

Corchorus olitorius L. can be collected from the nature and consumed as a freshly or the leaves are dried under the sun and consumed as a dried (Ummuhan, 2017). In some studies nutrient content of the plant investigated and it shows that C*orchorus olitorius* L. a higher rate of raw protein, iron (Fe), calcium (Ca) and magnesium (Mg) than spinach and cabbage. C*orchorus olitorius* L. also used as a medicinal plant besides of used as a vegetable (Gunsu Soykut, 2018).The consumed of the juice of the leaf that obtained from the fresh and dried leaves used as known as constipation relieving and the crèmes that obtained from plant extract are used in the treatment of various skin diseases (Gunsu Soykut, 2018).

Due to the fact that the leaves are rich in vitamins, minerals and antioxidants; which are known to be used in the treatment of diabetes, high blood pressure, cancer and heart diseases; In 100 g boiled leaf, 4.5-5.6 g protein, 7.6-12.4 g carbohydrate, 266-366 mg Ca, 97-122 mg P, 444 mg K, 11.6 mg Fe, 12 mg Na, 95 mg ascorbic acid, 6.3 IU vitamin A, 15 mg thiamine (vitamin B1), 28 mg riboflavin (vitamin B2) reported (Islam, 2013).

Its leafy vegetable is known as a folk medicine for the treatment of fever, chronic cystitis, cold and tumors (International Jute Study Group, 2018). The characteristics of the leaves are demulcent, diuretic, febrifuge and tonic. The plant is used in the treatment of chronic cystitis, gonorrhea, pain, fever, tumors and dysuria (Z.A. Zakaria, 2006). Seeds are laxative. From the extract of the plant, injections of olitoriside can be formed and used in significantly to improve cardiac insufficiencies and have no cumulative attributes. Therefore, it can be used instead of strophanthin (Plants For A Future, 2018).

1.6 Traditional Food in Northern Cyprus Knows as a Molokhia (Molehiya).

Corchorus olitorius L. is cultivated and is being used as green leafy vegetable known as molokhia in Northern Cyprus. The "Molehiya" meal, is among the traditional dishes of Turkish Cypriot cuisine which is made from the dried leaves of the plant. Plants are collected from cultivated field and then the leaves are separated from the roots. When they get dried, the prepared traditional dish is served with meat or chicken (Süzal, Istanbul).

Figure 4: "Molehiya" the traditional dishes of Turkish Cypriot Cuisine.

Chapter 2

MATERIALS AND METHODS

2.1 Collection of *Corchorus olitorius* L. Materials

Plant material was collected in the harvested period from the Çayönü Village (Famagusta, North Cyprus) in August 2018. Plant samples that collected from village were washed with water and then washed again with distilled water. The plant samples were transported in to the fume hood cupboard in the laboratory of Faculty of Pharmacy, Eastern Mediterranean University. Plant sample were dried inside the fume hood cupboard at room temperature for 7 days. After seven days, 100grams of dried stored *Corchorus olitorius* L. leaves are shredded by hand then powdered in the laboratory blender. After that plants that become powder stored in airtight closed bottles for analysis (Semra Ilhan, 2007).

Figure 5: *Corchorus olitorius* L. in Çayönü Village (Famagusta, North Cyprus)

Figure 6: Shredded by Hand Method of *Corchorus olitorius* L.

2.2 Microscopic Investigation of *Corchorus olitorius* L. Anatomical Structure

For anatomical structure of the plants, dried and fresh leaves were used. Both type of the plants were cutting with the blades in three different ways. The cross section of the leaf is getting from inner part, outer part and midrib. After the cross section, the piece of the plant that taken from different parts are placed on the microscope slides. After that one drop of the sartur solution placed on the slide and heated by hot plate for 10 seconds. Sartur solution is a preferred reagent because it offers the possibility to examine many elements in a drug (oil, cutin (waxy substance found in plants), citric acid, fungus, lignin, lignified walls, starch, etc.) with a single preparation. Finally these slide that prepared are investigated under the Olympus CX31and CX21 microscope in x10 and x40 magnification. Also, powder of the dried plants were investigated under microscope. Olympus CX31 light microscope with a camera was used to take the pictures of the cross section of the leaves.

2.3 Extraction of *Corchorus olitorius* L. Materials

Totally 100grams of dried stored *Corchorus olitorius* L. leaves are shredded by hand to use it in powdered. Totally 7 different solvent was used to make different extraction in same method. For each extraction, 10 gr of powdered plant samples and 500 ml solvents were used. The solvents that used for extraction was Chloroform ($CHCl_3$), Methanol (CH_3OH), Dichloromethane (CH_2Cl_2), Acetone (C_3H_6O), Hexane (C_6H_{14}), Ethyl acetate ($C_4H_8O_2$) and Water (H_2O)(Kopeliovich, 2018).

Figure 7: Extraction of *Corchorus olitorius* L. by Using the Soxhlet Equipment

The temperature of all solvents was adjusted to their specific boiling points and Soxhlet apparatus was started. The boiling points of $CHCl_3$is 61.2ºC, CH_3OH is 64.7ºC, CH_2Cl_2 is 39.6ºC, C_3H_6O is 56ºC, $C_6H_{14}$is 68ºC, $C_4H_8O_2$ is 77.1 and finally H_2O is 100ºC. The experiment process took total of 15-16 hours (James Redfern, May 2014) (Semra Ilhan, 2007). Once the experiment has finished, the solvents that used for the extraction evaporated using a rotary evaporator.

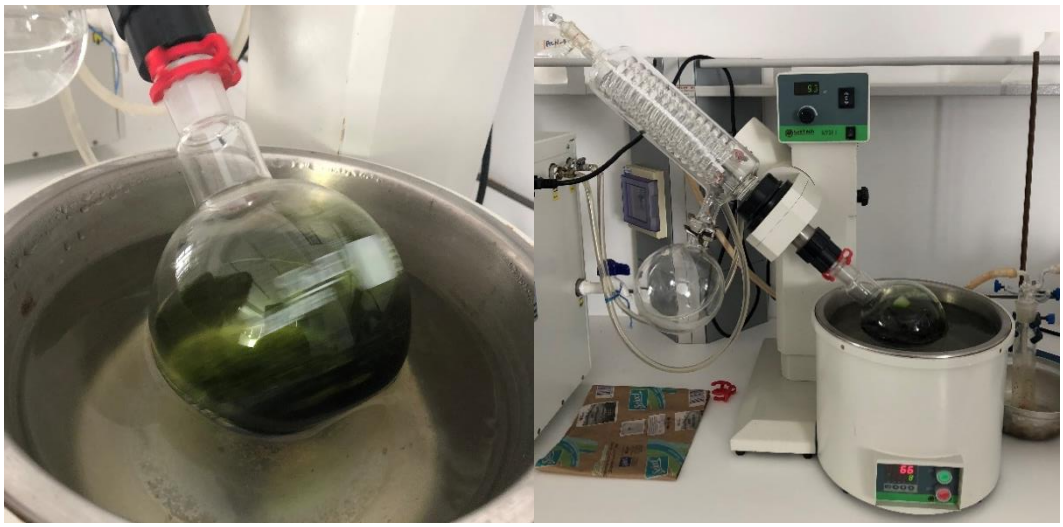

Figure 8: Rotary Evaporator to Evaporate the Solvents that Used

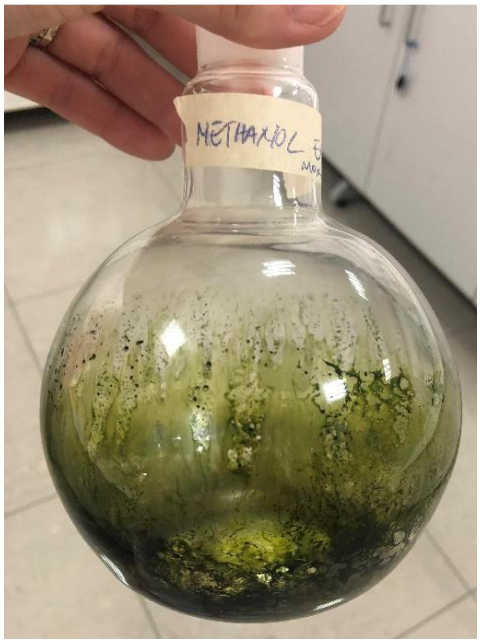

Figure 9: Example of the Extraction After the Rotary Evaporator

2.4 Anti-Microbial Activity of *Corchorus olitorius* L. Extracts

2.4.1 Test Organism

For determining the antimicrobial activity of *Corchorus olitorius* L., four different types of quality control test organisms were used. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains for Gram positive bacteria. As gram negative bacteria, *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as control strains for Gram positive bacteria.

All of the test organisms used in the study were obtained from the Department of Microbiology and Virology, Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, North Cyprus.

2.4.2 Preparation of Test Organisms

All the test organisms which had been frozen at -20 ˚C were subcultured onto 5% sheep blood agar (Biomerieux, France). Pure culture was obtained for all of the strains by subculturing one colony of each onto Mueller Hinton agar (Biomerieux, France).

2.4.3 Preparation of Mueller Hinton Broth Media for Macrodilution and Agar Well Methods

21 g of Mueller Hinton broth (Merck, Germany) was dissolved homogenously in 1000 mL of distilled water and the pH of the medium was arranged to 7.4±0.2. Then, the medium was sterilized by using autoclave at 1 atmosphere pressure and 121°C for 15 minutes. After sterilization, 5 ml Mueller Hinton broth was distributed into sterile test tubes.

2.4.4 Preparation of *Corchorus olitorius* L. Extracts for Antimicrobial Activity

Plant extracts were weighed by using the analytical digital balanced. For each of 7 different extracts, 1024 mg extract was weighed into the volumetric flask. All of the

plant extracts was dissolved in 25 ml Dimethyl sulfoxide (DMSO) in order to get a stock solution of 4096 mg/L.

2.4.5 Detection of Antimicrobial Activity of *Corchorus olitorus* L. by Macro Dilution Method

The antimicrobial activities of chloroform, Methanol, Dichloromethane, Acetone, Hexane, Ethyl acetate and Water extracts against *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922and *K. pneumoniae* ATCC 700603 were investigated by macrodilution method.

The concentration of test microorganisms were adjusted to 0,5 Mac Farland ($1.5x10^8$ in Mueller Hinton broth and then diluted 1:100 using Mueller Hinton broth to get a concentration of $1,5x10^6$ cfu/ml for each of the four strains.

Stock solutions (at the concentration of 4096 mg/l) of each of the extracts was diluted 1:16 by using Mueller Hinton broth so that the concentration of the each extract was reduced to 256 mg/l. Then, 5 ml of each extract at 256 mg/l was mixed with 5 ml of each test microorganism that has the concentration of $1,5x10^6$ cfu/ml so that the final concentration of extract became 128 mg/l. Then 1:2 dilution was done for determining the antimicrobial activity of each extract at 64 mg/l. The final DMSO concentration in the final solutions was 3% for extracts at 128 mg/l and 1,5% for extracts at 64 mg/l.

The minimum concentration of the extract that inhibit the growth of each strain was determined to be the Minimum Inhibitory Concentration (MIC) (CLSI, 2006) (Donna M. Hacek, 1999).

2.4.6 Detection of Antimicrobial Activity of *Corchorus olitorius* L. by Agar Well Diffusion Method

S. aureus ATCC 29213, *E. faecalis* ATCC 29212, *E. coli ATCC* 25922 and *K. pneumoniae* ATCC 700603 at the concentration of 1,5x108 cfu/ml (0,5 Mac Farland) was inoculated all over the surface of four Mueller Hinton agar plates (Biomerieux, France). Once the plates had been aseptically dried, wells with 6 mm diameter were bored by using the sterile glass rod. 100 µl of each extract at 128 mg/l, were placed into the wells. The plates were incubated under aerobic atmosphere at 37ºC for 24 hours and the inhibition zone diameter was measured in millimeters using a ruler (Mounyr Balouiri, 2016).

Chapter 3

RESULTS AND DISCUSSION

3.1 Anatomical Structure

Plant material was collected in August 2018 in from the Çayönü Village (Famagusta, North Cyprus). For anatomical structure of the plants, dried and fresh leaves were used. Both types of the plants were cutting with the blades in three different ways. The cross section of the leaf is getting from inner part, outer part and midrib. Also the cross, surface sections and powder of the leaves have been examined under the light microscope using the sartur reagent. The results of the leaves that examined under the microscope were the leaves are dorsi-ventral (bifacial) which means leaves having two surfaces, dorsal and ventral, are called dorsi-ventral. *Corchorus olitorius* L. has a one layered epidermis that found on both side. Outer wall of epidermis cells are thick and the cuticula is thin. Midrib is very prominent with 4 big secretion cells. The mesophyll tissues are divided into two cells which they are palisade and spongy parenchyma cells. The anatomical structure of this leaves are palisade parenchyma is 1- 2 layered, spongy parenchyma is 2-3 layered. Prismatic crystals are abundant and mainly run as one row along the veins.There isn't any anatomical structure analysis about the *Corchorus olitorius* L. to compare the results but there is a similar research about the closely related species which is *Corchorus capsularis* L. In this research, they have observed stomata and 2 layered palisade layer of the mesophyll in exposed leaf as well (Silverio, 2016).

Oxalate crystals are mostly found in plant-derived foods and inhibit the absorption of calcium minerals.Calcium oxalate crystals may accumulate in the kidneys and urinary bladder and this cause stone formation in these organs.On the other hand, calcium oxalate molecules are the most basic formation that causes kidney stones. As a result of the presenting of the prizmatic crystals in the leaves and using of this plant as a meal needs to be investaged further. Stomata are on the lower surface. There are no hairs but some small scabrous cavities are present.

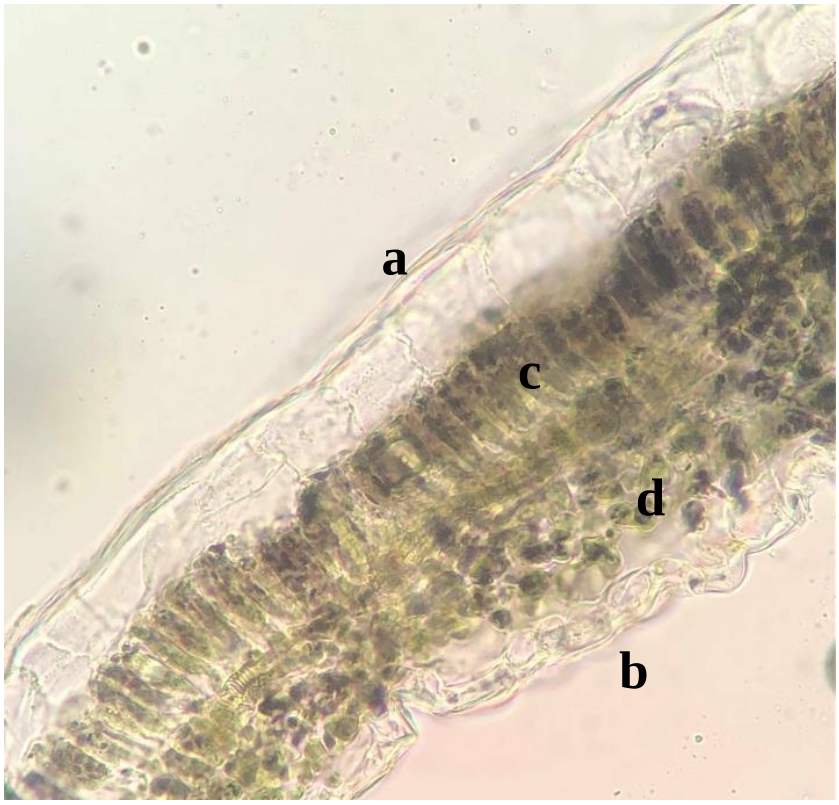

Figure 10: Cross Section of the *Corchorus olitorius* L. Exposed (a) Upper Epidermis (b) Lower Epidermis (c) Palisade Parenchyma and (d) Spongy Parenchyma

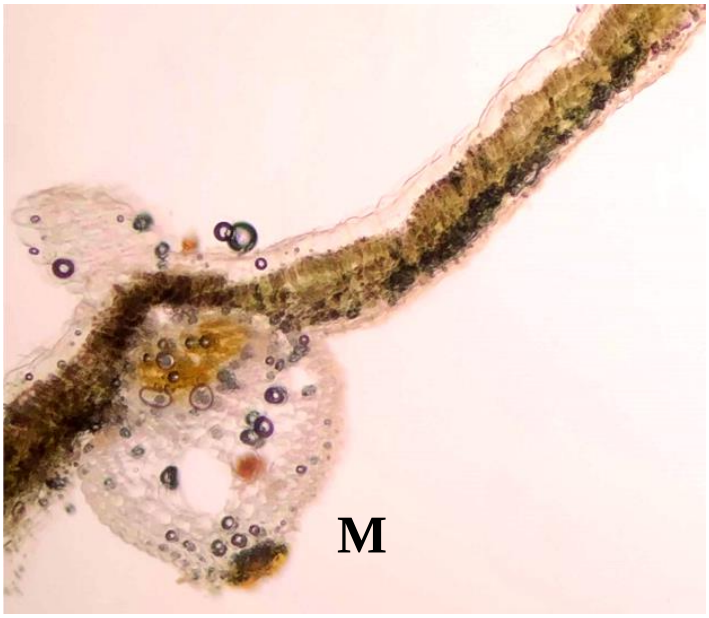

Figure 11: Cross Section of the *Corchorus olitorius* L. From the (M) Midrib

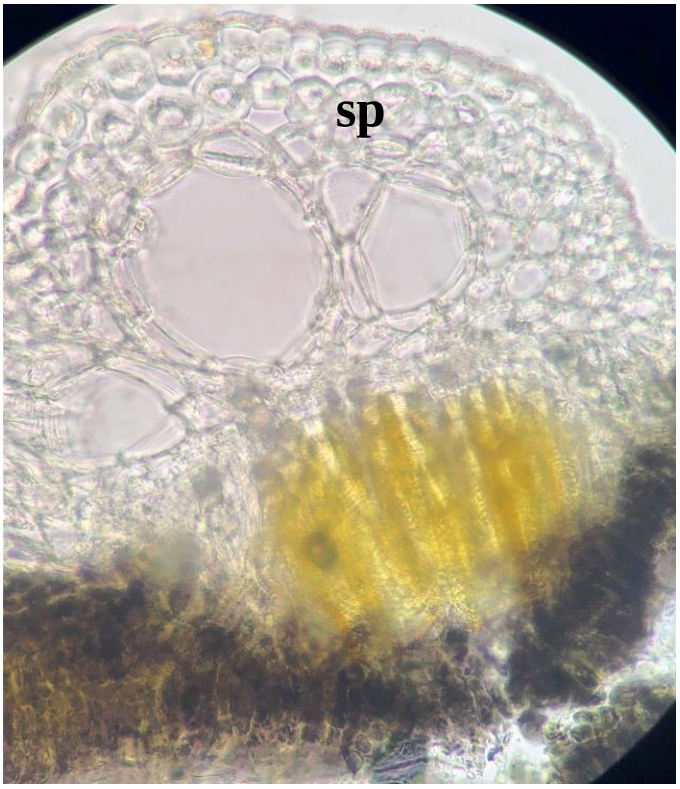

Figure 12: (sp) Spongy Parencyma of the *Corchorus olitorius* L. Leaf

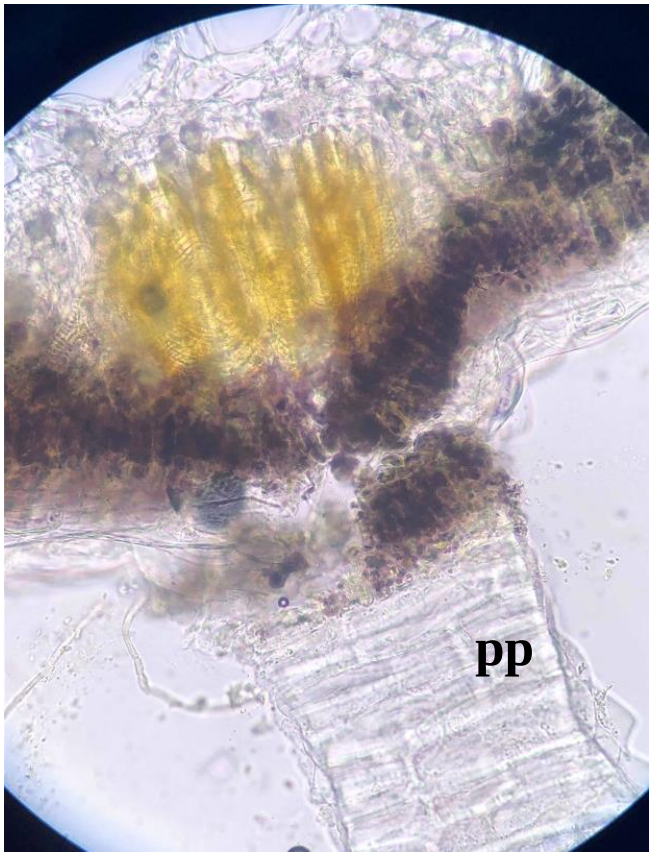

Figure 13: (pp) Palisade Parencyma of the *Corchorus olitorius* L. Leaf

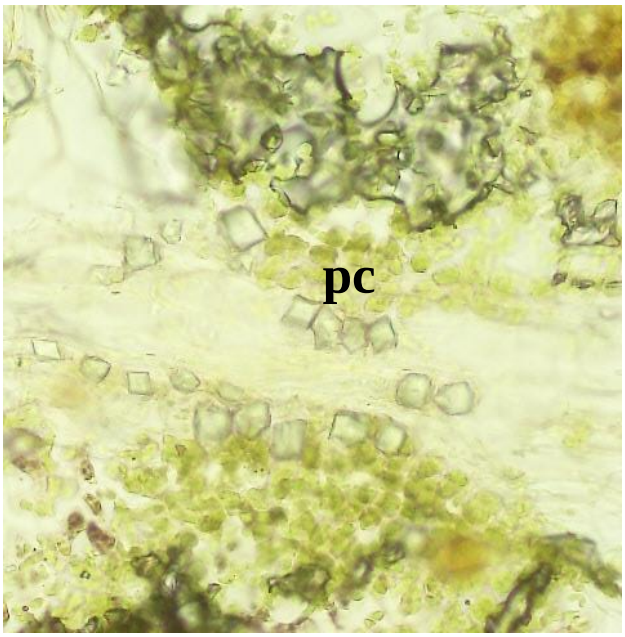

Figure 14: (pc) Prizmatic Crystals of the *Corchorus olitorius* L. Leaf

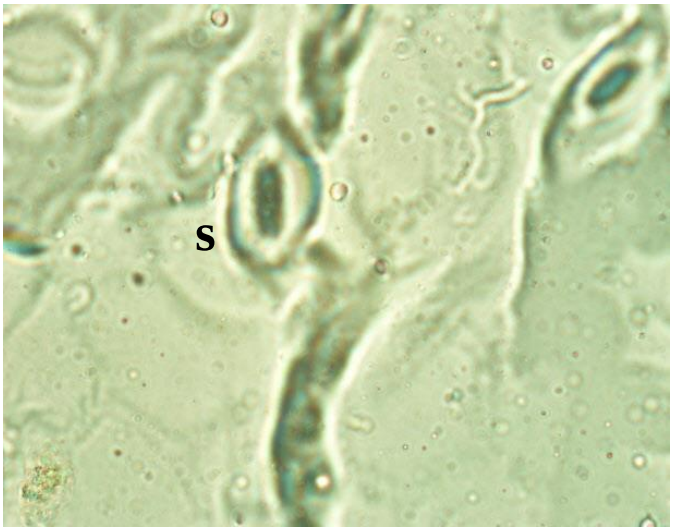

Figure 15: (s) Stomata of the *Corchorus olitorius* L. Leaf

3.2 Anti-Microbial Activity of the *Corchorus olitorius* L. Different Extracts by Macro Dilution Method

As shown in Table 8 and 9, MIC of methanol, chloroform, hexane and water extracts were found to be 128 mg/l for all of the Gram positive and Gram negative strains tested. MIC of dichloromethane extract was also found to be 128 mg/l against *S. aureus*, *E. faecalis* and *E. coli*. However, dichloromethane extract, acetone and ethyl acetate extracts inhibited the growth of *K. pneumoniae* at the concentration of 64 mg/l that deserves the further investigation at the concentrations of 32, 16 and 8 mg/L.

Table 8: Minimum Inhibitory Concentrations of Methanol, Chloroform and Dichloromethane extracts of *Corchorus olitorius* L.

Bacterial Isolates	**Minimum Inhibitory Concentration (mg/L)**		
	Methanol	**Chloroform**	**Dichloromethane**
S. aureus	**128**	**128**	**128**
E. faecalis	**128**	**128**	**128**
K. pneu7monia	**128**	**128**	*****
E. coli	**128**	**128**	**128**

*Will be further investigated

Table 9: Minimum Inhibitory Concentrations of Acetone, Hexane, Ethyl Acetate and Water extracts of *Corchorus olitorius* L.

Bacterial Isolates	Minimum Inhibitory Concentration (mg/L)			
	Acetone	**Hexane**	**Ethyl Acetate**	**Water**
S. aureus	**128**	**128**	**128**	**128**
E. faecalis	**128**	**128**	**128**	**128**
K. pneumonia	*****	**128**	*****	**128**
E. coli	**128**	**128**	**128**	**128**

*Will be further investigated

3.3 Anti-Microbial Activity of the *Corchorus olitorus* L. Different Extracts by Agar Well Diffusion Method

In the agar well diffusion method, none of the extract showed any zone (0 mm) against all strains tested.

There isn't any macro dilution method analysis about the *Corchorus olitorius* L. to compare the results but there is a similar research with agar well diffusion method. In this research, they have observed the methanol and ethyl acetate + water extracts from the *C. olitorius* L. plant which displayed antibacterial activity against the *S. aureus, E. faecalis* and *E. coli*. In our results, agar well diffusion didn't show any zone against the *S. aureus, E. faecalis* and *E. coli* due to differences of the concentrations. Our final concentration was used in this method was 256mg/L. However, in this research they used 100mg/mL (100000 mg/L) as final concentration (Semra Ilhan, 2007).

Chapter 4

CONCLUSION

As a conclusion of the anatomical structure, a large of calcium oxalate crystals have been observed and therefore the use of this plant as a meal needs to be further investigation. Calcium oxalate crystals may accumulate in the kidneys and urinary bladder and this cause stone formation in these organs.On the other hand, calcium oxalate molecules are the most basic formation that causes kidney stones.

Microbiologically, it can be concluded that the leaves extracts of *Corchorus olitorius* possess antibacterial activity. The Minimum Inhibitory Concentrations of all/some extracts were found to be 128 mg/l for all of the Gram positive and Gram negative strains tested. However, dichloromethane extract, acetone and ethyl acetate extracts inhibited the growth of *K. pneumoniae* at the concentration of 64 mg/l that deserves the further investigation at the concentrations of 32, 16 and 8 mg/L. This suggests that antibacterial compounds could be extracted and could be beneficial in the treatment of related diseases.

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