İZMİR KATİP ÇELEBİ UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

OLIVE LEAF POWDER AND BORON REINFORCED POLYPROPYLENE COMPOSITES

M.Sc. THESIS

Hilal KARA

Department of Material Science and Engineering

Thesis Advisor: Assoc. Prof. M. Özgür SEYDİBEYOĞLU

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İZMİR KATİP ÇELEBİ ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ZEYTİN YAPRAĞI TOZU VE BOR KATKILI POLİPROPİLEN KOMPOZİTLERİ

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Hilal KARA, a **M.Sc.** student of **İzmir Katip Çelebi University** student ID Y130111021, successfully defended the **thesis** entitled "**OLIVE LEAF POWDER AND BORON REINFORCED POLYPROPYLENE COMPOSITES**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my family,

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16 March 2016 Hilal KARA

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
cPP	Copolymer
DNA	Deoxyribonucleic Acid
FTIR	Fourier Transform Infrared Spectroscopy
HAA	Haloacetic Acids
HIV	Human Immunodeficiency Virus
OLE	Oleuropein
OLP	Olive Leaf Powder
SEM	Scanning Electron Microscopy
THM	Trihalomethanes
U	Ulexite
UV	Ultraviolet

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OLIVE LEAF POWDER AND BORON REINFORCED POLYPROPYLENE COMPOSITES

SUMMARY

Plastics have a wide application area in our daily life because of the properties such as being inexpensive, lightweight and easy to take shape. Plastics can lead to some problems such as endangering human health and reducing the life of the material, because the plastic surfaces are so available for microorganisms to form a biofim by holding onto the surface easily. Researches for antibacterial materials containing various natural and inorganic substances has been increased and become more important because of that people are often infected by microorganisms such as bacteria, molds, yeasts, and viruses in the environment in which they live. In recent years, increasing demand for antimicrobial materials and widespread use of these materials have necessitated the development of products with high quality through new studies.

Antimicrobial agents must have some important properties such as heat resistance, the solubilization of the plasticizer, compatibility with the polymer, low leakage rate, UV resistance, being harmless to the environment as well as being effective against the microorganisms to be applied in plastics. Polymer composites containing antimicrobial additives are widely used in many areas especially in hospitals, biocompatible implants, toy industry, food production and packing, medical device industry, home appliance industry and construction materials.

Microorganisms cause serious problems in the home appliance industry particularly in washing machines. Biofilm is composed by origin of tap water, human skin and contaminated textile products in detergent drawer and box of washing machines after a certain period of use. Biofilm formation consisted in the washing machine drawers endangers human health, reduces perceptual quality, shortens the machine life and requires constant cleaning and maintenance. This circumstance increases service requirements after-sales of the machine and creates customer dissatisfaction.

In this study, antimicrobial composite materials developed by adding ulexite (boron mineral) and olive leaf powder into the copolymer matrix material have been studied for using in washing machine detergent box and drawer.

In this thesis, antimicrobial agents are mixed as individually (5-10-15%) and jointly (10-15%) with the copolymer material by using a twin-screw extruder and then pelleted. Then the pellets were formed into plates in hot and cold presses by pouring into molds in certain sizes. JIS Z 2801 standard has been implemented to the plates in order to determine the antimicrobial activity. The Aging test was conducted to determine the antimicrobial activity time. Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to the composite plates in order to see structural

features. Mechanical properties were evaluated by tensile testing. Interface morphology was observed via Scanning electron microscope (SEM).

The results showed that the best antimicrobial effect is in the composite materials which ulexite and olive leaf powder are used together. The synergistic effect was created by using the combination of two additives together. But after the aging test, the antimicrobial activity of the composite materials is lost. The causes and possible solution methods were discussed under this study. It is seen in SEM analysis that the size of the ulexite and olive leaf powder is not homogeneous and also the agglomeration has occurred in the produced composites. It is seen that tensile strength of the composites is in closer values with the tensile strength of copolymer but, the elongation at break values were found to be decreased dramatically according to mechanical test results.

ZEYTİN YAPRAĞI TOZU VE BOR KATKILI POLİPROPİLEN KOMPOZİTLERİ

ÖZET

Plastik malzemeler ucuz olmaları, hafif olmaları ve kolay şekil alma gibi özelliklerinden ötürü günlük yaşamımızda oldukça geniş kullanım alanına sahiptirler. Fakat plastik yüzeyi mikroorganizmaların kolayca tutunup biyofilm oluşturmasına olanak tanıdığından, insan sağlığını tehlikeye atma ve malzemenin kullanım ömrünü azaltma gibi sorunlara sebep olmaktadır. İnsanoğlunun bakteriler, küfler, mayalar ve virüsler gibi mikroorganizmalar tarafından yaşadıkları çevrede sık sık enfekte olması çeşitli doğal ve inorganik maddeleri içeren antibakteriyel malzemelerin araştırılmasını yoğunlaştırmıştır. Son yıllarda antimikrobiyal malzemeye olan talebin artması ve bu malzemelerin kullanımının yaygınlaşması, yeni çalışmalar yaparak daha kaliteli ürün geliştirmeyi zorunlu kılmıştır.

Antimikrobiyal maddeler plastiklerde uygulanabilmeleri için mikroorganizmalara karşı etkili olmalarının yanı sıra ısıya dayanıklılık, plastikleştiricilerde çözünebilme, polimer ile uyum, düşük sızma hızı, UV'ye dayanıklılık, çevreye zararsız olma gibi özelliklere de sahip olmalıdırlar. Antimikrobiyal katkılı polimer kompozitler birçok alanda özellikle hastanelerde, biyouyumlu implantlarda, oyuncak endüstrisinde, gıda üretimi ve paketlenmesinde, medikal cihaz endüstrisinde, beyaz eşya sektöründe, yapı elemanlarında yaygın olarak kullanılmaktadır.

Beyaz eşya sektöründe mikroorganizmalar, özellikle çamaşır makinalarında ciddi problemlere sebep olmaktadır. Çamaşır makinaları deterjan çekmece ve kutularında belli bir kullanım süresinden sonra şebeke suyu, insan cildi ve kontamine olmuş tekstil ürünlerinden kaynaklı biyofilm oluşmaktadır. Çamaşır makinaları çekmecelerinde görülen biyofilm oluşumu, insan sağlığını tehlikeye atmakta, algısal kaliteyi düşürmekte, makina ömrünü kısaltmakta, sürekli temizlik ve bakım gerektirmektedir. Bu durum da hem mïşteri memnuniyetsizliği yaratmakta hem de satış sonrası makinanın servis gereksinimini artırmaktadır.

Bu çalışmada kopolimer matriks malzeme içerisine, bor minerali olan üleksit ve zeytin yaprağı tozu ekleyerek antimikrobiyal çamaşır makinası deterjan kutusu ve çekmecesi için kompozit malzeme çalışılmıştır.

Tez çalışmasında antimikrobiyal ajanlar tek tek (%5-10-15) ve kombin (%10-15) halinde kopolimer malzeme ile çift vidalı ektrüder kullanılarak karıştırılmış ve pellet haline getirilmiştir. Daha sonra peletler belirli boyutlarda kalıplara dökülerek sıcak soğuk preste plakalar haline getirilmiştir. Antimikrobiyal etkinliği belirlemek amacıyla plakalara JISZ 2801 standartı uygulanmıştır. Antimikrobiyal etkinlik süresini belirlemek için yaşlandırma testi gerçekleştirilmiştir. Yapısal özellikleri görmek amacıyla kompozit plakalara Fourier Kızılötesi Spektroskopisi (FTIR) analizi yapılmıştır. Mekanik özellikler çekme testi ile değerlendirilmiştir. Taramalı elektron mikroskopisi (SEM) ile arayüz morfolojisi gözlenmiştir.

Sonuçlar, en iyi aktimikrobiyal etkinin üleksit ve zeytin yaprağı tozunun birlikte kullanıldığı kompozit malzemelerde olduğunu göstermiştir. İki katkı malzemesinin birlikte kullanılmasıyla sinerjik bir etki yaratılmıştır. Fakat yaşlandırma testi sonrasında üretilen kompozit malzemelerin antimikrobiyal etkinliği kaybolmuş olup, çalışma kapsamında nedenleri ve olası çözüm yöntemleri tartışılmıştır. SEM analizi ile üleksit ve zeytin yaprağı tozu boyutlarının homojen olmadığı ve ayrıca üretilen kompozitlerde topaklanmanın meydana geldiği görülmüştür. Mekanik test sonuçlarına göre kompozitlerin çekme mukavemeti kopolimerin çekme mukavemeti ile yakın değerlerde olduğu fakat kopma uzama değerlerinin ciddi anlamda düştüğü görülmüştür.

1. INTRODUCTION

1.1 What is Microorganism

Microorganisms are kind of living creatures which are too small to be seen without a microscope, they are not visible to the naked eye [1]. Microorganisms make up a vast piece of the planet's living material and assume a noteworthy part in keeping up the Earth's biological system [2]. Microorganisms can be found in the body, air, soil and the all type of surfaces. They are able to proliferate quickly if appropriate conditions are met.

Microorganisms can be defined with six different category: bacteria, archaea, protozoa, algae, fungi, and viruses. Every type has a characteristic cell piece, morphology, mean of motivity, and replication. Microorganisms has advantages in creating oxygen, decomposing natural material, giving supplements to plants, and keeping up human well being, however some can be pathogenic and cause ailments in humans and plants [3]. The most important dangerous microorganisms for plastics are bacteria and fungi. Bacteria need to have optimum development conditions comprising enough food source with a sufficient humidity and temperature. In general, bacteria cause fetid odor; fungi cause biodegradation and staining [4]. It is important to understand how infection process is occurred by pathogens.

Infection is the process whereby the pathogen enters and detrimentally colonizes into its host. There are two essential steps in this process including:

1- entrance of the pathogen into the host, and

2- colonization of the pathogen within the host.

The achievement of these two stages will prompt the consequence of dynamic lesion [5]. Adherence of pathogenic microorganisms to host tissues is accepted to be the starting connection in the middle of pathogens and hosts, providing the colonization and consequent scattering or cell invasion [6]. The adherence likewise is a vital step in host cell slaughtering and poison conveyance by the pathogens. Also, it is the first step for composition of biofilm, a destructive structure which is observed to be

included in wide mixed bag irresistible ailments and connected with anti-infection treatment disappointment or antimicrobial resistance in numerous microbial infections [7, 8]. Therefore, the inhibition of microbial adherence to host tissues is the new perfect focus on that may prompt the improvement of new methodologies for the treatment or prophylaxis in irresistible diseases [8].

Table 1.1: Names of microorganisms that some of are pathogenic or nonpathogenic [9].

Name of Microorganism	**Pathogenic Nonpathogenic**	**Effects**
Bacillus subtilis	Generally nonpathogenic	Causes deterioration of food
Escheria coli	Low-Pathogenic	Causes food spoilage and urinary infections
Klebsiella pneumonia	Pathogenic	Causes pneumonia and urinary tract infections
Pseudomonas aeuroginosa	Low-Pathogenic	Causes various infection
Proteus vulgaris	Low-Pathogenic	Causes inflammation
Staphylococcus epidermis	Low-Pathogenic	Causes surgical wound infection
Staphylococcus aureus	Pathogenic	Causes toxic shock, pus collection, abscess, fibrin clots, endocarditis

1.2 Biofilms

Biofilms are defined as a community of microorganisms that live in a gel-like layer has polymeric structure produced by themselves via adhering to a surface [10]. In respect to another definition of the biofilm is an activity that bacteria embed into an organic polymer matrix [11].

Extracellular matrix [EPS] comprised by polysaccharide, protein, DNA and water provides that biofilm cells can adsorb. Bacteria hold tightly to the surface firstly form microcolonies by multiplying and then microcolonies form biofilm surface by growing and expanding. EPS production is required that organism adsorbs irreversibly to the surface and an indication of the biofilm forming [12]. EPS also protect the organism against to osmotic stress, the phages waste, toxic compounds and antibiotics [13]. EPS constitutes 75-90% of the mass of a ripe biofilm [12].

Biofilm formation is not a random event occurs in the way that bacteria adsorb to a particular surface by just coming together and they continued to live together with other species on the surface. In order to coordinate their activities, many organisms use small diffusible molecules while sending signal to each other. Bacteria can measure the intensity of the signal they produced, can feel the amount of other microorganisms in the environment and can transmit the data to the others thanks to the process called as "Quorum Sensing [QS] [14, 15].

In other words, bacteria determine the density of bacterial population in the environment via QS. Bacteria adsorbed to a surface secrete a molecule that gives 'I'm here' message. While increasing of the number of bacteria adsorbed to the surface, the local concentration of this signal increases. Along with the increasing of this signal molecule concentration, a series of biofilm formation processes are initiated. In this matter, bacteria within the biofilm communicate via precursors has low molecular weight and intercellular characteristic [15, 16].

Biofilm forms when bacteria adsorb to surfaces in moist environments by discharging a slimy, glue-like substance. Sites for biofilm formation comprise a wide range of surfaces: natural materials above and below ground, plastics, metals, medical implant materials even plant and body tissue. Wherever finding a combination of moisture, nutrients and a surface, biofilm formulation can be likely found [17].

As in the case of biofilm formation could occur on living cells as in vivo and inanimate surfaces, there is a high possibility that we may exposure to microorganisms within the day. There is a strong relationship between the biofilm-forming organisms and infectious diseases. These microorganisms especially can cause diseases such as heart inner membrane inflammation, periodontitis, cystic fibrosis [18].

Therefore, use of antimicrobial materials is becoming very common day by day to prevent biofilm formation and lots of studies has been performed in this regard of the subject.

Biofilm formation can be studied in five steps. First, cells hold the surface alternately by interacting in close surfaces. While alternately hanging on the surface, they explored whether there are the nutrients that will allow them to live on the surface. Then they hold on to the surface irreversibly with the cell organelles through the dipole-dipole interaction which is short-range interactions with the surface, hydrophobic interactions, ion-dipole interaction, ionic and covalent interactions and hydrogen bonds. Bacteria attached to the substrate is developed and form colonies. Microcolonies grow over time and are transformed into high qualified structures. Mature biofilm is formed at this stage. A single bacteria or bacterial clusters spread to environment by breaking off from biofilm surfaces at the stage of deletion from biofilm development or separation [19].

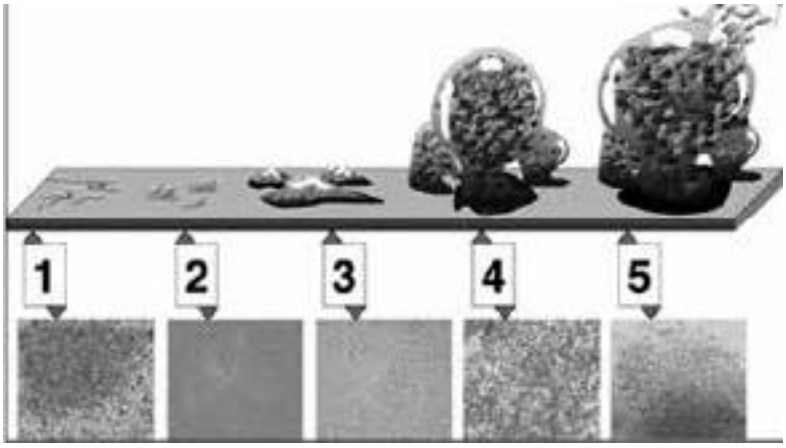

Figure 1.1: Stages of biofilm formation [20].

Explanation of the stages defined in the Figure 1.1:

1. Reversible adsorption

2. Irreversible adsorption
3. Colony development
4. Biofilm maturation
5. Separation of biofilm cells by breaking off

Table 1.2: Microorganisms effected on biofilm infections [21].

Infections or Diseases	**Microorganism**
Tooth decay	Streptococci
Otitis media	Untypeable *Haemophilus influenzae*
Chronic tonsillitis	A variety of aerobic and anaerobic bacteria
Endocarditis	*Viridans group streptococci*, staphylococci
Cystic fibrosis pneumonia	*Pseudomonas aeruginosa*, *Burkholderia cepacia*
Biliary tract infections	Enteric bacteria
Infectious kidney stones	Gram-negative bacilli
Bacterial prostatitis	*Escherichia coli* and other gram-negative bacteria
Foreign Body infections	
Central venous catheter	KNS, *Staphylococcus aureus*, enterococci
Urethral catheters	*Escherichia coli, Candida spp.*, KNS
Artificial heart valves	KNS, *Staphylococcus aureus*, streptococci
Coronary stents	*Staphylococcus aureus*, KNS, *Pseudomonas aeruginosa*, *Candida app.*
Erythosine dialysis catheters	*Staphylococcus aureus, Pseudomonas aeruginosa* and other gram-negative bacteria

Orthopedic prostheses	Staphylococci, *Staphylococcus pneumoniae,* other streptococci, *Propionibacterium acnes*
Endotracheal tubes	Enteric gram-negative bacilli
Breast implants	Staphylococci, *Escherichia coli, Peptostreptococcus spp., Clostridum perfringens*
Cochlear implants	*Staphylococcus aureus, Pseudomonas aeruginosa,* staphylococci, *Neisseria meningtidis,* fungi

1.3 Antimicrobials

Antimicrobial is a general name for natural or synthetic compounds that contains concentrations restrain the development of or destroy microorganisms completely [22].

Antimicrobials are compounds present in or added to plastics, foods, food processing environments, food packaging or all human skin contact surfaces, or to restrain microbial development or annihilate microorganisms.

Besides the effectiveness of antimicrobials, antimicrobial resistance is an important global issue. Antimicrobial resistance in bacteria is a growing problem for antimicrobial agents that are being used in medical, agriculture and other related industries. With an increase in the prevalence of microbial resistance to classic antiseptics and antibiotics, antimicrobial agents derived from natural origin compounds [23].

In this matter, it is needed to define and discuss characteristics of antimicrobials [derived from plants, microorganisms, animal sources and natural sources-mine], as well as why there is a need for these compounds. Antimicrobials are categorized according to effect levels to the microorganisms, besides origin compounds they derived from. According to the effect levels of antimicrobial agents to microorganisms, they are categorized into 2 classes as biocide and biostatic. Biocides

are antimicrobial agents that has lethal effect on microorganisms and antimicrobial agents that has inhibitory effect on microorganisms called as biostatic [1, 24].

1.3.1 Characteristics of antimicrobials

1.3.1.1 Antibacterials

Antibacterial agents are referred as substances that inhibit the growth and development of bacteria [25].

Bacteriostatic are called as substances which inhibit the proliferation of bacteria. After removal of the bacteriostatic from the environment then bacteria are begun to reproduction [26].

Bacteriocidal is called as substances that can kill bacteria. The difference of the effect between bacteriocidal and bacteriostatic is that this is irrevocable transaction. Even a completely killed organism is removed from the agent, it is no longer reproducible. In some cases it has the melter effect on the active cell. In some other cases, cells may remain intact and even they may remain active form with respect to metabolic [26].

The difference between the bacteriostatic and bactericidal agents are not obvious, because it can be a bacteriostatic agent in a low concentration or it can be bactericidal at higher concentrations. Bacteriostatic agents should exist constantly on the environment to be effective, if it is removed or the activity is neutralized, organisms may show growth in case environment conditions are convenient [27].

Microorganisms which cause problems due to existing in specific surface area, they are pathogenic bacteria rather than fungal organism. Therefore, biocide should be highly active against bacteria [28].

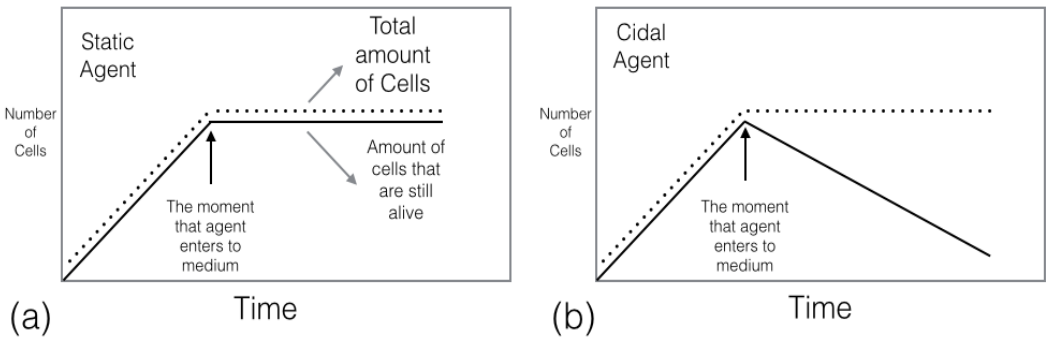

Figure 1.2: Change in quantity of bacteria after treatment with antibacterial agent a: "-static" agent, b: "-cidal" agent [29].

To get the required antibacterial activity, antibacterial agents can be used individually or in combination depending on the application conditions and requirements [25].

Action mechanisms of antibacterial agents can be analyzed as follows [30];

1- Those that prevent synthesis of cell wall.
2- Those that affect the cytoplasmic membrane
3- Those that prevent protein synthesis.
4- Those that disrupt the function of nucleic acids and synthesis.
5- Those that affect various of cell functions.

1.3.1.2 Antifungals

Fungal infections cause continuous and serious effects on human health and life. These fungal infections in humans can be classified into three groups [31]:

1. allergic reactions to fungal proteins,
2. toxic reactions to toxins present in certain fungi
3. infections [mycoses].

An antifungal agent is a drug that selectively eliminates fungal pathogens from a host. Their activity proceeds with minimum toxicity to the host [32].

Substances and extracts of different natural resources especially plants have strong ability to control the fungal infections and spoilage [33]. So they are used as antifungal agents to prevent many diseases. Most antifungal agents affects either the formation or the function of ergosterol, an important component of the fungal cell membrane [34].

Antifungals are classified in three groups depending on their site of action:

1. Azoles: inhibits the synthesis of ergosterol [the main fungal sterol]
2. Polyenes: interacts with fungal membrane sterols physicochemically
3. 5-fluorocytosine: inhibits macromolecular synthesis.

Antifungal agents reacts with different types of mechanisms including; alteration in drug target, alteration in sterol biosynthesis, reduction in the intercellular

concentration of target enzyme, and overexpression of the antifungal drug target [34].

The number of agents used to inhibit fungal infections has increased by 30% since the turn of the millennium.The recent development of new antifungal agents has significantly contributed to the successful treatment of fungal diseases [35].

1.3.1.3 Antiviral

Viral diseases, including emerging and chronic viruses, are an increasing worldwide health concern that causes to discover of new antiviral agents from plants more urgency than in the past. Although there are a number of plant-origin medicines known to have antimicrobial property, their antiviral properties have not been studied yet [36].

Viruses utilize the host cell environment to propagate new viruses unlike bacterial cells. Each virus has its own unique configuration of surface molecules. These surface molecules enables viruses to enter into hosts by precisely fitting the molecules on their surfaces to those on the membranes of target cells. Viruses can be easily adapted to all forms of life resulting in widespread diseases in humans, livestock and plants [36].

There have been many traditional medicinal plants studied and reported as strong antiviral plants. The extracts of 40 different plant species have been used in traditional medicine and were investigated for antiviral activity against many viruses [37].

1.3.1.4 Antiprotozoal

Protozoa are responsible for a number of serious tropical diseases including amoebiasis,leishmaniasis, malaria, and trypanosomiasis [38].

Human protozoal infections are common in worldwide and pathogens of protozoal can cause both symptomatic and asymptomatic infections and affect nearly all humans. The long term studies of antiprotozoal chemotherapy show the effect of diseases resulting from these pathogens and revealed the modern antibiotic era. Many antiprotozoal agents are used currently in many cases. There have been increasing level of interest in protozoal infections since 1990s because of

international travel and immigration, a growing awareness of antiprotozoal drug resistance and the significance of acute and recrudescent protozoal infections in immunosuppressed hosts [39].

1.3.2. Mechanism of antimicrobial action

An antimicrobial agent has particular inhibitory action and mechanisms against each microorganism. In this way, the selection of antimicrobial agents is dependent on their adequacy against a target microorganism. There is no "Specific shot" antimicrobial agent that will work successfully against all spoilage and pathogenic microorganisms because all antimicrobial agents have diverse exercises that influence microorganisms differently. This is because of the characteristic antimicrobial systems and the distinctions in physiology of the microorganisms. Basic

categorization of microorganisms may be very useful to choose particular antimicrobial agents, which may be sorted by oxygen requirement [aerobes or anaerobes], cell-wall composition [gram-positive and gram-negative], development stage [spores or vegetative cells], ideal growth temperature [thermophilic, mesophilic, or psychrotrophic], or corrosive/osmosis resistance [40].

1.3.2.1 Effect on nucleic acid

Some physical or chemical agents show their effect by DNA damage. Ionizing radiation, ultraviolet light and chemicals effective to DNA exist in this group. Chemicals including alkylating agents and other compounds bind to purines and pyrimidine bases by strong links, so they cause DNA adhesion and generation of cross-links between chains. Radiation damages to DNA in several ways. For example, ultraviolet light cause pyrimidine dimers forming by formation of crosslinks between adjacent pyrimidine bases in one or two chain. Ionizing radiation creates chain break in single or double chains. DNA damages formed by radiation or chemical action cause cell death by affecting DNA copying in the cell [41, 42].

1.3.2.2 Effect on protein

Proteins are folded due to weak bonds and three-dimensional shapes like hydrogen bonds, hydrophobic interactions, intramolecular strict disulfide bonds or ionic interactions. This is called as tertiary structure of the protein. This structure can be

easily disrupted by various physical and chemical agents and therefore proteins may become nonfunctional. Damaging the tertiary structure of the protein is called as breaking the nature of the protein [41, 42].

1.3.2.3 Impact on the cell wall or membrane

Bacterial cell membrane constitutes a selective barrier that is permeable to some of substances and impermeable to some. Some of substances concentrate inside by transporting actively from the membrane. Membranes also contain the enzymes involved in the production of cell membrane element. Substances accumulating on the cell surface can interfere the normal function of the membrane by disrupting the physical and chemical properties of the membrane, thereby causing cell death or suppression [42].

The cell wall is a rigid layer that protects the cell against osmosis degradation. Thus the substances damaging the cell wall or inhibit the synthesis of the cell wall cause degradation of the cell [42, 43].

1.3.2.4 Impact on free sulfhydryl group

Some of proteins which includes cysteine and has enzyme characterictics have side chains end with sulfhydryl groups. Coenzymes such as coenzyme-A and dihidrolipoat also carry free sulfhydryl groups. Therefore such enzymes and co-enzymes have no functions unless sulfhydryl groups are free and reduced. Oxidizing agents, disturbs the metabolism by forming disulfide bonds between adjacent sulfhydryl groups. Oxidizing agents and metals cause widespread damage, because of presence of so many sulfhydryl enzymes in the cell [42].

1.3.2.5 Chemical antagonism

When a chemical agent decomposes a chemical reaction between the specific enzyme and its substrate, it is called as chemical antagonism. Chemical agent blocks binding of the substrate by linking with a part of holoenzyme. Antagonism chemical agents connects o the enzyme's active region due to its chemical affinity. Enzyme fulfills its function by showing an affinity to the natural substrate. Therefore, any compound with similar substrate structural outline may show affinity to the enzyme. Many holoenzyme carry mineral ions forming bridges between enzyme-coenzyme or

enzyme and its substrate. Chemicals easily connect these minerals can also prevent the binding of coenzyme or substrate [42].

1.3.3 Types of antimicrobials

1.3.3.1 Natural antimicrobial agents

Substances used to control microbial growth are referred to as antimicrobials. However in recent years, synthetic origin substances used for antimicrobial purpose caused unwanted and unforeseen side effects in the human body. Besides this, resistance of microorganisms to these antimicrobials has led to the search for alternative natural antimicrobial agent [44].

Antimicrobials derived from plants

Nowadays, while new drugs are rapidly being developed in the world, on the other hand, infections occured due to rapidly arised drug resistance of microorganisms are reported and the extent of this problem is growing [45].

On the other hand, microorganisms are not able to gain resistance against to plants and herbal products which have antimicrobial activity. The reason is that drugs which are synthetically produced comprise only an active substances. Whereas it makes complicated that bacteria develop "multiple" resistance to active substances due to active substances in plants are found as complex structures with other substances. Therefore, in recent years scientists have focused their work to investigate the antimicrobial activity of plants [46].

The healing effect of the plants are derived from chemicals and different combinations of these chemicals called as secondary metabolites that are found in their natures. Secondary metabolites are consisted from primary metabolites [amino acids, simple character lipids and fats, simple sugars] which are essentials for vital activities. It is occured by enzymatic means [47].

12,000 of these compounds that are produced as secondary metabolites could have been isolated so far and this number constitutes only 10% of all the aromatic compounds. The majority of these compounds is necessary for the defense system of plants. Terpenes, quinones and tannins played a role in the formation of odor and pigment are used in antimicrobial research [23, 48].

Table 1.3: Some plant components having antimicrobial activity [49]

Class	**Subclass**	**Examples**	**Mechanism**
Phenolics	Simple phenols	Catesol Epikatesin	loss of substrate destruction of membrane
	Phenolic acids	Cinnamic acid	?
	Quinones	Hypericin	Binding to the adhesins Cell wall complex Enzyme inactivation
	Flavonoids	Chrysin	Binding to the adhesins
	Flavones	Abisinon	Cell wall complex Enzyme inactivation Inhibition of HIV reverse transcriptase
	Flavonoids	Totarol	?
	Tannins	Ellagitanin	Binding to proteins Binding to the adhesins Enzyme inactivation Loss of substrate Cell wall complex Destruction of membrane Complex of metal-ion
	Coumarins	Warfarin	Interaction with eukaryotic DNA [antiviral activity]
Terpenes **Essential oils**	-	Capsaicin	Destruction of membrane
Alkaloids	-	Berberine	Intercalation with the

		Piperine	cell wall or DNA
Lectins and Polypeptides	-	Mannose-specific agglutinin	Blocking of viral fusion or adsorption
	-	Falksatin	Disulfide bridge formation
Polyacetylenes	-	8s-heptadeca-2[Z],9[Z]-diene-4,9-diyne-1,8-diol	?

So how do these plant compounds having antimicrobial activity interact with microorganisms? Several hypotheses have been proposed regarding the response of this question. According to researchers, natural compounds in the cells affect the biochemical processes of cells directly or indirectly and impairs the physicochemical integrity. Terpenes particularly that have hydrophobic structure damage integrity of the cell wall through the interaction with the cell wall. Hydrophobic property of terpenes leads aggregation of the lipids and increase membrane permeability. Naturally, degradation of physicochemical structure will cause movement of the proton, electron flow in the cell. Thus it causes the coagulation of the cell contents. It is known that antimicrobial compounds also affect the proteins found in the cell wall [48].

The first process to determine the antimicrobial activity in plants is the extraction of active ingredients. Extraction is the process of separating the antimicrobial active substances in the plant tissue by using various solvents [50].

Phytochemicals showing antimicrobial activity can be studied under several main groups.

Simple phenols and phenolic acids: One of the simplest bioactive phytochemicals containing one phenolic ring. It is considered that binding site and the number of

hydroxyl groups on phenol groups are associated with toxicity over microorganisms. It is proved that increasing of hydroxylation increases the toxicity. In addition, some researchers have identified that a higher proportion of oxidized phenols are better inhibitor [23].

Quinones: Quinones has aromatic ring containing two ketone. These compounds cause inactivation and function loss of proteins by forming an irreversible complex with nucleophilic amino acids. Potential antimicrobial effect of quinones has wide range. The targets in microbial cells are cell wall polypeptides and enzymes with membrane dependent [23].

Flavones, flavonols and flavonoids: Flavones are phenolic structures containing a carbonyl group. flavonol is formed by adding 3-hydroxyl group on this structure. Flavonoids are hydroxylated phenolic compounds. These compounds are produced by plants in response to microbial infection. Therefore, it is not surprising that they are not effective against most of microorganisms in vitro medium. Antimicrobial activity of these compounds is effective due to their ability of forming complex with the cell wall of bacteria and soluble proteins out of the cell [23].

Tannins: It is is the general name of a group of polymeric phenolic compounds that have property of blood coagulation, used for tannery and known with the acrid taste. They are usually found in bark, wood, leaf, fruits and roots of many plants. Molecular weights range between 500-3000 dalton. They can be analyzed within two groups; hydrolyzable tannins and condensed tannins. While hydrolyzable tannins are gallic acid based, condensed tannins are derived from 17 flavonoid monomers [Cowan, 1999]. These compounds provide their antimicrobial effect by destroying the electronic delivery systems in the cell membranes [23, 51].

Coumarins: They are phenolic compounds formed by the fusion of benzene and α-pyrrol ring. They are known with their antithrombotic, anti-inflammatory, anti-viral and vasodilator activities. It has been identified that compounds in this group stimulate macrophages [23].

Terpenoits and essential oils: These oils known as terpenes are secondary metabolites consisting of isoprene structures. Classification of terpenes occured by growth of chains that are consisting of isoprene units are hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes and tetraterpenes. These

compounds are active against bacteria, fungi, viruses and protozoa. Although antimicrobial activity mechanism of the terpenes has not been understood yet, it is estimated that it is effective by penetrating into lipophilic compounds found in cell membrane [23].

Alkaloids: Alkaloids contain a heterocyclic ring and the nitrogen atom. "Morphine" which is the first recognized the alkaloid, was isolated from the plant of Papaver somniferum [opium poppy] in 1805. Codeine and heroin are morphine derivatives. It is one of the wide ranged classes with 6500 compounds among secondary metabolites. Natural and modified forms of alkaloids are still used today in modern medicine. Berberine is an alkaloid known with its antimicrobial activity. It is known that it has effect on inhibition of replication by penetrating into DNA chains. It is likely available to use in the treatment of AIDS, Because Berberine inhibits HIV-1 reverse transcription [52].

Table 1.4: Plant by-products as antimicrobials [53].

By-products	**Major component**	**Target organisms**	**References**
Pomegranate fruit peels	Phenolics and flavonoids	*L. monocytogenes*, *S. aureus*, *E. coli*,*Yersinia enterocolitica*, *P. fluorescens*	[54, 55]
Pomegranate juice byproducts	Phenolics, flavonoids, tannins	MRSA ATCC 43300 and *E. coli* ATCC 35218	[56]
Apple peels	Polyphenolic compounds	*S. aureus*, *P. fluorescens*	[54]
Almond skin extracts	Polyphenols	*S. aureus*, *L. monocytogenes*	[57]
Coconut husk	Phytochemica	*L. monocytogenes*,	[58]

	l including phenolics and tannins	*S. aureus* and. *cholera*	
Green tea waste	Tannins	*S. aureus*, *E. coli* *L. monocytogenes*, *Bacillus coagulans*,*Shigell a flexneri*	[59]
Acorn, chestnut, and persimmon hull	Tannins	*S. aureus*, *E. coli* *L. monocytogenes* *B. coagulans*, *S. flexneri*	[59]
Tomato seeds	Metabolites such as fatty acids, carotenoids, saponins, phenolic compounds	*S. aureus*, *S. epidermidis*, *Micrococcus luteus*, *E. faecalis*, *B. cereus*, *Candida albicans*	[60]
Quince fruit peel	Polyphenolic compounds such as chlorogenic acid, catechin, quercetin and kaempferol	*S. aureu*, *P. aeruginosa*, *E. coli*,*C. albicans*	[61]
Potato peels	Chlorogenic, caffeic, gallic, and	Bacteriostatic effect on *E. coli* and *S.*	[62]

	protocatechuic acids	*typhimurium*	
Walnut green husk	Antioxidants such as phenolic compounds	*S. aureus, B. cereus, B. subtilis*	[63]
Mango seed kernel extract	Phenolic compounds, saturated fatty acids, mono-unsaturated oleic acid, tocopherols, squalene, and different sterol fractions	Inhibited total bacterial count, coliforms, and *E. Coli*	[64]
Peels, seeds, and pulp of mexican lime	Phytochemicals: flavononeS, polymethoxylated flavones, tannins	*E. coli* O157:H7, *S.typhimurium,Shigella sonnei*	[65]
Grape pomace	Phenolic acids, flavonoids, stilbenes	*S. aureus*, *Salmonella*, Enterococci, Total aerobic mesophilic and psychrotrophic bacteria, yeasts and molds	[66]

Olive pomace	Phenolic compounds including oleocanthal, deoxyloganic acid lauryl ester	*E. coli* O157:H7, *S. enterica*,*L. monocytogenes*, and *S. aureus*	[67]
Beet root pomace extract	Phenolics, flavonoids betacyanins, betaxanthins	*S. aureus*, *B. cereus E. coli*,*P. aeruginosa*	[68]
Buckwheat hull extracts	Phenolics, flavonoids, antioxidants comprising tocopherols, rutin, quercetin derivatives	Gram-positive [*B. cereus*, *S. aureus*,*Enterococcus faecalis*] and Gram-negative bacteria [*SalmonellaCholeraesuis*, *E. coli* and *Proteus mirabillis*]	[69]
Legume hulls [*Vigna radiate*,*Cicer arietinum*and *Cajanus cajan*]	Polyphenolic compounds, flavonoids	*B. cereus*, *S. aureus*	[70]
Grapefruit seed extracts	Phenolic compounds such as catechins, epicatechin,	*Pesudomonas* spp.	[71]

	epocatechin-3-O-gallate, dimeric, trimeric and tetrameric procyanidins		
Oriental mustard [*Brassica juncea*L.] seed meal extracts	Phenolic compounds [sinapic acid and several sinapoyl conjugates]	*B. subtilis*, *E. coli*, *L. monocytogenes*,*Pseudomonas fluorescens*, and*S. Aureus*	[72]
Coffee pulp	Polyphenols such as flavan-3-ols, hydroxycinnamic acids, flavonols and anthocyanidins	Total viable count, coliform, *E. coli*, and fungal count	[73-75]

Olive leaf

Olive is a member of Oleaceae family. There are many species of this family and oil is produced from some of these. These species connected to the Olea genus are usually grown in regions of subtropical and tropical climates and in the regions dominated by Mediterranean climate. Olive has 27 types and around 600 varieties in the world [76].

In terms of olive production, Turkey is one of the important olive producers in the European Union and in the world. Turkey is second country in the world in the production of table olive and is in 4th position in production of olive oil. World production of olives is around 20 million tons. Turkey has a share of 16.7% in olive production. Turkey is also in the 4th rank in terms of the parameters such as number of trees, field presence and manufacturing [77].

Olive tree is rich in phenolic compounds have important biological properties. Phenolic substances contained in olives are mainly phenolic glycosides such as oleuropein, verbascoside, ligrosit and flavonoids, flavonol glycosides, anthocyanins and its glycosides, phenolic acids and other components [78].

The most important one of these phenolic compounds is oleuropein. The structure of this compound discovered for the first time in 1908 by Bourquelot and Vintilesco were identified in 1960. OLE naturally presents in olive leaves and fruits. The main reason of the bitterness felt in olive is OLE and this bitterness taste prevents the expendability of olives soon after the harvest. The main reason for the long life of olive trees is this particular phenolic compounds [79-82].

Table 1.5: Phenolic compounds and ratios in olive leaf extract [83]

	Phenolic Compounds	**Ratio**
Oleropoesids	Oleuropein	24,54
	Verbascoside	1,11
Flavones	Luteolin-7-glukosid	1,38
	Apigenin-7-glukosid	1,37
	Diosmetin-7-glukosid	0,54
	Luteolin	0,21
	Diosmetin	0,05
Flavanol	Rutin	0,05
	Catechins	0,04
	Tyrosol	0,71
Flavan-3-ol	Hydroxytyrosol	1,46
	Vanillin	0,34
Phenols	Vanillic acid	0,05

	Caffeic acid	0,63

Oleuropein decomposes to glucose and oleuropein aglycone with ß-glucosidase enzyme. Hydroxytyrosol composed after hydrolysis is highly bioactive phenolic molecule. The structure of the aglycone enters to elenolic ring and enables increasing of reactions such as decarboxylation, methylation and oxidation and creates new phenyl aglycone structure. In the enzymatic hydrolysis method, hydroxytyrosol and elenolic acid are composed with the effects of esterase enzyme after decomposition of glucose and oleuropein to aglycone with beta-glucosidase enzyme activity [84, 85].

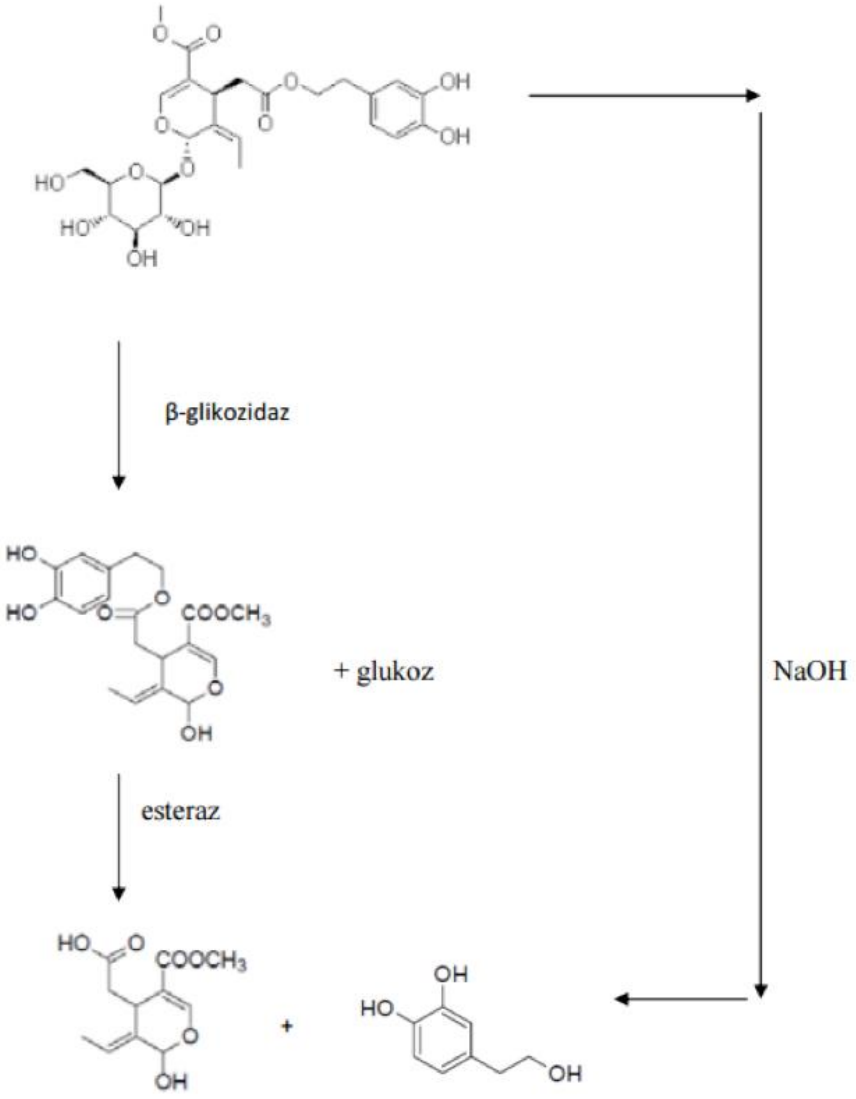

Figure 1.3: Hydrolysis of oleuropein with the beta-glucosidase enzyme [86].

Biological activities of oleuropein [87-90]:

• Antimicrobial
• Antioxidative
• Antiatherogenic
• High antioxidant potential
• Anti-inflammatory effect [by inhibiting the 5-lipoxygenase enzyme]
• Hypotensive activity [blood pressure lowering]
• Cardioprotective [inhibition of LDL oxidation and platelet cell aggregation]
• Hypoglycemia [blood sugar lowering]
• Antihypertensive [drugs used in vasodilators-hypertension treatment]
• Antiviral [even effective against HIV]
• Cytostatic [against McCoy cell]
• Molluscicidal [toxic effects against snails]
• Endocrinal-hormonal
• Enzyme modulator

Antimicrobial properties:

Antimicrobial compounds are compsed as a result of the hydrolysis of oleuropein. Although the effect mechanism of antimicrobial compounds has not fully clarified, it is indicated that phenolic compounds are capable of denaturing proteins and affect the cell membrane permeability negatively. In general, these compounds classified as surface active agents carry out their antimicrobial activities, by damaging cell membranes or by crashing cell peptidoglycan; causing leakage of the cytoplasm compounds such as proteins, inorganic phosphate, glutamate or potassium [82, 91-93].

In many studies conducted on this subject matter, it is stated that phenolic glycoside oleuropein and degradation products have the inhibitory effect on *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Lactobacillus plantarum, Moraxella catarrhalis, Pseudomonas frag, Salmonella enteritidis, Salmonella typhi, Staphylococcus aureus, Staphylococcus carnosus, Vibrio parahaemolyticus, Vibrio cholerae, Vibrio alginolyticus* and molds [92-96].

It has been proven in USA that OLE shows antiviral activity against mononucleosis herpes, hepatitis viruses, rotavirus, bovine virus, parvovirus in dogs and leukemia virus in cats [82, 97].

In another study, phenolic compounds present in aqueous extracts of powdered olive leaves were analyzed using HPLC / DAD and antimicrobial properties have been investigated. It is reported that the various concentrations of obtained extract has inhibit effect on microorganisms in order of *Bacillus cereus ~ Candida albicans> Escherichia coli> Staphylococcus aureus> Cryptococcus neoformans ~ Klebsiella pneumoniae ~ Pseudomonas aeruginosa> Bacillus subtilis* [98].

It has also been identified in studies that the phenolic compounds obtained from aqueous extract of olive leaf show anti-HIV properties. It is also identified that separately or together usage of OLE and cleavage product hydroxytyrosol interferes entry of the virus to cell and adaptation to the cell inside and outside of the cell [99-102].

Antimicrobials derived from animals

Many inhibitor for microorganisms can be found naturally in animal products. Inhibitors affect microorganisms by having impact on cell, cell wall and cell membrane, by causing damage to enzyme systems and the genetic mechanism of the cell or by linking the important food factors [103].

Chitosan

Chitosan is an important amino-polysaccharide obtained by the N-deacetylation in the alkaline medium of biopolymer kit that is the most widespread found in nature after cellulose [104].

Chitin is usually found in algae, protozoa [flagellates, amoeba ciliates etc.], selentere, mollusks, arthropoda, bacteria, fungi, insects and some plants in natural life. The richest sources of chitin are shells of crab, shrimp, lobster and crawfish [crayfish] [105].

Structure of chitin is converted to chitosan, as a result of distillation in a heterogeneous medium containing sodium hydroxide or potassium hydroxide at high temperature [106].

Chitosan was first discovered in 1811 by Henri Braconnot. Braconnot tried to dispersed the chitin which is found in mushrooms in the sulfuric acid, but he was unsuccessful. In 1894, Hoppe-Seyler proceed the chitin in potassium hydroxide at 180 C degree [deacetylation] and he obtained "chitosan" that has a reduced acetyl content. In 1934 two patents were granted on subject matter of film production from chitosan and obtaining chitosan fibers. In the same year, very well oriented chitosan fibers were produced successfully by Clark and Smith [107].

Molecular weight of chitosan can vary between 50 to 2000 kilodaltons [kDa]. Chitosan can be classified as lower, medium and high according to its molecular weight. Chitosan is insoluble in water, but it is is quite good soluble in acidic medium [pH <6.5]. It is well soluble particularly in dilute solutions of formic, stearic and acetic acid. Usually, acetic acid solution is used as standard solvent [108].

The biggest advantage of chitin and chitosan is that they are renewable resources and environmentally friendly natural biopolymer [109].

In general perspective, chitosan has field of use opportunitiy in many industrial applications due to the following properties [110];

• It is renewable and there are plenty
• It is non-toxic, bio-compatible and biodegradable,
• It has bioeffects such as antacids, antiulcer, antitumor, antimicrobial, antioxidant, antibacterial and antifungal effects,
• No need for harmful organic solvents to dissolve,
• It is cationic polymer that easily interact with negatively charged surfaces

Table 1.6: Field of uses for chitin, chitosan and derivatives [111]

Sectors	**Field of use**
Food	Natural thickener
	Food preservatives
	Food additive including animal feeds
	Food processing [eg sugar processing]
	Filtering and cleaning
	Hypocholesterolemic material [attenuation material]

	Reprocessing of waste food
Agriculture	Plant additive
	Antimicrobial and antifungal agents
	Coating of plant seed
	Fertilizer production
	In insecticides and nematicides
Medical	Making adhesive plaster for animals and humans
	Making bandages and wound treatment [accelerates wound healing by 30%]
	Blood coagulating agent
	Making hydrogels
	Anticoagulants and antithrombogenic materials
	Hemostatic agent
	Contact lens production
	Drug release
Cosmetics	Making hair styling
	The skin hydration
	Anti-cholesterol and fat binder material
	Anti-odor substances in aftershave and deodorants
Biotechnology	Chromatographic methods
	Enzyme immobilization
Water Treatment	Coagulation and flocculation in contaminated wastewater
	Removal and recovery of metal ions in waste water

Antimicrobial mechanism

Even so, antimicrobial mechanism of chitosan is not yet fully clarified, three mechanisms have been proposed to explain the antimicrobial properties. The most feasible hypothesis is interacting with negatively charged residues of macromolecules [proteins and lipopolysaccharide] in microbial cell membrane [112, 113].

In the second mechanism, chitosan acts as chelating agents by selectively connecting to trace metals and thus it inhibits the microbial growth and toxin production [114].

According to the third mechanism, ow molecular weight chitosan is prone to enter the cell nucleus. It leads to inhibition of mRNA and protein synthesis and also inhibits the effect of various enzymes by binding DNA [112-114].

It has broad antifungal properties because it degrades cell wall of mold and it stimulate chitinase which is plant-defense enzymes in plant tissues [115].

Chitosan usually has a strong antimicrobial activity against molds rather than bacteria. Chitosan is used as potential natural food preservative against fungus, yeast and bacteria due to its antimicrobial activity [112].

High grade distillation increases the solubility and charge density of chitosan. These are two important factors of chitosan for the adhesion of bacterial cells. Antimicrobial activity of chitosan increases at low pH values [up to 5.5].Protonation and solubility are higher in the acidic pH range. Antimicrobial activity of chitosan is better at 37 °C rather than the refrigeration temperature [116].

Lactoferrin

Lactoferrin [LF], is first Discovered in 1939 by Sorensen and Sorensen [117], it is decomposed as one of the major components of bovine and human milk in 1960 by Groves [118].

Serous is a protein that is produced in cells and has antibacterial, antimycotic, antiviral, antineoplastic and anti-inflammatory effect. Lactoferrin is one of the non-enzymatic antimicrobial proteins located in the secretions of the exocrine glands and some special neutrophil granules. It is an iron binding glycoprotein secreted by the serous cells of large and minor salivary gland [119].

This glycoprotein present in Plasma, amniotic fluid, tears, saliva, semen, bile, urine, vaginal, nasal, bronchial and the gastrointestinal secretions is prevailed in milk and colostrum. With its concentration; It is the most prominent protein present in milk after of the casein in terms of [120, 121].

The most important function of lactoferrin on mucosal protection is to have antimicrobial activity [122].

Antimicrobial effect of lactoferrin:

Lactoferrin also has a bacteriostatic effect in addition to its bactericidal activity against gram [+], gram [-] bacteria and aerobes, anaerobes and yeasts. There are two mechanisms of this [123]:

1] Lactoferrin inhibits the use of ferro by bacteria. When ferro is removed from the environment by lactoferrin, it is suppressed the growth of bacteria. Because ferro is an essential element for the growth of almost all pathogenic microorganisms.

2] Lactoferrin leads to release of lipopolysaccharide from the outer membrane of gram [-] bacteria.

Although antiviral activity is not become exactly clear, this is the accepted mechanism; LF blocks by binding to viral glycosaminoglycan receptors such as specifically heparan sulfate, therefore virus is unable to adhere to host cells and prevent infection. Antifungal effect of lactoferrin is realized as demonstrated by electron microscopy like that: cell fractionation by air bubbles and swelling in cell Wall [124].

Lysozyme

Lysozyme is an enzyme that catalyzes hydrolysation of the glycosidic bonds between N-acetylglucosamine [NAG] and N-acetylmuramic acid [NAM] that exist in the peptidoglycan heteropolymers of prokaryotic cell wall [125, 126].

Lysozymes are proteins that exhibit anti-microbial properties directly. First, it was discovered by Alexander Fleming in nasal secretions in 1922 [127]. While Fleming was working in his lab, his nasal flow accidentally fallen into the petri dish containing the bacteria culture and he discovered that it killed Micrococcus lysodeikticus. Lysozyme that has the structure of mucopolysaccharides and also known as muramidase can be found in human tears, sweat, nasal secretions, many secretion in the gastrointestinal tract wall and the gingival sulcus fluid. It derives from leucocytes and the granulocytes [127].

Lysozyme also can be found in egg white, milk various animal tissues [103, 128].

Nakimbugwe and colleagues [103] worked on the article which is about lysozyme effect on Gram negative and Gram-positive bacteria in milk and banana water under high pressure shows that lysozyme has supressor effect on gram-positive bacteria L. monocytogenes and can be used as natural antimicrobials in food systems.

Lysozyme has antibacterial activity by taking part in the digestion of polysaccharides found in the cell walls of many bacteria shows. It shows this effect by catalyzing a water molecule that inputs to the glycosidic bond [129].

Table 1.7: Antimicrobials of animal origin [53].

Antimicrobial agent	**Major component**	**Major source**	**Target organisms**	**Ref.**
Lactoferrin	Iron binding glycoprotein	Milk	*E. coli* O157:H7, *L. monocytogenes*,*Salmonella*, *Pesudomonas*	[130]
Chitosan	Polycationic biopolymer compound	Exoskeletons of crustaceans and arthropods	*E. coli*, *S. aureus Pseudomonas* spp., *E. coli*, and *L. monocytogenes*	[131]
Lipids	Eicosapentaenoic acid [EPA] and Docosahexaenoic acid [DHA]	Fish and shellfish	*B. subtilis*, *L. monocytogenes*, *S. aureus*ATCC 6538, *S. aureus* KCTC 1916, and *Enterobacter aerogenes*, *E. coli*, *E. coli*O157:H7, *Pseudomonas aeruginosa*, *S.Enteritidis*, and *S. Typhimurium*	[132]
Defensins	Cationic peptides	Mammalian epithelial cells of	Gram-positive and Gram-negative	[133]

		chickens, turkeys	bacteria, fungi, viruses	
Lactoperoxidase	Glycoprotein	Raw milk, colostrum, saliva and other biological secretions	*Salmonella*, *E. coli*, *S. aureus*,*L. monocytogenes*, *Y.entericolitica*	[134]
Ovotransferrin	Glycoprotein [peptide OTAP-92]	Egg albumin	*Micrococcus*, *Bacillus* spp., *E. coli*O157:H7, *L. monocytogenes*, *S. aureus*	[135]
Lysozyme	Glutamic acid, aspartic acid	Hens' eggs, mammalian milk	*C. tyrobutyricum*, *Bacillus*, *Micrococcus*,*L. monocytogenes*	[136]
Pleurocidin	Polypeptides	Myeloid cells and mucosal tissues of many vertebrates and invertebrates	*L. monocytogenes*, *E. coli* O157:H7, and pathogenic fungi	[137]
Protamine	Cationic antimicrobial peptides	Salmon	*L. monocytogenes*, total bacteria, coliforms	[136]
Casein and whey	Bioactive peptides [Isracidin α_{s1}casein f1-23;	Milk protein	*Enterobacter sakazakii* ATCC 12868,*E. coli* DPC5063, *S.*	[138]

	Casocidin-I f150-188; Kappacin]		*aureus,L. monocytogenes, S. typhimurium,B. subtilis*	

Antimicrobials derived from bacteria

Microbes, particularly lactic acid bacteria produce a good vary of chemicals with antimicrobial activity. Among these, the proteinaceous compounds referred to as bacteriocins such as nisin have been shown to inhibit the expansion and development of different microorganism species. Likewise, reuterin produced from glycerol by some strains of Lactobacillus reuteri is another widely used broad-spectrum antimicrobial agent. Reuterin is also effective against several pathogenic and spoilage microorganisms. In this part, we have a tendency to discuss two widely known antimicrobials of bacterial origin: bacteriocin and reuterin as natural preservatives. Some other antimicrobials that are derived from bacterials are listed in Table1.8 [53].

Table 1.8: Antimicrobials of bacterial origin [53]

Antimicrobial Agent	**Source**	**Target Organisms**	**References**
Nisin	*Lactococcus lactis*	*Clostridium app., L. monocytogenes*	[139]
Pediocin	Strains of *Pediococcus acidilactici* and *P. pentosaceus*	*L. monocytogenes, Enterococcus faecalis, S. aureus, C. perfringens*	[133]
Natamycin	*Streptomyces natalensis*	Fungi	[136]
Lactocin	*Lactobacillus curvatus CRL705*	*E. coli* strains	[140]

Acidophilin	*L. acidophilus*	*B. cereus, B. subtilis, Listeria ivanovii, S. aureus*	[141, 142]
Bulgaricin	*L. bulgaricus*	*L. monocytogenes, S. aureus, B. subtilis, Helicobacter pylori*	[142, 143]
Helveticin	*L. helviticus*	*L. monocytogenes, Clostridium botulinum*	[142, 144]
Plantaricin	*L. plantarum*	*L. monocytogenes, S. aureus, S. typhimurium, E. coli*	[142, 145]
Reuterin	*Lactobacillus reuteri*	*L. monocytogenes, S. aureus, E. coli O157:H7, C. jejuni*	[146]

Bacteriocin

Bacteriocins, which is exist in protein structure and they are antibacterial compound produced by bacteria [147].

Generally, Bacteriocins are produced by the type of bacteria Gram-pozitive but the last searches are shown that Bacteriocins might be produced by the member of Arke [148].

Gratia first reported that *E. coli* V produce an antibiotic substance that inhibits the development of *E. coli* at 1925. This antimicrobial agent defined as a high molecular weight protein and it is called as colisin. Then, researchers discovered that nearly 17 colisin might be produced by the different types of *E. coli* species. The description of bacteriocin were made by Jakob et al. at 1953. This definition composed of the antagonistic relationships between different species and the proteins of colisin. The definition of bacteriocin has been expanded after the new discoveries. According to the definition of Klaenhammer, bacteriocin generally defined as relationship with the inhibitory effect on the bacteria producing microorganisms with protein or protein complex.

Bacterial antimicrobial peptides, i.e. bacteriocins are believed to be synthesized by many bacteria. According Klaenhamm, most of the bacteria synthesizes at least one

bacteriocin and these bacteriocins have not been defined yet. The reason of this, few researchers have made research about the bacteriocin [149].

Classification of bacteriocins

Although different classifications are used in order to categorize bacteriocins, the classification of Klaenhammer are commonly used .The classification made on the basis of biochemical properties, bacteriocins molecular size, chemical composition, are generally divided into four classes according to their mechanism of action and heat stability. However, the first three classes are considered more in terms of biochemical identification [150].

GROUP I bacteriocins

The bacteriocins in this group, it was called as antibiotics due to the containing of lanthionine. On the other hand, the amino acids found in structure of group I bacteriocin contain the amino acid derivatives.These are lanthionine [Lan] and methyllanthionine [MeLan]. Nisin is the most well-known member of this group [151, 152].

GROUP II bacteriocins

Bacteriocins in this group is small, they have 30-60 amino acid residues, and they are peptide which do not contain heat-resistance and lanthionine [Lan]. Their molecular weights lower than 10 kDa. Pediocin AcH [153], sakasin A [154] and Leucocin A [155] can be considered as examples of bacteriocins, which are included in this group.

GROUP III bacteriocins

Bacteriocins have larger molecular weight [>30kDa] in this group and they are composed of heat-sensitive peptide chains [156] . Helvetisin J and V [157], lactasin B [158] are the most known member of this group.

GROUP IV bacteriocins

The bacteriocins in this group are large and complex molecules, carbohydrate or lipid components they require for their activities. The information about the bacteriocins is insufficient and their biochemical property has not been characterized yet [150].

Effect mechanism of bacteriocins

Bacteriocins have different effect mechanisms on sensitive microorganisms. They connet the cytoplasmic membranes of cells and they generate pores in the membrane. Thus, the cell components of low molecular weight can be penetrated the out of the cell. However, leak out of the ions especially K + ions which are effective on loss of ATP and balance of intracellular, causes energy consumption in the cell. These changes occurring in cells leads to degradation of macromolecules such as DNA and RNA, which is vital for the cell. Also it leads to inhibition of biological processes such as protein and peptidoglycan [151].

Reuterin

Reuterin [h-hydroxypropionaldehyde] is a broad spectrum antimicrobial compound produced by some strains of Lactobacillus reuteri during anaerobic fermentation of glycerol. It is soluble in water, active at a wide range of pH values and resistant to proteolytic and lipolytic enzymes [146].

Reuterin is an active antimicrobial against Gram-positive and Gram-negative bacteria, yeasts, moulds and protozoa. It is proposed as a food biopreservative due to its water solubility. It's chemical structure makes it active at a wide range of pH values and resistant to proteolytic and lipolytic enzymes [159].

Reuterin has bacteriostatic activity against *Listeria monocytogenes*, whereas its activity was slightly bactericidal against *Staphylococcus aureus* at 37 °C. Bactericidal activity against *Escherichia coli*, *Salmonella choleraesuis* subsp. *choleraesuis*, *Yersinia enterocolitica*, *Aeromonas hydrophilasubsp.hydrophila* and *Campylobacter jejuni* is higher [146].

The antimicrobial effects of reuterin is complex and has not proven yet, despite 20 years of investigation, Inhibiting bacterial growth by modifying thiol groups, which indicates that reuterin negatively affects a large number of cellular targets. is one of its mechanisms that have been investigated [160].

Antimicrobials derived from natural resources [Mine]

Antimicrobial substances containing heavy metal

Metals are radiant surface elements which usually conduct heat and electricity. Heavy metals are often used as an antiseptic and disinfectant. When metals have specific gravity value is greater than 4 or 5, they are called heavy metals. However, this definition is ambiguous and there is no precise chemical identification. In the water, metals are in the form of positive ions [cations] so it is the basis of the anti-microbial and toxic effects [161].

Metal ions stop the proliferation of microorganisms according to two different mechanism hampers. First mechanism is that metal ions damage to cell membranes or metal ions go through the cell membrane and the enzyme – connects SH groups. Continuously decreased enzymatic activity leads to change the microbial metabolism. Secondly, metal ions catalyze the production of oxygen radicals which are damage to bacteria molecular structure [9].

Many metal has been used as traditional biocide in the past, but it is limited their application because of bioaccumulation and toxicity concerns. For example, only the liquid metal mercury in various forms [pure mercury, organic compounds, and inorganic compounds] has been used as an antiseptic, disinfectant and protective. Mercury, which is in the form of mercury chloride [sublimation], was used in the treatment of skin diseases by The Greeks and Romans. It is very toxic and when it is coupled with the other organic materials, the antimicrobial activity falls. Organic mercury compounds are effective as antiseptic for the treatment of minor wounds; as protector for serum and vaccine. Organic mercury is bacteriostatic and relatively not toxic. First organic mercury is mercurochrome [merbromin], known as antiseptic, has limited bacteriostatic effect. However, metaphen and merthiolate are more effective than mercurochrome [161-163].

Other metal ions, such as copper, zinc, titanium, gold, is known as anti-microbial, but the silver has the best activity against to bacteria, viruses and other eukaryotic micro-organisms [164].

Copper is the essential element that is required in small amounts to plants, animals and other life forms [161].

Copper is used by human civilization for more than 10000 years. Copper metal known as mechanisms of implementation, the effect of its creatures is not exactly as described. Between 2600 and 2200 B.C., copper was used for chest wounds to

sterilize and purify drinking water, written in Egypt Papurus. Hippocrates suggests the copper for local application in the treatment of leg ulcers and in the century of 19 and 20, before the antibiotics were not known, copper drugs was used in the treatment of skin diseases, syphilis and tuberculosis [165].

Elemental copper is rarely used, while copper sulphate [CuSO4], and other copper-containing compounds [copper chloride, copper-8-kinolinat, copper naftalate and copper oxide] is used more often [161].

Copper is used as fungicide in agriculture, in the form of copper sulphate [CuSO4]. In addition, it can be used for the disinfection of the water and the algae control. Copper compounds are added to paints, fabrics, and adhesives as well as material preservatives or antimicrobial property. In normal concentrations, copper is a cost-effective, easy-to-use and odorless. But in high concentrations, it is toxic and it can be bioaccumulate in aquatic live [161, 166].

Metal ions for their resistance against micro-organisms ranking; Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn. Silver metal is used more often than other metals, due to that it is the most resistant metal for bacteria, cheaper than most of materials, has easy manufacturing process [167].

Silver compounds

Silver is the most useful metal than other heavy metals, has high antimicrobial activity and low human toxicity. It is known since ancient times as metal [168]. The oldest silver finds was based in 4 BC century. Silver is used in medical purposes as it is estimated that Egypt first. In the middle ages, the use of silver was influenced by ancient and Arab alchemists. In the 14th century, Konrad von Megenberg wrote detailed and educational information about silver in the "Book of Nature". In this book, the empowerment effects of metabolism of silver are explained [169].

Ancient civilizations had used this precious metal for medicine, utensils, plates, cups, food storage containers, jewelry, coins, clothing, building materials, water disinfectant and human diseases [170]. The first modern definition of silver antimicrobial activity was made in 1869 by Raulin who have observing that Aspergillus niger were not grow in silver vessels [171].

Colloidal silver is effective for the treatment of wounds, according to U.S. Food andDrug Administration [FDA] by the 1920s. However, after the introduction of penicillin in the 1940s, antibiotics have become the standard treatment for bacterial infections and decreased use of silver. After 1940, the use of synthetically manufactured antibiotics has become common because they are cheaper. However, alternative antibiotics needs and importance of silver increases due to that the bacteria enhancements resistance against to the synthetic antibiotics, which is failed to bacteria, and these antibiotics have side effects more than the benefits [172].

Antimicrobial mechanism of silver

For bacteria, silver ions react with nucleophilic amino acid residues of proteins, and bound to sulphydryl groups, amino, imidazole, phosphate and carboxyl group of membrane of enzyme protein to protein denaturation [173].

Generally, it is known that the heavy metals emit ions which react with thiol groups of the bacteria surface. These proteins go outside of the cell membrane of bacteria and allow the passage of nutrients through the cell membrane. Silver interacts with proteins of thiol groups and neutralizes, reducing the permeability of the membrane, replacing with hydrogen cations so that bacteria are known to cause the death of cells [174].

In addition, silver inhibits succinate uptake and E.coli respiratory chain by some of the oxidative enzymes, membrane vesicles, as well as cause the metabolite leaking and inhibit DNA replication. Silver has been associated with the cell wall, cytoplasm, cell membrane. Chappell and Greville reported that the driving force of proton has been crashed at low levels of Ag+ in the bacteria membrane. This report supported with Mitchell's work in 1961 and 1966. Dibrov et al. determined that low Ag+ concentrations cause massive proton leak from bacterial membrane. The most important mechanism in occurring bacteria death with Ag+, is connecting to a surface and damaging membrane function [173].

Nano-silver

Reducing the size of the particles is efficiency and reliability method to increase the biocompatibility of materials. Indeed, nanotechnology helps resolve the size

constraints. Besides, nanomaterial can be modified to achieve a better efficiency in different application areas like bioscience and medical [175].

Nano-scale metal particles have important physical, chemical and biological features, such as large surface area and high reactivity, compared to the normal metals. There is growing interest in the use of nano Ag as a special class of biocidal agents [176]. Silver nanoparticles are sets of silver atoms from 1 to 100 nm in diameter [177].

The antimicrobial effect and inert nature of nano silver has become an attractive option for strong food processing and medical equipment industry. There are two main reasons for using nano silver in medical plastics and food processing. One of them is to stop the effects of bacteria and fungi on physical properties of the object. Another is to prevent the development of harmful bacteria that may be the source of infection [178].

Silver nanoparticle has antimicrobial effects at low concentrations. Their antibacterial properties are related with nanoparticle surface area. Since the particle size is reduced, the increase in surface area provides more impact on antibacterial activity [179].

But nowadays, the use of nano silver causes discussions. For example;

In December 2013, the report was presented to the Commission by the Scientific Committees of the European Commission:

- Typical use of nano silver has negative effects on human health and the environment,
- Widespread and long term usage of silver nano particles [Ag-NPs] -nano silver- causes additional adverse effects,
- Nano silver has other negative health and environmental effects,
- More data was needed for better understanding of bacterial reaction against ionic Ag and Ag-nanoparticle exposure.

At the beginning of the third group of the periodic system and atomic number 5 is boron element, consists of the two stable isotopes, have mass numbers 10 and 11. Chemical symbol is B, is the only element in the periodical table that is not a group of metal ruler 111A [180].

Although boron compounds have been known for thousands of years, elemental boron was discovered by Sir Humphry and Gay Lussac in 1808. Firstly, 4000 years ago, the Babylonians imported borax from the Far East and used in gold operations. It is known that Egyptians use the borax in pipe, medicine and mummification, metallurgical applications. The first source of borax has been getting from the Tibetan Lake. Ancient Greeks and Romans used the borax as a cleaning agent. In the year 875 A.D., borax was used firstly as medicine by the Arab doctors [181].

Boron is an important industrial raw material. Balıkesir, Bursa, Eskişehir and Kütahya province to scattered boron beds, the largest known reserves in the world. The total world boron reserve is estimated and 1.2 billion tons, 63% of total reserves are in Turkey. In contrast, both in production as well as the share of the economic basis are not commensurate with the reserves in Turkey. For this reason, Turkey has declared as strategic mineral boron ore [www.boren.gov.tr].

Table 1.9: Boron reserves in the world [ETI Mine Report, 2012]

Countries	**Total Reserves [1000 tons B_2O_3]**	**Distribution [%]**
Turkey	864,500	72
Russia	100,000	8
USA	80,000	7
China	47,000	4
Chile	41,000	3
Peru	22,000	2

Boron minerals are kind of minerals containing different proportions of boron oxide [B2O3]. In our country, commonly found boron minerals are tincal, colemanite and ulexite [182].

Ulexite is the most important boron mineral used in the manufacture of boron compound. Between boron minerals, it is the most abundant and common in Turkey. Its chemical formula is Na2O·2CaO·5B2O3·16H2O and it is a kind of sodium-

calcium borate which has a complex structure. Commercially the most used boron compounds are boron oxide and boric acid, sodium perborate. Ulexite is one of the main raw materials for the production of these compounds [183].

Colemanite, $2CaO \cdot 3B_2O_3 \cdot 5H_2O$ chemical formula, is the most common monoclinic crystalline boron mineral. The largest known ore in the world is in Turkey. Colemanite ores are used in the production of boric acid [H_3BO_3] and glass fiber, mostly. Colemanite ores includes clay minerals significantly, strontium borate, also includes other minerals such as calcite [184].

Application areas are; glass, ceramics, nuclear, electrical and electronics and computer industries; communication tools; construction-cement, metallurgical and energy sectors; automobile industry; textile industry; chemistry, cleaning and whitening industry; the agricultural sector; paper industry; photography; production of composite materials and sporting goods; magnetic devices and mummification [185].

In addition, pharmaceutical and cosmetic industry [disinfectants, antiseptics and production of toothpaste] and there are also areas in medicine. Osteoporosis, allergic disorders, psychiatric disorders, arthritis and menopause treatment, multiple myeloma in the diagnosis of intracranial tumors in the treatment, diagnosis and treatment of infectious disease takes place in [185].

Literature information about antimicrobial properties of boron

The first biomolecule, containing boron, is a kind of antibiotics, called as boromycin, is produced from streptomyces antibioticus. Boromisin, gram [+] are effective against certain fungi and protozoa, but gram [-] ineffective against bacteria. Boric acid ester is comparable with antibiotics such as used in the clinic, erythromycin, gentamicin and streptomycin has been reported [186].

Boron is attracted the attention of researchers in the field of Periodontology, with antibacterial property, and known effects on bone and immune response. In general medicine, antibacterial and anti-inflammatory effect of boric acid compound has been reported [187].

Boric acid esters have been shown to inhibit the essential enzyme of, microorganisms. DNA methyltransferase in gram-negative, and menaquinone

methyltransferase, HIV-1 protease, s. griseus glycohydrolaz and NAD-belonging to ADP-ribosyltransferaz in gram-positive are prime examples [186, 188, 189].

Boric acid is recommended in local alternative antimicrobial agents in the treatment of bacterial infections. It is known that the effectiveness of the boric acid also contains different types of bacteria, Staphylococcus [190].

1.3.3.2 Synthetic antimicrobial agents

There has been an increasing interest in pharmaceutically active compounds consisting of many synthetic antimicrobial agents because of their high activity. As a consequence of that scientific studies have been carried out to destroy these compounds' toxic properties to use them inhibiting the bacteria. Human and animal infections are treated by synthetic antimicrobial agents [191]. Synthetic antimicrobial agents are grouped as listed below.

Sulphonamides

Sulfonamides are synthetic antimicrobial agents derived from sulfanilic acid. They are also known as sulfa drugs and widely used to treat human and animal infections. They are used as food additives in livestock production. The mechanism of sulphonamides in bacteria is to inhibit folic acid synthesis by competitive inhibition of the enzyme dihydropteroate synthetase [191]. Sulphonamides have high effect on bacteria such as Strep. pneumoniae, bhaemolytic streptococci, Escherichia coli and Proteus mirabilis. Sulphonamides are important antimicrobial agents for the treatment of uncomplicated urinary tract infections caused by E. coli, meningococcal meningitis and superficial eye infections [192].

Diaminopyrimidine derivatives

Compounds derived from diaminopyrimidine are highly active against human cells, protozoa or bacteria. They could be used to heal cancer and malaria [192].

Co-trimoxazole

Co-trimoxazole is formed by the mixture of sulphamethoxazole [five parts] and trimethoprim [one part]. Cotrimoxazole is used to treat pneumonias caused by Pneumocystis carinii, a yeast [although it had been classified as a protozoan].

Pneumocystis carinii is a common cause of pneumonia in patients receiving immunosuppressive therapy and in those with AIDS [192].

Dapsone

Dapsone is especially used for the treatment of leprosy. Since the resistance to dapsone is well known, it should be used with rifampicin and clofazimine [192].

Antitubercular drugs

There are 3 types of drugs used to treat tuberculosis: streptomycin, p-aminosalicylic acid [PAS] and isoniazid [isonicotinylhydrazide, INH; synonym, isonicotinic acid hydrazine, INAH]. The tubercle bacillus becomes resistant to streptomycin so fast, and PAS is used to prevent this resistance. There is a new approach for healing tuberculosis. Combination of these three drugs should be used to reduce the bacterial level and then the process should be continue by the combination of two drugs [192].

Nitrofuran compounds

The antimicrobial property of nitrofuran compounds was found 40-years ago. It is known that a nitro group in the 5 position of 2-substituted furans gives these compounds antibacterial activity. Although many compounds of nitrofurans have been synthesized, only a few are used in current treatments.

The nitrofurans have antibacterial activity against a wide spectrum of microorganisms. However, furaltadone is not recommended to use as antimicrobial agent because of its toxicity. Furazolidone is highlyactive against most members of the Enterobacteriaceae and used for the treatment of diarrhoea and gastrointestinal disturbances of bacterial origin. Another nitrofuran compound, nitrofurantoin is used in the treatment of urinary tract infections. Nitrofurazone is used mainly as a topical agent in the treatment of burns and wounds and also in certain types of ear infections. The nitrofurans are believed to be mutagenic [192].

4-Quinolone antibacterials

There have been Over 10000 quinolone antibacterial agents synthesized. Nalidixic acid is known asthe progenitor of the new quinolones. It has been used in the treatment of urinary tract infections for several years. It has become as a clinically

important drug. Some of 4-quinolone antibacterial agents that have been synthesized shows high antibacterial property [192].

Imidazole derivatives

The imidazoles are known to have antibacterial [metronidazole], antiprotozoal [metronidazole], antifungal [clotrimazole, miconazole, ketoconazole, econazole] and anthelmintic [mebendazole] activity.Metronidazole is effective against the protozoa Trichomonas vaginalis, Entamoeba histolytica and Giardia lamblia even in low concentrations and inhibits the growth of pathogenic protozoa. The other imidazole derivatives such as clotrimazole, miconazole and econazole are the antimicrobial agents used topically [192].

Flucytosine

Flucytosine has the greatest antifungal activity against yeasts such as Candida, Cryptococcus and Torulopsis [192].

Synthetic allylamines

Terbinafine, a member of the allylamine class of antimycotics, is used to inhibe the enzyme squalene epoxidase in fungal ergosterol biosynthesis. It is orally active, fungicidaland effective against a broad range of dermatophytes and yeasts. It is also used topicallyas a cream [192].

Synthetic thiocarbamates

The synthetic thiocarbamates such as tolnaftate inhibit squalene epoxidase. Although tolnaftate inhibits this enzyme from C. albicans, it is inactive against whole cells because of its inability to penetrate the cell wall. Tolnaftate is used topically in the treatment or prophylaxis of tinea [192].

Oxazolidinones

The oxazolidinones were produced in 1987 as a new class of synthetic antimicrobial agents. They are especially active in vitro against antibioticsusceptible and -resistant cocci [192].

Triclosan

Triclosan [2,2,4'trihidrokloro-2'-kidroksidifenilet's] that is non-ionizing synthetic and with broad spectrum is already in use antimicrobial agent for more than 30 years. It is firstly used to reduce dental plaques within the category of health products and used for the surgical field painting [193, 194].

Today, Triclosan is used in hand soap, surgical site cleaning, shower gel, deodorant, hand hygiene for health cares staff, hand lotions, hand creams and toothpaste. United States Food and Drug Administration [FDA] officially stated that Triclosan has no harmful effect on human health [195].

In 1986, The European Union has approved in accordance with Cosmetics Directive that Triclosan can be used up to 0.3% concentrations in cosmetic products as preservative, up to 5mg/kg in materials contact with food, up to 3% consantrations in textile materials [especially sport wear] and plastics [plastic packages, brushes] materials [196].

Triclosan effects by inhibiting the synthesis of bacterial cytoplasmic membrane, RNA, lipid and protein. When its effect mechanism is analyzed, it is observed that it shows static effect against bacteria at low concentrations and it inhibits fatty acid synthesis of bacteria. In the concentrations used in soap, it shows lethal effects on organisms by affecting a large number of the cytoplasmic membrane targets. The removal of Triclosan from the body is occured by conjugating with urine and stool [197, 198].

In addition to bacteriostatic effect of Triclosan on gram-positive and gram-negative bacteria, it is known that it has antifungal and antiviral properties [199].

Adverse negative effects of triclosan

In the course of contacting with products containing Triclosan, it is taken into body by skin, nose and mouth [199].

According to the report prepared by the Australian government in 2009, triclosan is identified as irritating for eyes, respiratory system, skin and toxic for inhalation [200].

In addition, triclosan is taken to human body via sea foods as a result that triclosan has reached to the food chain by merging to the sea, lakes and groundwater [199]. A series of studies conducted in the United States, it is stated that significant amount of triclosan was found in mothers' milk in the study conducted on 36 nursing mothers who use personal care products very much that contain triclosan [199]. In the recent studies, it is stated that triclosan effect androgens in the male body and estrogen in the female body. It is shown that Triclosan effects the transport between placenta and the fetus in pregnant sheep. It has been reported that it may cause abnormal development and Triclosan may stimulate breast cancer, especially in females. In a series of studies conducted on rabbits, it is reported that the triclosan reduces the number of sperm, leads to tissue destruction in the reproductive organs, disrupts the male hormone in the male rabbits [199]. It is known that the thyroid has vital impact on development and metabolism. Thyroid hormone is a highly effective hormone that effects on the development of fetuses and young children. Studies shows that triclosan decreases the thyroid hormone levels on rabbits and changes metamorphosis time on frog [199].

Large companies such as Procter & Gamble, Johnson & Johnson and Walmart areforced to find biocompatible and non-toxic solutions instead of using triclosan based products because of new regulation of FDA stated that triclosan and triclocarban are harmful to health. And also according to a new law in US state of Minnesota that will enter into force on 1 January 2017, triclosan can not be used as an active ingredient in any hygiene products.

Such changes in regulations and prohibitions will make easier to create value of nontoxic biocompatible solutions rather than the content that is harmful to the human body. It is expected that the growing user awareness against harmful content will support the rise of biocompatible nontoxic solutions in the market in the short and medium term.

1.3.4 Applications of antimicrobials

Microbial contamination is one of the most serious problems in various fields especially such as medical devices, drugs, healthcare services, hygienic applications, water treatment systems, hospital and dental surgery equipment, textiles, food packaging and food storage. Antimicrobials has attracted interest in academic research and the industry because they bring quality and safety to many materials. In addition to this, organic antimicrobial agents with low molecular weight are toxic to the environment and they have disadvantages such as short-term antimicrobial capabilities. Antimicrobial functional groups integrate into the polymer molecules to overcome these kind of problems associated with antimicrobials. Use of antimicrobial polymers is promising to expand the influence of some existing antimicrobial agents, to minimize environmental problems, to improve the effects, selectivity and lifetime of antimicrobial agents. Research on the development of these polymers have a great importance for both academic research and industry [201].

1.3.4.1 Industrial fields

Paper industry:

Microorganisms form a film with other fibrous or inorganic material by adhering in both surfaces of the machine elements in paste preparation and paper production lines. A typical paper factory is easily capable of providing nutritional needs of a large portion of the microorganisms. The basic raw materials for the production of paper provide a natural feeding medium to microorganisms because of their contents such as through cellulose, hemicellulose, soluble sugars, starches, extractives. The growth of microorganisms during paper production process may lead to significant technical, economic and hygiene problems due to slime formation. Biocides are added at the end of wet the process to prevent the formation of slime. Also biocides are used to protect the materials used during the processes applied in the pulp and paper industry [202, 203].

Paint industry:

A broad range of water-based products containing paint, polymer emulsions and construction materials are open to harmful effects of many different microorganisms. As these effects can result with significant economic losses, measurements of the

institutions which can prevent this conditions may become very important. The use of biocides are very important to prevent the economic losses that may result from paints and other industrial products [204].

Water-based paints are more indurable to microbiological effects and decay due to depending on their raw materials, the production and storage conditions. Microbial contamination leads to many adverse conditions such as reduced viscosity, pH change, color change, gas-off and bad odor formation in these products. It is even not enough to use active biocides by increasing their concentrations to eliminate these problems. Instead, it is needed to assess carefully the stages of production in terms of microbiologically with the help of biocide manufacturers. Improvements in the cleanliness of the production field, possible improvements related with the field, washing with biocidal products etc. will help to eliminate the problems [203].

Sterilization of water:

The chlorination of drinking water reservoirs containing natural organic substances causes to formation of disinfection by-products [205].

Trihalomethanes [THM] and haloacetic acids [HAA] are the main disinfection byproducts commonly found in drinking water [206].

Scientific studies related to disinfection by-products are stated that these harmful compounds close relations with diseases such as risk of cancers, growth retardation in children, miscarriage in women and congenital challenge heart defects [207].

Disinfection by-products occurring as a result of the chlorination have other acute and chronic effects on human health besides the risk of developing cancer [208].

Therefore, these residues are involved in the food chain nearby. This handicap will be eliminated by removing of microorganisms in water with using a number of insoluble substances. Polymeric disinfectants, water filters, surface coatings and fibrous are ideal materials to use in disinfectants, because they can be produced as water-insoluble with using many different techniques. The last few years, many researchers have focused on producing water insoluble polymeric disinfectants to be used in water treatment [201].

Healthcare and medical materials:

Three major factors affecting "health" which is defined by The World Health Organization as physical, mental and social well-being, are human, constructive disease factors and environment. The environment is a medium that we're always in contact with microorganisms that are one of the most important factors causing to the disease [209].

Hospital infections are one of the most important problems of our era. Every 3-10 of 100 people hospitalized [vary according to volumes] get an infection while waiting for healing in the hospital. Prolongation of hospital stay, increased cost of treatment, even a part of coming face to face with death are not enough to show the exact size of the problem [210].

The most widely used method is the purification process is carried out with the help of chemical substances to overcome this situation. Chemical materials are used in the form of liquid and gas. Alcohols, phenol compounds, hydrogen peroxide, hypochlorite, chlorine, strong oxidising systems such as iodine, etiloksit, lipid-containing detergents and mercury, heavy metal salts such as copper and silver are widely used antimicrobial chemical agents. It is a known fact that these chemicals cause many health problems including cancer for human health, and theirs wastes lead to environmental pollution in recent years. After purification process of the bacteria by using chemical agents, this chemical effect is going through after a period and bacteria can reproduce itself again in the surface. In other words, continuous purification can not be achieved [211]. Therefore studies on biocides that have both no dangerous effect for human health and the environment have been increasing in recent years, long-lasting results.

Polymers can be used as material in the medical field such as soft contact lenses, artificial tendon materials, and the Bioadhesive substances used in wound healing, artificial kidney membranes, artificial leather and plastic surgery. Most of pharmacologically active substances which can easily penetrate into all cell types and can be disposed very quickly by the body are compounds with small molar mass. Therefore, continued use and intense dose can cause therapeutic effects and serious side effects. Antimicrobial agents and associated drugs such as polymers carrying them change their rate of elimination from the body. And they provide a controlled release over a longer period. Controlled release systems provide secured release of

drugs according to the needs of physiological environment. And also they improve therapeutic efficacy [201].

Food industry:

Microbial contamination shorten the shelf life of foods and increases the risk of acquired diseases caused by foods. Conventional methods including some applications such as heating, freeze drying, cold storage, irradiation, adding antimicrobials and salts are used to preserve the effects of microbial growth. Antimicrobials can be synthetic antimicrobials obtained in the laboratory [acetic acid and acetate, benzoic acid and benzoates, lactic acid and lactate, nitrate and nitrite, sorbic acidand sorbates, sulfites] and also may be natural compounds derived from animals, plants and microbials found in biological systems. Consumers prefer to use natural antimicrobials derived from animal, plant and microbial sources instead of synthetic additives animal. Some part of natural antimicrobials are used for food preservation and some of are still under investigation. The antimicrobial polymers are used in many food-related applications including packaging. One of these applications is used for solid foods like meat and cheese, and liquid foods contain milk or meet fluids to increase the extension of the food safety and the shelf life of foodmeats by inhibiting the growth of distinct microorganisms. One of the other applications is antimicrobial packaging application to prevent recontamination in the food [201, 212, 213].

Textile industry:

Bacteria can also cause health-related problems such as infections, disease and odor as well as the deterioration and staining of textile products. Natural fibers such as cotton are more sensible rather than synthetic fibers to microorganism origin problems because of its porous and hydrophilic structures. On the other hand, the human body provides heat, moisture and nutrients to bacteria in the garments contacting directly to itself. So, it provides an excellent environment and favorable conditions for bacterial growth. Applications in this field are also old because harm of microorganisms in textile products is well known since time immemorial old.

Inorganic salts, spices and herbs used by Egyptians to protect fabric that wrap the mummies are one of the oldest practices in this regard [214].

Antimicrobial agents commonly used in the textile sector are listed as triclosan, quaternary ammonium salts, polibiguanid, N-Halamines, chitosan, metal and metal-oxides. Many antimicrobial materials used in the textile industry have been developed in recent years. These materials have many differences according to their chemical structures, the operating mechanisms, effects on people and the environment, adhesion characteristics to products which they apply, their resistance to various external influences, prices and interactions with microorganisms [25].

In self-awared societies, gradually increasing needs for human-environment friendly antimicrobial textiles have attracted the attention of developing composite technologies. Consequently, use of particles such as chitosan which is natural or silver, zinc oxide and titanium dioxide which have low toxin effects has been raised. Producing textile materials that have more persistent antimicrobialactivities by joining of these particles to structure of polymer materials become one of the more interesting research topics. The aim of gaining antimicrobial property to textiles is to protect both textile products and consumers from damages caused as a result of microbial attacks [215].

1.4 Objective

Polymer composites with antimicrobial additives are widely used in many areas especially in hospitals, biocompatible implants, toy industry, food production and the packaging, medical device industry, white appliances industry, construction materials. However, many agents used in recent years endanger human health and increasing of resistance that pathogenic bacteria has against antimicrobial agents are the major problems [216]. Therefore, development of new types of reliable and cost effective biocidal materials comprising natural and inorganic substances is very important. In order to use antimicrobial agents in plastics, As well as they must be effective against to microorganisms and they must have some of properties such as heat resistance, soluble by plasticizers, compatibility with the polymer, low leakage rate, UV resistance, harmless to the environment [28].

Microorganisms such as bacteria, fungi and algae effect aesthetic and physical properties of plastic by causing the black spots or fading, the odor and polymer degradation [178]. These problems are also quite big problems for home appliences industry. The biofilm caused by using of tap water, contaminated textile products and human skin is occured in detergent drawer and box of washing machines after a certain period of use. Biofilm formation has a negative impact on human health as well as it reduces the lifetime of the material and increases the service burden.

The aim of this study is to produce antimicrobial washing machine parts by adding natural antimicrobial agents into copolymer in order to minimize or preferably completely eliminate biofilm formation.

2. MATERIALS AND METHOD

2.1 Materials

2.1.1 Copolymer

Heterophasic polypropylene copolymer *Moplen* 2000 HEXP [medium high fluidity, T_m=165^{o}C, ρ=0.9 g/cm3, Melt flow rate [230^{o}C/2.16 kg]=16g/10 min] produced by Lyondell Basell Company, was used as the polymeric material. The product features an outstandingly high impact performance, particularly at very low temperature, combined with stiffness. It is especially designed for injection molding applications where either a very high impact at deep-freeze conditions or a heavy duty impact performance at room temperature is critical.

Figure 2.1: Granular heterophasic polypropylene copolymer

2.1.2 Olive leaves

Olive leaves were collected from the Aegean region, Torbalı. They were collected in summer [June] and properly prepared for drying process in the day they were collected.

Figure 2.2: Olive leaves

2.1.3 Ulexite

Ulexite [$NaCaB_5O_9.8H_2O$] was provided by Eti Mine Works General Directorate.Ulexite is powder form and contains %43,0 concentration of B_2O_3.

2.2 Method

2.2.1 Preparation of the olive leaves powder

Olive leaves were washed with deionized water and dried at 37 ° C for 3 days. Then, dried olive leaves were powdered to 50-90 μm particle size by vibratory disc mill. Finally, dried olive leaves powder were conserved in the glass storage container until the extrusion process.

Figure 2.3: Olive leaf powder

2.2.2 Preparation of the copolymer

Copolymer was grinded by using micronized device with 1400 rpm speed during 15 minutes in order to decrease the particle size of the pellets to obtain a homogeneous mixture due to ulexite and olive leaves used as reinforcing material in the powder form.

2.2.3 Extrusion process

Ulexite and olive leaves filled composites were manufactured by using twin-screw extruder branded Labtech [Figure 5]. The prepared mixture was placed to the hopper

of extruder and feed with 20 rpm. Zone temperatures of extruder were selected between the temperatures 170^{o}C and 185^{o}C. Screw speed is adjusted as 190 rpm.

Figure 2.4: Twin-screw extruder

After the extruder die, composite was cooled with water in cooling bath and transferred to pelletizer to obtain pellet type composite materials. At the exit of the pelletizer, composite pellets produced had moist due to cooling process after extrusion. The oven was used in order to eliminate the moisture of the composite pellets. After extrusion, composite pellets were dried in oven for 1 day at 37^{o}C.

Ulexite and olive leavesfilled composites were manufactured based on 1 kg. The amounts of materials are shown in Table 2.1.

Table 2.1: Ulexite and olive leaves filled composites produced in the study

Composites	**Copolymer amount**	**Ulexite amount**	**Olive leaf powder**
cPP5U	950 g	50 g	-
cPP10U	900 g	100 g	-

cPP15U	850 g	150 g	-
cPP5OLP	950 g	-	50 g
cPP10OLP	900 g	-	100 g
cPP15OLP	850 g	-	150 g
cPP10U-10OLP	800 g	100 g	100 g
cPP15U-15OLP	700 g	150 g	150 g

2.2.4 Thermokinetic mixer

Melt mixing of the pellets was achieved using a Gelimat [Figure 2.5], a high-speed laboratory thermokinetic mixer branded as Gülnar Makina. 50 gr composite samples were mixed for 15-20 seconds with 2000 rpm speed in order to get mixture in the form of dough.

Figure 2.5: Thermokinetic mixer

2.2.5 Hot &cold press

Hot press [Figure 2.6] was used to form the composite panels from compounded pellets. After heating the hot press until processing temperature [200^{o}C], produced composite pellets were placed into the mold cavity [15 cm x 15 cm] between Teflon sheets which were used to avoid direct contact of PP composites with the hot press metal platens during heating and pressing. Plates were obtained from the mixture by

using Hydraulic Laboratory Press [Labtech] via heating and cooling platens. Samples were pressed under 39 bar pressure with 200^{o}C upper and lower temperature of heating platens for 20 seconds in hot part and 2 minutes in cold part of the press.

Figure 2.6: Hot and cold press

2.3 Characterization

2.3.1 Antimicrobial analysis

Bacteria to be used for the test: E.coli [ATCC 25922] and S.aureus [ATCC 6538] cultures were used. Cultures were obtained from Micromed Company.

Culture medium: Baird Parker Agar [BPA] was used as culture medium for *S.aureus* and Rapid *E.coli* 2 agar was used as culture medium for *E. coli* counting.

Antimicrobial test standard: Antimicrobial tests were evaluated according to "JISZ 2801: Antibacterial products -Test for antibacterial activity and efficacy" standard.

According to this standard 5x5 cm^2 plates were treated by *E. coli* and *S. aureus* bacteria and waited for 24 hours in a humidified atmosphere. At the end of the test period, amount of bacteri from test materials and control samples were calculated using standard plate assay. With respect to Standard, the value over 2 log reduction is considered as "Sufficient Antibacterial Efficiency".

Aging test was carried out for 3 composites [cPP15U, cPP15OLP and cPP15U-15OLP] having the best results at the end of the antimicrobial test.

Neutralizer liquid content: The sterile physiological saline solution was used as neutralizer liquid during tests. Neutralizer liquid was prepared by adding 8.5 g / L NaCl (Merck) into 1 liter of distilled water.

It is distributed to intended containers (tube, flask, etc.) was sterilized for 15 minutes at 121 °C in the autoclave. We made use of this solution while diluting the samples that contain antimicrobial agents. Briefly, 9 ml physiological salt solution was distributed to each tube for the dilution. SCDLP broth was used to transfer microorganisms which are on the sample plaque into the solution environment. Casein peptone (17g), Soybean / Soymeal peptone (3g), NaCl (5g), disodium hydrogen phosphate (2.5 g), glucose (2.5 g), 1 g lecithin were weighed and 1L sterile water was added during preparation of SCDLP broth. Non-ionic YAM (7g) was added to the mixture. pH 6.8 and 7.2 was set. After taken from the incubator, starter plates, and after-24h plates were whipped for 30 seconds in stomacher by adding 10 ml SCDLP Broth solution; plates were considered as -1 dilution. Then other serial dilutions were prepared.

The Amount of Dilution: Freshly prepared *E. coli* and *S. aureus* cultures were prepared as 10^5- 10^7cfu/ml in Densimat device. Microorganism solution was prepared in the separate tubes for both of *E. coli* and *S. aureus*, not as a mixture. The starting solution was prepared as -3*, -4 and -5 dilutions to be added into Rapid *E. coli* 2 agar (BIO-RAD) and Baird Parker Agar (BPA) (MERCK) for inoculation. It was added to 0.1 mL of BPA's and it was left for 48 hours at 36-38 °C in incubation. The pour plate technique was applied for plantation on Rapid *E.coli* 2 agar. 1 ml of dilutions was added to the blank Petri dishes and 15-20 ml of Rapid *E.coli* 2 agar was poured on it. Homogeneous mixture in the Petri dish was provided by "8" shaped movement by slightly shaking. After the nutrient agar was solidified in the biosafety cabinet, Petri dishes were left in the 36-38 ºC for 24 hours in incubation.

-2, -3, -4 dilution ratios was used for initial examples of the plate samples to start. The dilution ratio applied for the treated and untreated samples after-24h was identified as -1 (1ml), - 1, -2, -3.

*(- 3) dilution=Solution diluted 103 times.

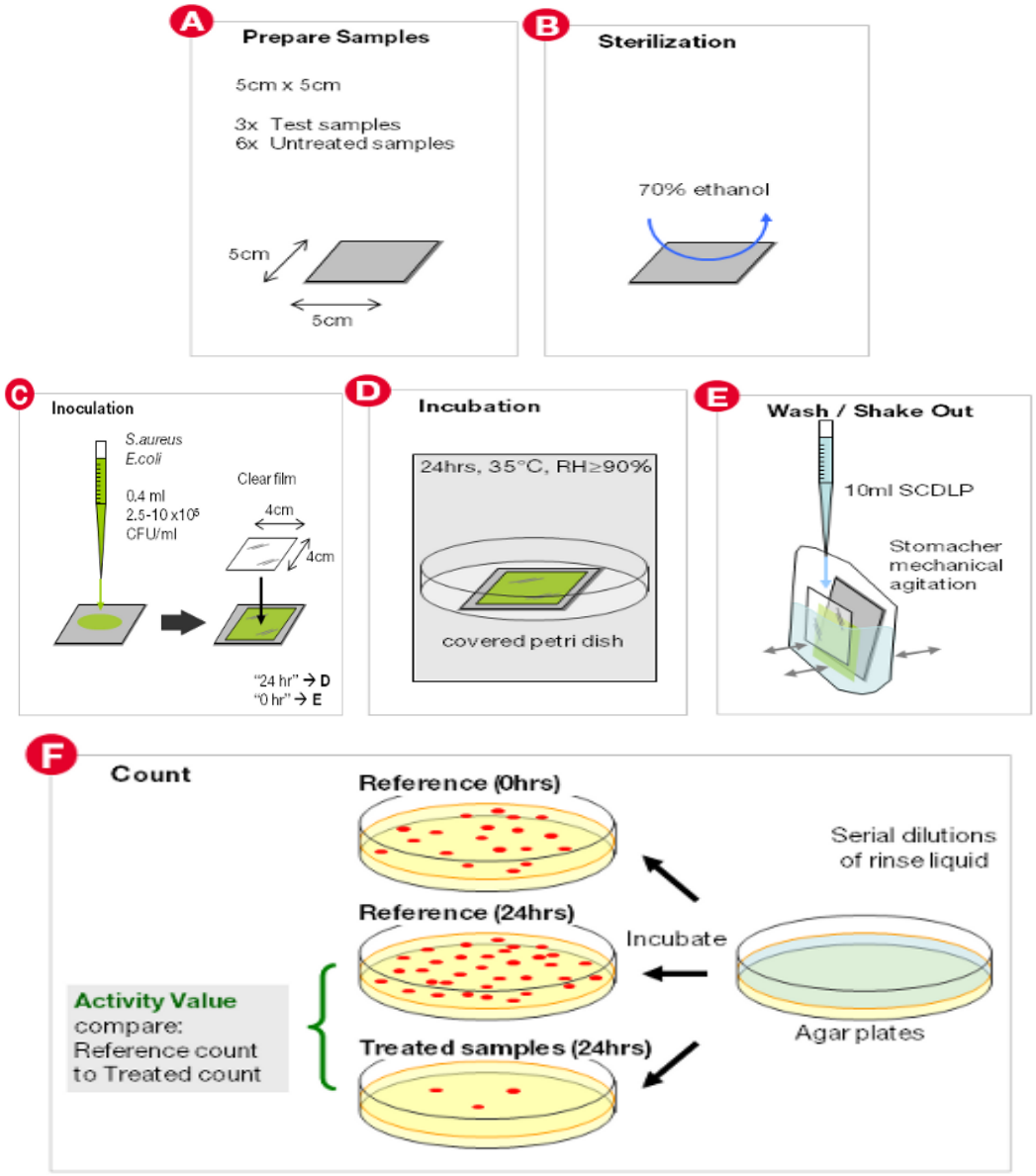

Figure 2.7: JIS Z 2801 Standard [Graphics by Dr Murray Height]

2.3.2 Aging testing

Samples of washing machine detergent aging test was carried out with 0.7% detergent solution, at 95 C for 1 week. Detergent solution was replaced every day.

2.3.3 FTIR analysis

The FTIR technique was used to study the main functional groups present in materials used in this study. Thermo Scientific™ FTIR spectrometer in Attenuated

Total Reflection Infrared [ATR-IR] mode was used to obtain the spectra of ulexite, olive leaf powder, copolymer and polymer composites. Attenuated total reflection infrared [ATR-IR] spectra of the materials were collected at a resolution of 4 cm^{-1} with a range of 400–4000 cm^{-1} and a number of 16 scans per sample. Spectral outputs were recorded in transmittance mode as a function of wave number.

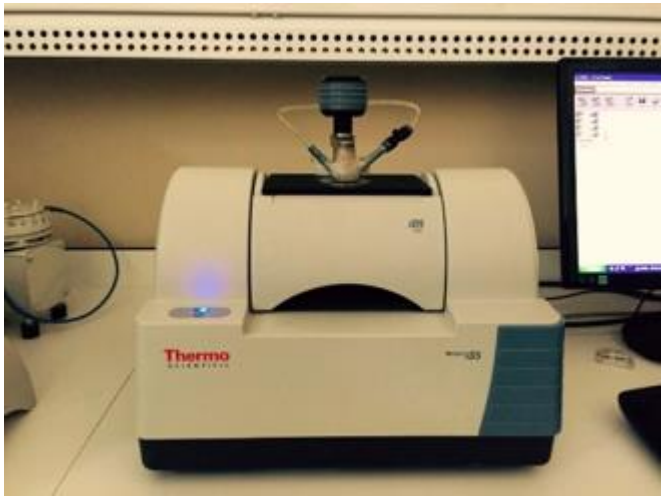

Figure 2.8: FTIR device

2.3.4 Tensile testing

Tensile testing samples were obtained by using a mold which cut the plate with blades according to ASTM D638 standard. SHIMADZU AGS-X 5kN tensile test machine [Figure 2.9] was used for ulexite and olive leaf powder reinforced composites at room temperature with a crosshead speed of 50 mm/min. 5 specimens were tested for each composite formulation to obtain a reliable average of tensile properties as well as their corresponding standard deviations. The strength (σ_{max}) and the elongation at break were evaluated.

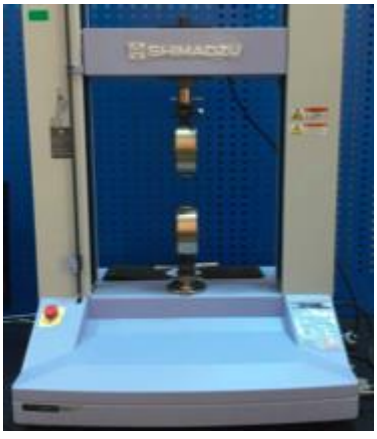

Figure 2.9: Tensile tesing device

Figure 2.10: Samples of tensile testing obtained from the composites.

2.3.5 SEM analysis

Scanning electron microscopy (SEM) was used to observe the microstructure of olive leaf powder, ulexite, copolymer, and polymer composites. SEM images were taken from the fracture surfaces of the tensile specimens. FEI Quanta FEG 250 was used as SEM. The samples were coated with gold.

Figure 2.11: SEM analysis device

3. RESULTS AND DISCUSSION

3.1 Antimicrobial and Aging Analysis

When analyzing the table;

- The neat material shows inactivation to *E. coli* with 0.69 log and *S. aureus* with 1.92 log. It can be said that it does not have desired antimicrobial activity because these values are remained under log2.
- When inactivation values observed in the additive-free plates were taken as a reference;
    - It was observed that both of two plates containing only ulexite can not provide adequate efficacy against *S. aureus* bacterial species but the cPP10U composite material provides antibacterial activity against the bacteria *E. coli.*
    - It was seen that both of two plates containing only OLP could not provide adequate efficacy against *E.coli* bacteria but the cPP10OLP composite material provided high antibacterial activity against *S. aureus* bacteria.
    - On the other hand, it was confirmed that both of cPP10U-10OLP and cPP15U-15OLP composite materials have antibacterial efficacy against bacteria *E.coli* and *S.aureus.* This was an expected result. The reason for using a combination of Ulexit and OLP additive material was to create a synergistic effect. The results proved that this effect was obtained.

Table 3.1: Results of antimicrobial analysis

Sample	Microorganism	N	Initial Value (cfu/ml)	Initial Value (log/ml)	After 24 hours (cfu/ml)	After 24 hours (log/ml)	Standard Deviation	Log Reduction
cPP	*E. coli*	3	6,65E+05	5,8228	1,35E+05	5,1303	0,870	0,69
	S. aureus	3	3,77E+06	6,5763	4,55E+04	4,6580	0,102	1,92
cPP5U	*E. coli*	3	4,47E+05	5,6503	6,75E+04	4,8293	0,486	0,82
	S. aureus	3	1,13E+06	6,0531	2,02E+05	5,3054	0,129	0,75
cPP10U	*E. coli*	3	4,47E+05	5,6503	7,00E+01	1,8451	0,198	3,81
	S. aureus	3	1,13E+06	6,0531	2,95E+05	5,4698	0,366	0,58
cPP15U	*E. coli*	3	5,93E+04	4,7733	1,00E+01	1,0000	0,000	3,77
	S. aureus	3	1,11E+07	7,0440	3,67E+01	1,5643	0,314	5,48
cPP5OLP	*E. coli*	3	1,00E+03	3,0000	1,16E+06	6,0645	0,072	Unidentified
	S. aureus	3	3,53E+06	6,5478	5,25E+04	4,7202	0,985	1,83
cPP10OLP	*E. coli*	3	1,00E+03	3,0000	4,85E+02	2,6857	0,065	0,31
	S. aureus	3	3,53E+06	6,5478	1,00E+01	1,0000	0,000	5,55
cPP15OLP	*E. coli*	3	5,93E+04	4,7733	4,47E+04	4,6500	0,1412	0,12
	S. aureus	3	1,11E+07	7,0440	1,00E+01	1,0000	0,000	6,04
cPP10U-10OLP	*E. coli*	3	6,65E+05	5,8228	8,33E+01	1,9206	0,440	3,90
	S. aureus	3	3,77E+06	6,5763	1,00E+01	1,0000	0,000	5,58
cPP15U-15OLP	*E. coli*	3	5,93E+04	4,7733	1,00E+01	1,0000	0,000	3,77
	S. aureus	3	1,11E+07	7,0440	1,00E+01	1,0000	0,000	6,04

It is required that antibacterial activity should be continued after aging test in order to use cPP15U, cPP15OLP and cPP15U-15OLP composite materials that have high antibacterial activity into detergent drawer and box of the washing machine. Therefore, aging test was carried out for these two composites. The antibacterial activity results before and after aging test are shown in Table 3.2 and Table 3.3.

Table 3.2: Antimicrobial efficacy before aging test

Sample	Microorganism	N	Initial Value (cfu/ml)	Initial Value (log/ml)	After 24 hours (cfu/ml)	After 24 hours (log/ml)	Standard Deviation	Log Reduction
cPP	*E. coli*	3	5,93E+04	4,7733	9,27E+03	3,9669	0,723	0,81
	S. aureus	3	1,11E+07	7,0440	5,43E+02	2,7351	0,645	4,31
cPP15U	*E. coli*	3	5,93E+04	4,7733	1,00E+01	1,0000	0,000	3,77
	S. aureus	3	1,11E+07	7,0440	3,67E+01	1,5643	0,314	5,48
cPP15OLP	*E. coli*	3	4,47E+05	4,7733	4,47E+04	4,6500	0,141	0,12
	S. aureus	3	1,13E+06	7,0440	1,00E+01	1,0000	0,000	6,04
cPP15U-15OLP	*E. coli*	3	5,93E+04	4,7733	1,00E+01	1,0000	0,000	3,77
	S. aureus	3	1,11E+07	7,0440	1,00E+01	1,0000	0,000	6,04

Table 3.3: Antimicrobial efficacy after aging test

Sample	Microorganism	N	Initial Value (cfu/ml)	Initial Value (log/ml)	After 24 hours (cfu/ml)	After 24 hours (log/ml)	Standard Deviation	Log Reduction
cPP	*E. coli*	3	2,04E+06	6,3096	3,57E+05	5,5531	0,094	0,76
	S. aureus	3	7,07E+06	6,8492	3,72E+05	5,5702	0,311	1,28
cPP15U	*E. coli*	3	2,04E+06	6,3096	3,88E+05	5,5888	0,465	0,72
	S. aureus	3	7,07E+06	6,8492	2,91E+05	5,4639	0,436	1,39
cPP15OLP	*E. coli*	3	2,04E+06	6,3096	Unidentified			
	S. aureus	3	7,07E+06	6,8492				
cPP15U-15OLP	*E. coli*	3	2,04E+06	6,3096	2,97E+05	5,4732	0,283	0,84
	S. aureus	3	7,07E+06	6,8492	1,92E+05	5,2825	0,390	1,57

cPP15U, cPP15OLP and cPP15U-15OLP composite materials showed high antibacterial activity against both of *S. aureus* and *E. coli* before aging test. However, when the reinforced material and the reference material were compared after aging test, it has been shown that antibacterial activity could not be achieved and the effect was disappeared. This situation can be explained by two reasons. The first one of these, additive agents were released quickly and antimicrobial effect has lost its influence. The second one, surface agents was depleted by dissolving in liquid media during aging test and other agents were immobilized and stayed in the polymer. This is consistent with the data obtained by KALYON et al. [217]

3.2 FTIR Analysis

The FTIR spectra of OLP, cPP and cPPOLP composites are shown in Figure 3.1. FTIR spectra of olive leaf showed that there is a OH band at 3272 cm^{-1}. Fathia et al. [218] stated that there is O-H (oleuropein, cellulose, organic acids, etc.) stretching vibration. This stretching vibration was not observed in FTIR spectra of copolymer itself. But, OH band became less visible in olive leaf powder filled composites. Moreover, after the OLP addition to cPP, two bands at 3000-2800 cm^{-1} correspond to symmetric and asymmetric CH stretching vibrations disappeared in the composites. On the other hand, the region of olive leaf powder around 1800 $cm^{-1}$and 1500 cm^{-1} became visible although not clearly apparent in the composites. This region corresponds to C=O, C=C (esters, acid, carboxylate, aromatic ring) and OH stretching vibration, confirms the structure of OLP. Furthermore, the intensity of these groups slightly increases while increasing amount of OLP. In the study made

by Fathia et al. [218], it was seen that typical absorbance bands of OLP at the 1500-1200 cm^{-1} range was very complex with especially CH (CH, alkyl) and OH deformation vibration. These bands came from OLP were observed in composites. In the FTIR spectra of the composites, it was seen that the intensity of these groups increases while increasing amount of OLP. Furthermore, the intense bands between 1150 and 950 cm^{-1} correspond mainly to the endocyclic and exocyclic C-O stretching vibrations of carbohydrates [218].

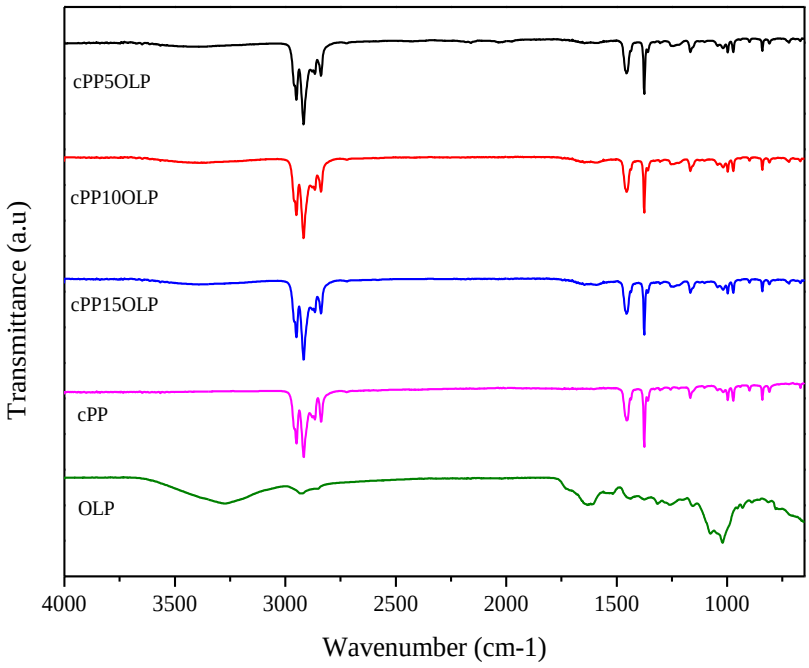

Figure 3.1: The FTIR spectra of OLP, cPP and cPPOLP composites

The FTIR spectra of ulexite, cPP and cPPU composites are shown in Figure 3.2. The region observed between 3000 cm-1 and 3600 cm-1 became visible in ulexite filled composites. In the literature, it is mentioned that this region indicates the OH band [219]. Ulexite has also a broad band between 1691 $cm^{-1}$and 1612 $cm^{-1}$and in the study performed by Stuart, B., and W. O. George [219] thought that the band at 1625 cm^{-1}assigned to free H2O band. This band came from ulexite itself became visible in ulexite filled composites. Addition to these bands, the band at 1400 cm^{-1} was assigned to the asymmetric stretching mode of B-0 in BO_3. Ulexite has three bands at 1355 cm^{-1}, 1315 cm^{-1} and 1211 cm^{-1}. The bands between 1352 and 1209 cm^{-1}were assigned to the in-plane bending band of OH [220]. This broad band peaks region was observed after the Ulexite addition in the composite samples and the intensity of

these peaks increase while increasing amount of Ulexite. In addition to this, the bands at 1049, 973, and 854 corresponded to asymmetric stretching mode of B-0 in BO_4 [221].

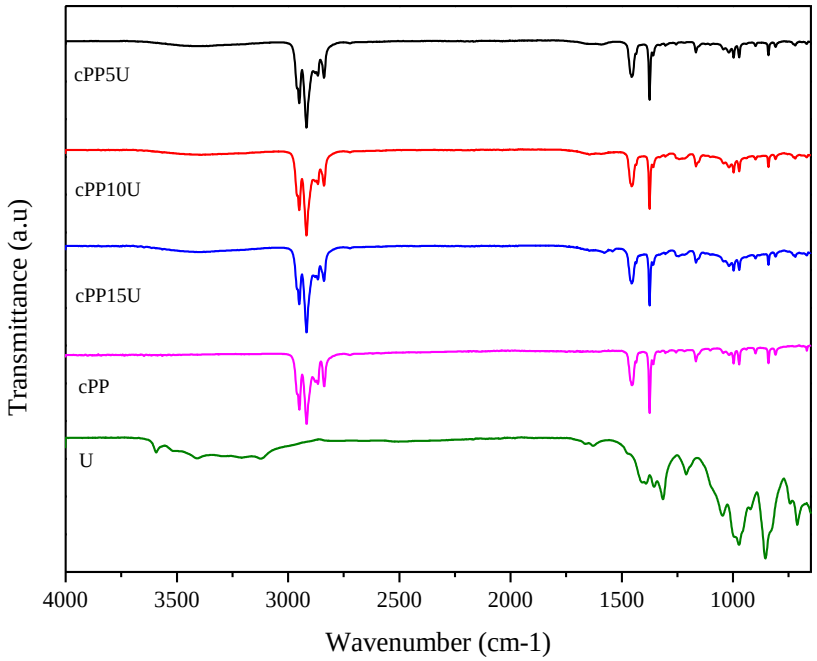

Figure 3.2: The FTIR spectra of Ulexite, cPP and cPPU composites

The FTIR spectra of the Ulexite, OLP, cPP,cPP10U, cPP10OLP and cPP10U-10OLPcomposites, cPP15U, cPP15OLP and cPP15U-15OLPcompositesare shown in Figure 3.3 and Figure 3.4. Typical intense bands between 3000 and 3600 cm^{-1} were observed and these band regions (OH) were observed both OLP and ulexite FTIR spectra. However, typical absorbance peaks of OLP and ulexite were observed in this study. It was seen in the study performed by Fathia et al. [218] that typical absorbance bands of OLP at the 1500-1200 cm^{-1} range were very complex with especially CH (CH, alkyl) and OH deformation vibration. In these range, also it was seen in FTIR spectra of ulexite powder, the bands between 1352 and 1209 cm^{-1} were assigned to the in-plane bending band of OH in the study made by Piskin S. [220]. OH band of olive leaf and ulexite were seen markedly in the composites filled with olive leaf powder and ulexite powder.

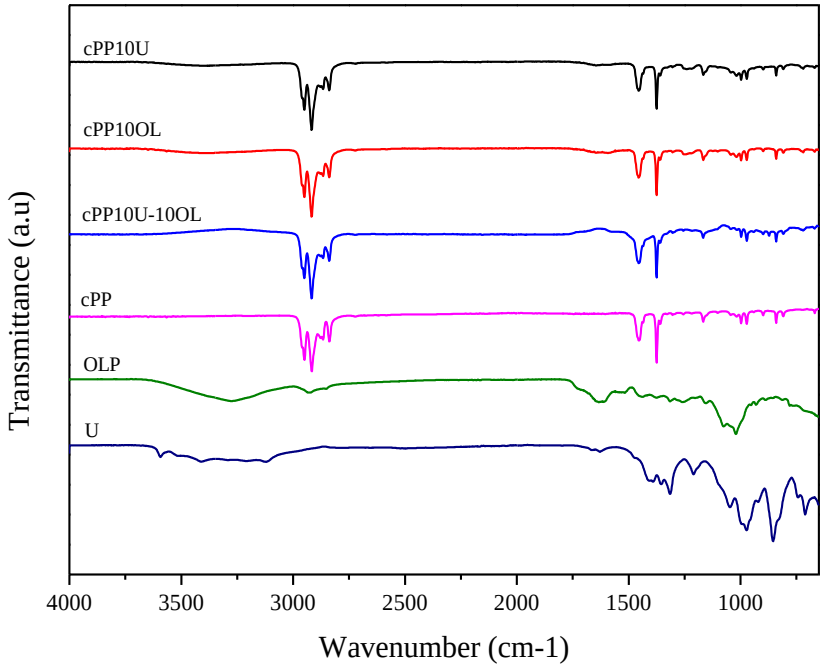

Figure 3.3: The FTIR spectra of Ulexite, OLP, cPP, cPP10U, cPP10OLP and cPP10U-10OLP composites

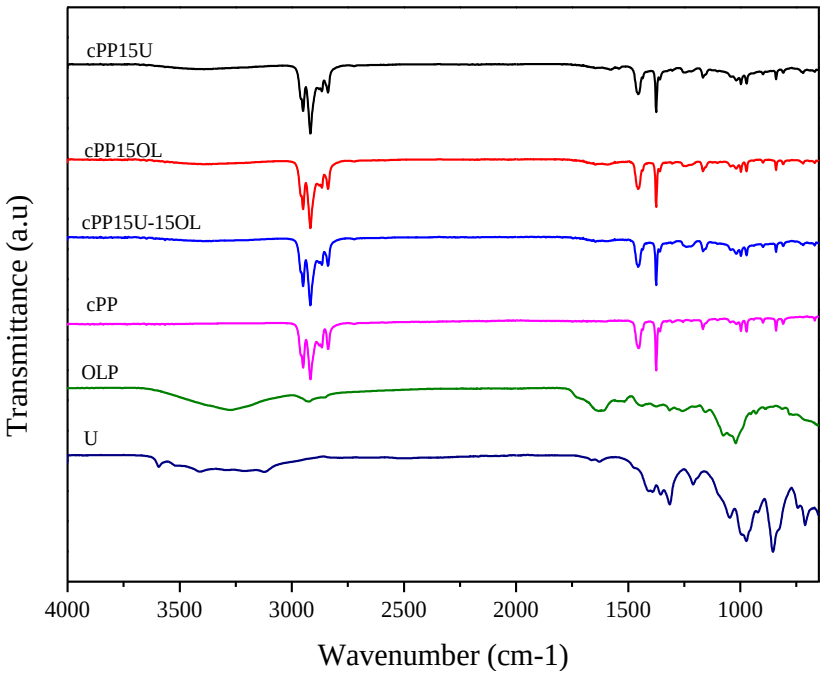

Figure 3.4: The FTIR spectra of Ulexite, OLP, cPP, cPP15U, cPP15OLP and cPP15U-15OLPcomposites

3.3 Tensile Testing

Tensile strength and elongation at breakvalues of pure copolymer and composites containing Ulexite are shown respectively in Figure 3.5 and Figure 3.6. According to Figure 3.5, it is observed that addition of 5%-10% and 15% concentration of Ulexite did not change the tensile strength of the composite. While tensile strength of the pure copolymer is 13,88 MPa, It has been seen that tensile strength of 5% Ulexite-reinforced composite is 14,6 MPa, tensile strength of 10% Ulexite-reinforcedcomposite is 14,2 MPa, tensile strength of 15% Ulexite-reinforcedcomposite is 13 MPa. These values were remained in the range of the standard deviation (2,150) of the tensile strength of pure copolymers. When we compared the composite materials itself; if the amount of additive material increased, the tensile strength decreased. The reason of this decreasing was that the dust agents were agglomerated in the matrix material and surface of the matrix material tended to decrease due to not using any binding agents. SEM images also support this conclusion.

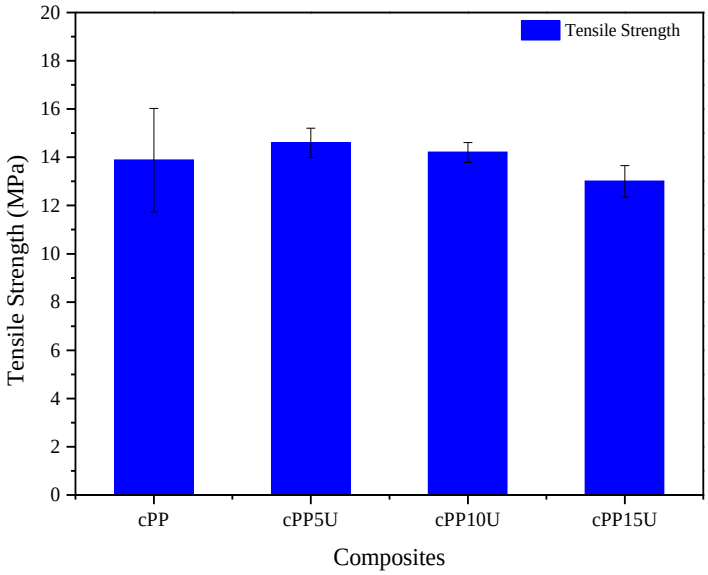

Figure 3.5: Tensile strength values of cPP, cPP5U, cPP10U, cPP15U

According to Figure 3.6, it was observed that the elongation at break values of the material is severely reduced by the addition of ulexite additive material. It was determined that this reduction increases while the amount of ulexite was increasing.

The reason of this situation is because ulexite particles weaken chemical bonds among polymer molecules.

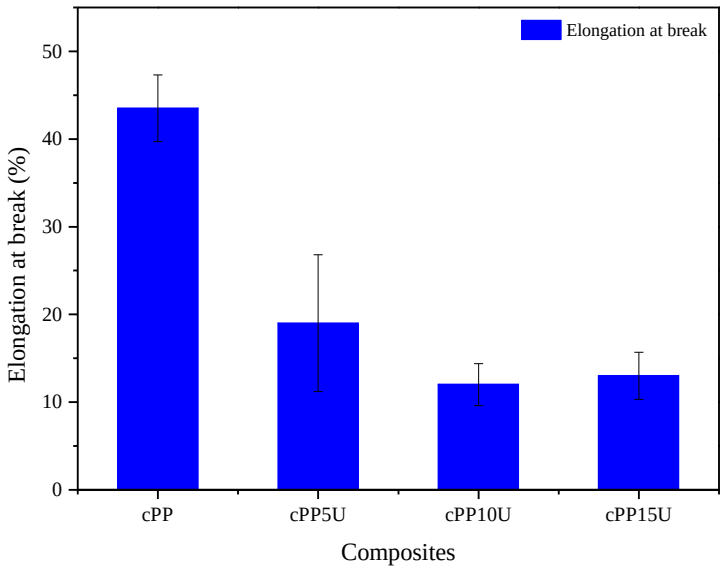

Figure 3.6: Elongation at breakvalues of cPP, cPP5U, cPP10U, cPP15U

In some of the studies conducted by using mineral colemanite, decreasing of tensilestrength value could not provide increased strength because of the powder form of the dope additive. It was considered that tensile value has been decreased as a result of the reduction in the matrix percentage of cross-section. It was emphasized that as a result of decreasing the percentage of the matrix depends on the increasing contribution rate (reduction in resistance unit to tensile strength in cross-section) and increasingly attaining brittle structure, the elongation at break value decreased [222].

Pure copolymer and OLP-reinforcedcomposites tensile strength and elongation at breakvalues are shown respectively in Figure 3.7 and Figure 3.8. According to Figure 3.7, it was seen that 5% OLP-reinforcedcomposite material has the highest tensile strength. The lowest values were observed in 15% OLP-reinforcedcomposite material. It was observed that the tensile strength of the olive leaf-reinforcedcomposites did not change compared with the pure copolymer. The tensile strength values of olive leaf-reinforcedcomposites remained in the range of the standard deviation of the tensile strength of the pure copolymer. When we compare the tensile strength of olive leaf composites within their own category; if the amount of the olive leaf powder increases, its tensile strength decreases.

This is because, homogeneous mixing of powder additives within the main material can not be ensured and, therefore agglomeration accordingly occurs. The SEM images confirm this conclusion.

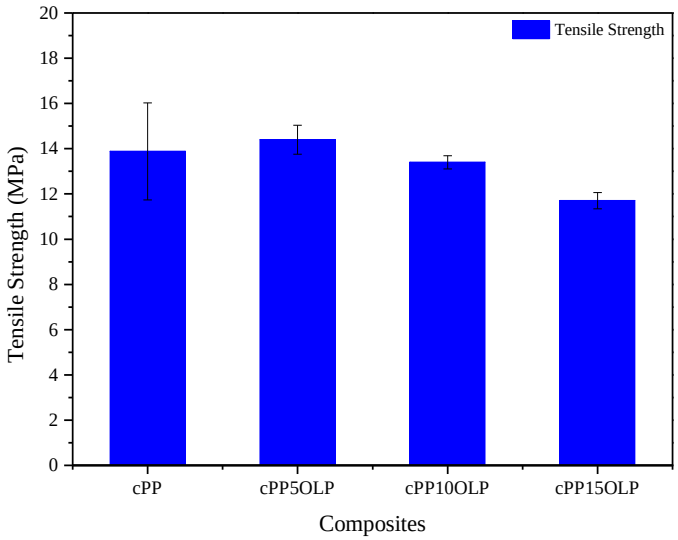

Figure 3.7: Tensile strength values of cPP, cPP5OLP, cPP10OLP, cPP15OLP

According toFigure 3.8, a significant decreasing in elongation at break value of the material with OLP additive was observed. And, as expected, when the amount of additive increases, elongation at break value reduces in direct proportion. Therefore most significant decreasing was seen at 15% OLP-reinforcedcomposite material.

OLP is a natural material and is some of compounds contained can not resist to the high temperature. Due to some compounds in OLP within the extrusion space operating temperature may burn, it causes spaces in the polymer. Therefore, it is expected that elongation at break value may decrease depending on increasing in the percentage of OLP. SEM images supported that the bond between the copolymer and OLP can not be occured without any chemical process. In this study, the copolymer and OLP were not able to cohere due to not using any chemical agents. The elongation at break values decreased dramatically because OLP particles entered among the polymer molecules and weakened chemical bonds.

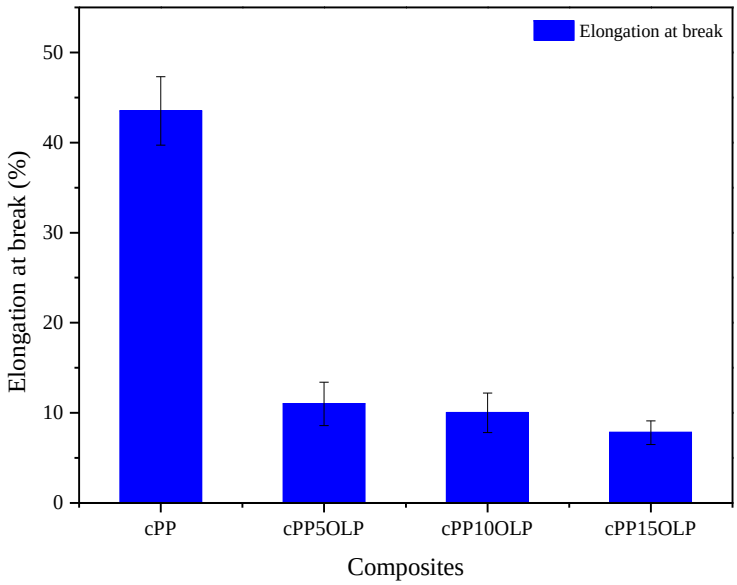

Figure 3.8: Elongation at break values of cPP, cPP5OLP, cPP10OLP, cPP15OLP

The tensile strength and elongation at break values of pure copolymer, ulexite-reinforced, olive leaf powder-reinforcedand ulexite-olive leaf powder-reinforcedcomposites are shown respectively in Figure 3.9, Figure3.10 and Figure 3.11 and Figure 3.12. When we analyzedFigure3.9 and Figure3.10, it was observed that the tensile strength of the composite materials with both of two additives decreased in comparison with the composite materials that additives are used individually. It was observed that the tensile strength decreased in composite materials both additives used in combination when the amount of additive materials increased. While the tensile strength of cPP10U-10OLP composite material was 12.78, the tensile strength cPP15U-15OLP composite material was 10.8. This reduction was caused because the additives did not have homogeneous distribution in the copolymer and they were agglomerated.

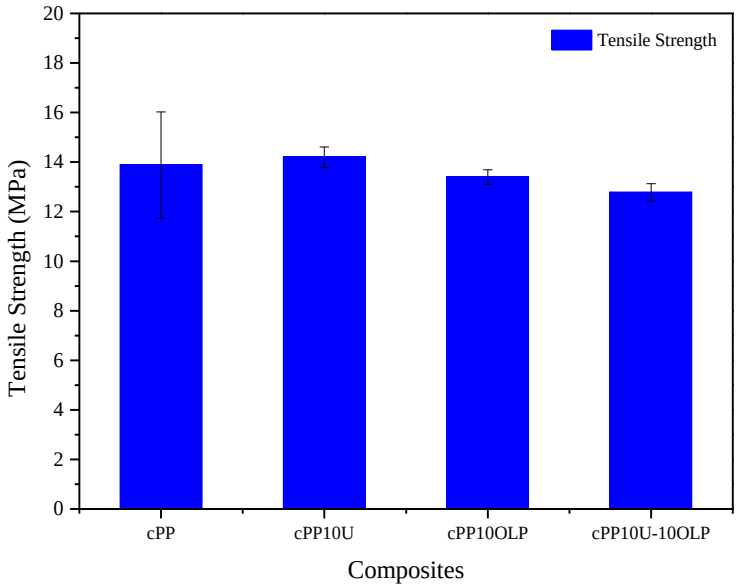

Figure 3.9: Tensile Strength values of cPP, cPP10U, cPP10OLP, cPP10U-10OLP

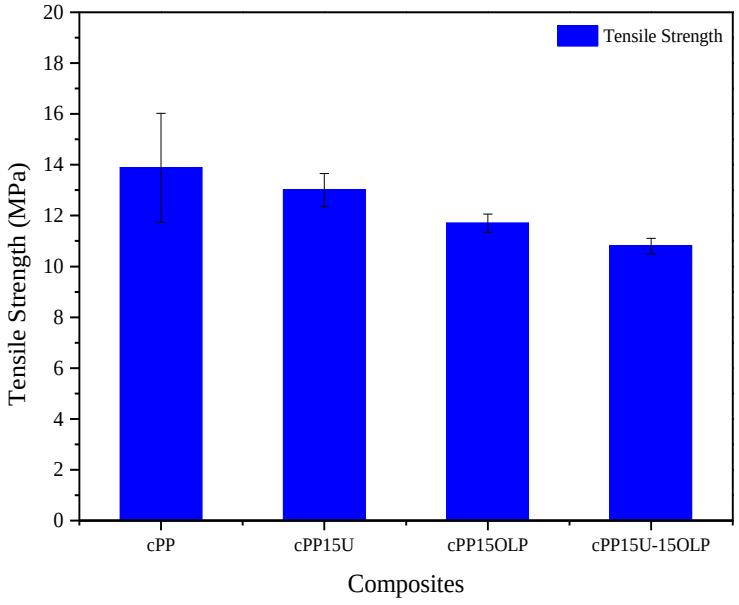

Figure 3.10: Tensile Strength values of cPP, cPP15U, cPP15OLP, cPP15U-15OLP

According to Figure 3.11 and Figure 3.12, the elongation at break value of the composite material that two additives used as combination had dramatically declined in comparison with the elongation at break value of pure copolymer. This decline was increasing proportional to the amount of additives. It has been observed that the

elongation at break value of the composite material that two additives used as combination tended to decrease in comparison with the composite materials that additives used individually. It has been observed that the elongation at break value of the composite materials that two additives used as combination tended to decrease when the amount of additives increases. While the elongation at break value of cPP10U-10OLP composites was 9.86, the elongation at break value of cPP15U-15OLP composite materials was 7.3. What the reason of this decreasing in composite materials was that both OLP and ulexite particles intervene in polymer molecules and weaken or break the bonds as mentioned above.

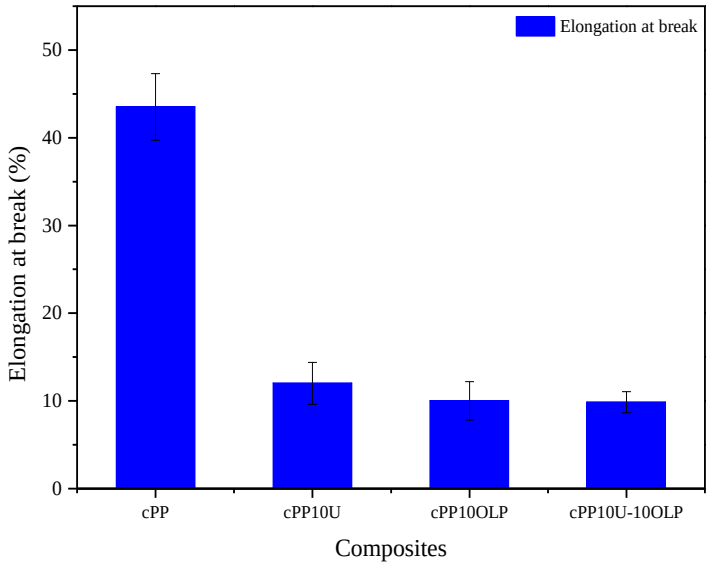

Figure 3.11: Elongation at break values of cPP, cPP10U, cPP10OLP, cPP10U-10OLP

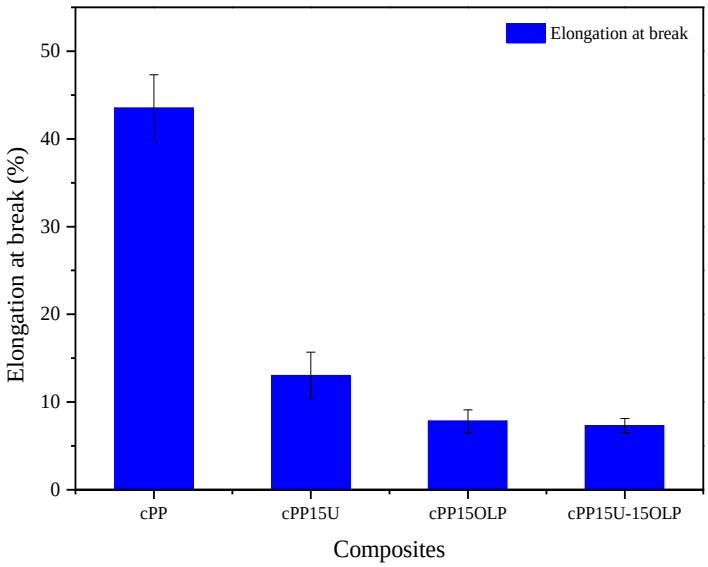

Figure 3.12: Elongation at break values of cPP, cPP15U, cPP15OLP, cPP15U-15OLP

3.4 SEM Analysis

SEM images of pure copolymer, ulexite and olive leaf powder are shown in Figure 3.13. When the SEM image of the pure copolymer was examined, it has been observed that it was similar to the rupture characteristic of the elastic materials. When SEM image of ulexite mineral was examined, it has been seen that the distribution of particle size varies and generally had a striped structure. When the SEM images of OLP, it has been observed that the grain size distribution was not homogenous as similar to mineral ulexite.

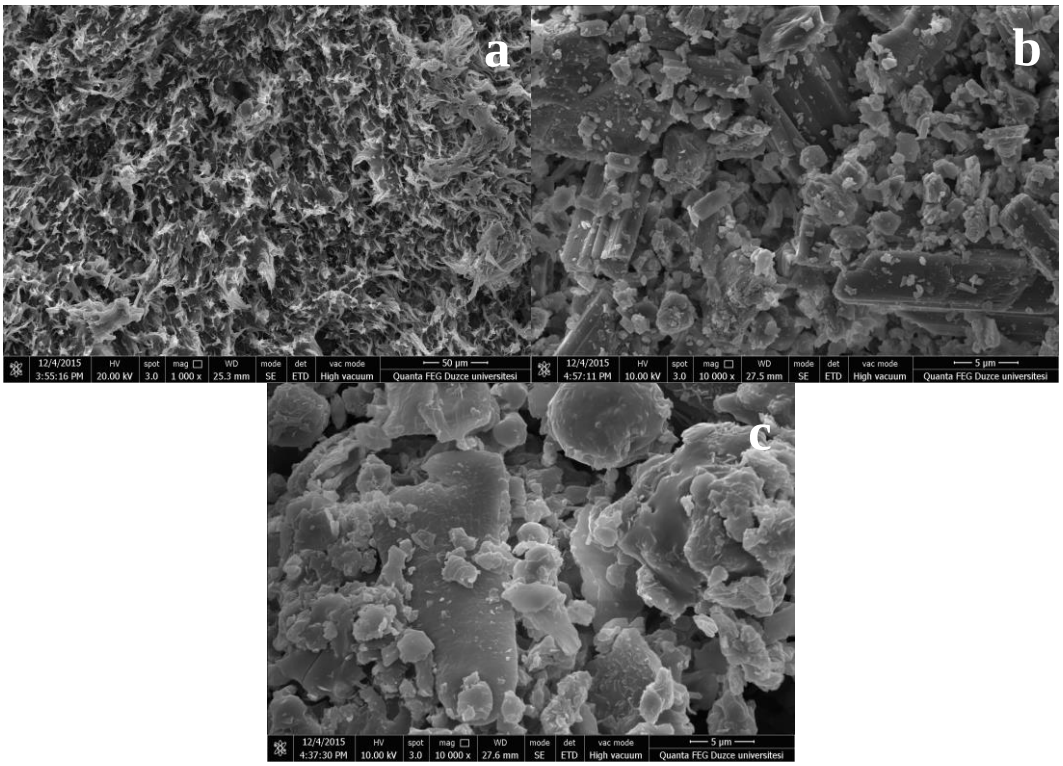

Figure 3.13: SEM images of a) cPP b) OLP c) U

SEM images captured from rupture surfaces after the tensile test of ulexite-reinforcedcomposites are given in Figure 3.14. When the images were examined, it was observed that the amount of ulexite mineral in the polymer matrix visibly increased after the amount of additives increased. Also when SEM images of ulexite-reinforcedcomposites were examined, it has been determined that ulexite particles did not show a homogeneous distribution with the polymer matrix. In particular, the agglomeration in the matrix has been occurred in parallel to increase the amount of additives in 10% and 15% ulexite-reinforcedcomposite materials. This case can be explained with that ulexite minerals not subjected to any surface modifications or binding agent was incompatible with the matrix. The agglomeration decreased the chemical bonding force between molecules besides restricting the interaction between the polymer molecules. This case supported the results obtained from tensile test analysis.

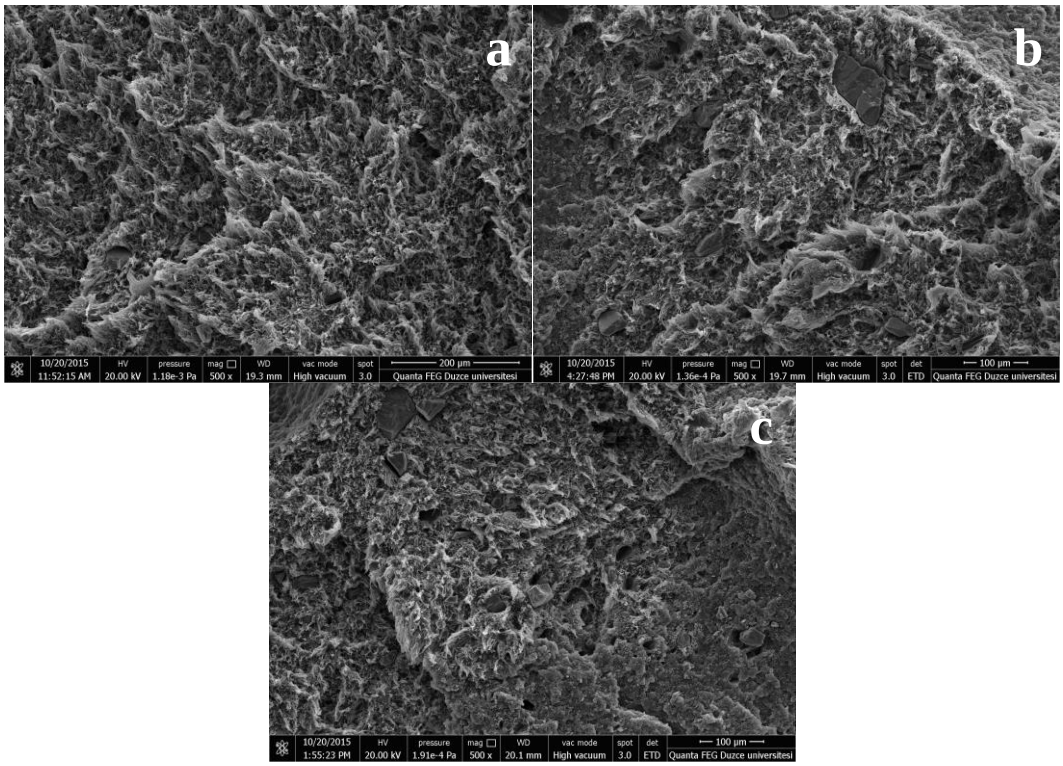

Figure 3.14: SEM images of a) cPP5U b) cPP10U c) cPP15U

SEM images captured from rupture surfaces after the tensile test of OLP-reinforcedcomposites are given in Figure 3.15. When SEM images were examined, it was observed that OLP distribution in the matrix increased in proportion to the amount of OLP added to the matrix structure. It was also observed that OLP showed a heterogeneous distribution in the matrix material depend on increasing in the amount of OLP. It was observed that OLP aggregated in the material matrix due to used without subjected to any modification despite of its organic structure. Likewise with the situation of ulexite-reinforcedcomposites, aggregated OLP particles intervene among polymer molecules and weaken the structure of chemical bonds. This case confirmed the tensile test analyzes.

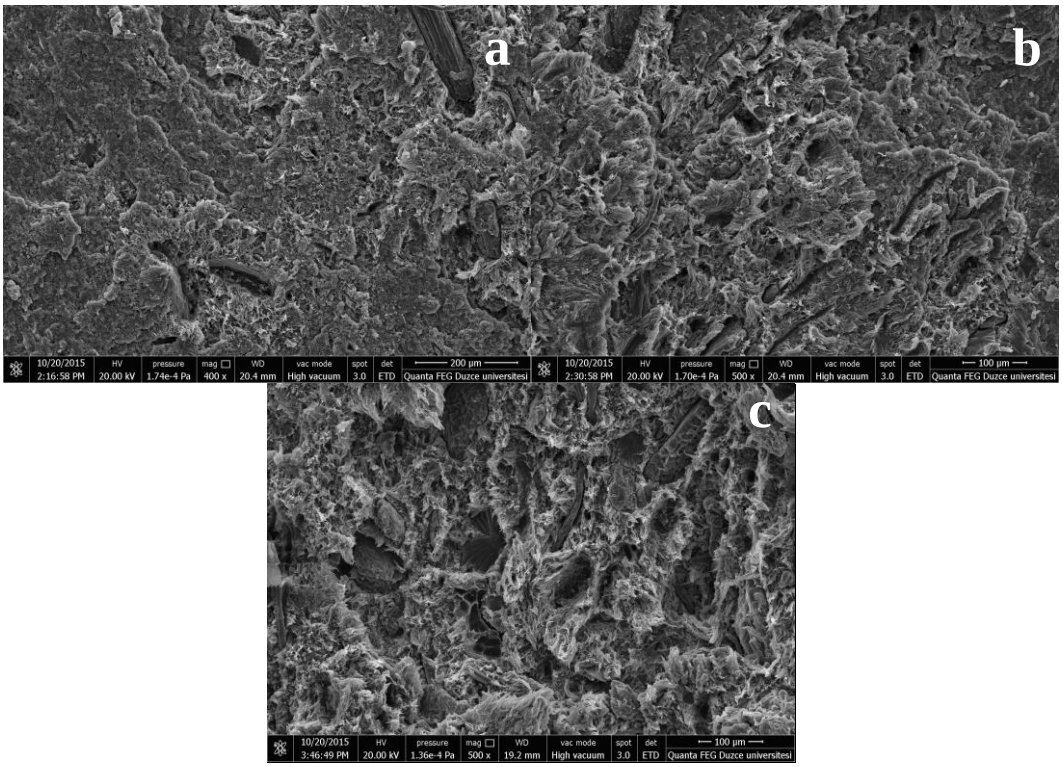

Figure 3.15: SEM images of a) cPP5OLP b) cPP10OLP c) cPP15OLP

SEM images taken from the rupture surfaces after the tensile test of Ulexite, Olive leaf powder and Ulexite-Olive leaf powder-reinforcedcomposites and are given in Figure 3.16. When the images were examined, it was observed that the distribution of OLP and ulexite in the polymer matrix visibly increased in case the amount of additives was increased. When SEM images of OLP-reinforced composites were analyzed, it has provided a more homogeneous appearance according to the individual use of additive particles with the polymer matrix. It was observed in SEM images that more agglomeration has occurred compared to the situation that they separately contributed, when the amount of additives increased depending on not using bonding agents with additive materials. This case can be the reason of the decline in tensile test results.

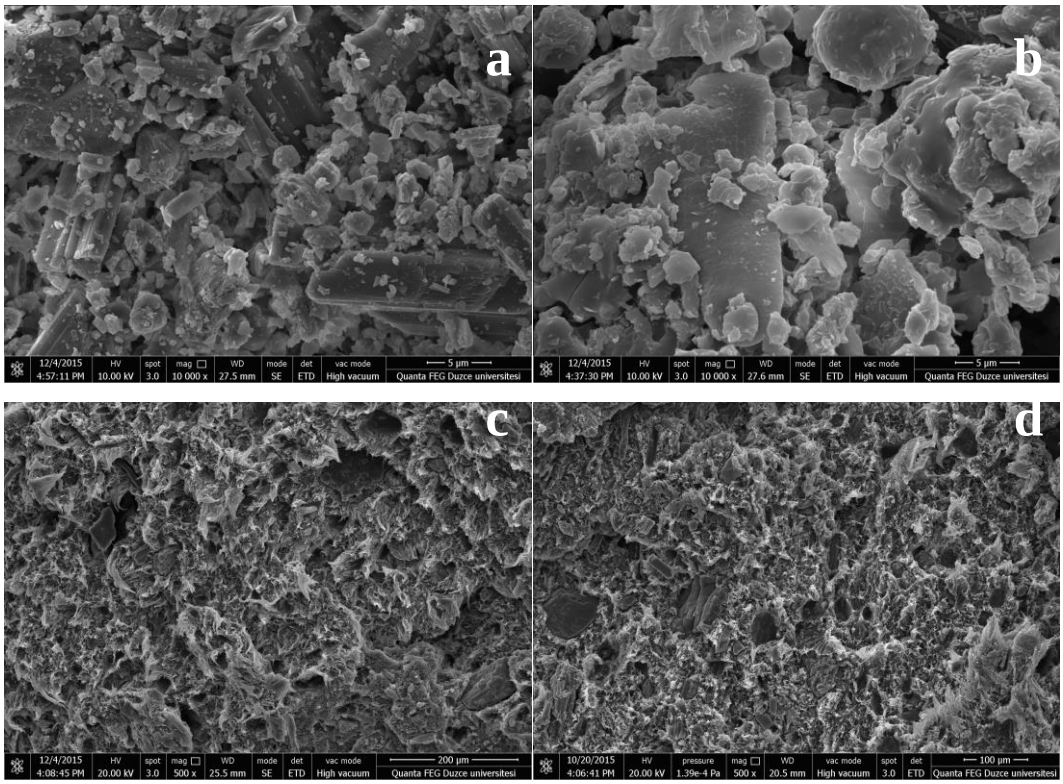

Figure 3.16: SEM images of a) U b) OLP c) cPP10U-10OLP d) cPP15U-15OLP

4. CONCLUSION

The scope of this study was to produce composites which have strong antimicrobial activity, especially highly effective against bacteria.

It was observed that composite materials, cPP10U and cPP10OLP, provided high antibacterial efficacy against bacteria, *E. coli* and *S. aureus*, respectively [Table 3.1]. On the other hand, high antibacterial effects of cPP10U-10OLP and cPP15U-15OLP composite materials against in both *E. coli and S. aureus* bacteria have been confirmed. A synergistic antimicrobial effect was achieved by utilizing both additive materials together. However, after aging test the efficiency of the microbial effect was reduced since there are no antimicrobial agents on the surface and used agents did not show their antibacterial properties without contacting the bacteria.

There are two approaches to illustrate this case;

1) All of the additive materials in copolymer were released easily and antibacterial effect has been lost during aging test because the agents that enable cohering of additive materials and copolymer have not been added. Chemical agents that will bond agents and matrix can be used to prevent this situation.
2) Additives materials [ulexite and olive leaf powder] on the composite material surface dissolved in the liquid medium during the aging test and thus remain without any effect. And rest of the agents remained within the polymer by being immobilized. Therefore, these fact can be one of the reasons for that situation. To prevent this case, controlled release of agents to surface of material can be carried out by adding biopolymers such as starch or cellulose into composite materials.

FTIR analysis results of manufactured composite materials, copolymers and additive materials (olive leaf powder and ulexite mineral) are examined and it was observed that the olive leaf powder and ulexite mineral interact with the matrix copolymer. This composite material production as a result of obtaining the desired

matrix/reinforcement material and both the matrix and reinforcement materials supports features such as protection of the properties.

As a result of the tensile testing analysis of the composite materials produced, tensile strength values did not change with adding ulexite mineral in 5%, 10% and 15% concentrations. When composite materials were compared with the other composite samples; the amounts of reinforcement materials increase with reduction in tensile strength the material. With the addition of the ulexite-reinforcedmaterial, reduction on elongation and at break value was observed. It was determined that this reduction increases by increasing of the amount of ulexite mineral.

The tensile strength of composites with olive leaf powder compared to the pure copolymer does not change. When the tensile strength values of the composites with olive leaf powder compared to the other composites; olive leaf powder ratio increases with tensile strength reduction. Elongation at break values were demonstrated a serious decline in pure copolymer.

For both additives were used in composite materials, the amount of the contribution material increased with reduction the value of the tensile strength and elongation at break.The reasons of reduction in tensile strength values of composite materials depending on increasing contribution rates were that the surface incompatibility of matrix/reinforcement and not disperse the reinforcement materials inside matrix materials homogeneous as a result of the use of high amounts of reinforcing material. Due to the heterogeneous distribution, by making a matrix material in lumps of material contributions, reduced percentage of the matrix unit cross section, the value of the stress caused to the fall. SEM images supported this result. The reasons of reduction in elongation at break values were the percentage of matrix decreased with the amount of reinforcement increased and polymer structure changed to crunchy because of that the chemical bonds could weaken between reinforcement powder and matrix polymer molecules.

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EĞİTİM

2013-2015	İzmir Katip Çelebi Üniversitesi Malzeme Bilimi ve Mühendisliği Bölümü
2007-2012	Mersin Üniversitesi, Fen-Edebiyat Fakültesi Biyoloji Bölümü (not ortalaması: 3.64 / 4)- Bölüm birincisi.
2001-2005	Naci Şensoy Lisesi (not ortalaması: 3.88 / 5)

Yabancı Diller: İngilizce

Bilgisayar Bilgisi: Microsoft Office; Excel, Word, PowerPoint, Mendeley, Endnote

Diğer Yetkinlikler: Avrupa Birliği projeleri, Santez,TEYDEB projeleri ve KOSGEB projeleri yazma, Patent araştırması yapma

PROJELER

YIL	**PROJE ADI**	**KURUM**
2013-2015	Çevreye Duyarlı ve Antimikrobiyal Malzeme Geliştirilmesi	ARÇELİK *Bilim Teknoloji ve Sanayi Bakanlığı (SANTEZ) Projesi Kapsamında*

EĞİTİMLER, SEMİNERLER VE KURSLAR

YIL	**ETKİNLİK ADI**	**KURUM**
2015	TEYDEB 1501/1507 Proje Yazım Atölyesi	Ege Üni. EBİLTEM Teknoloji Transfer Ofisi
2015	Buluş Değerlendirme Eğitimi	Staeger-Sperling
2015	AB Horizon 2020 Yeni KOBİ Aracı (New SME Instrument) Eğitimi	Ege Üni. EBİLTEM Teknoloji Transfer Ofisi
2015	Makale Yazım Atölyesi	ELSEVIER
2015	3.Ege Kozmetik Günleri	Ege Üniversitesi
2014	Fourier dönüşümlü kızılötesi spektrometresi (FTIR)	İzmir Katip Çelebi Üniversitesi
2014	Thermogravimetry (TGA)	İzmir Katip Çelebi Üniversitesi

2014	Reoloji	Anton Paar
2014	Dinamik mekanik analiz (DMA) Q800 operatör eğitimi TA Instruments	İzmir Katip Çelebi Üniversitesi
2013	X-Işını Kırınım yöntemi (XRD)	İzmir Katip Çelebi Üniversitesi
2013	Ekstrüzyon Yöntemi	İzmir Katip Çelebi Üniversitesi

STAJLAR

YIL	**BÖLÜM**	**KURUM**
2013	Biyokimya laboratuvarı	İzmir Dr. Suat Seren Göğüs Hastalıkları ve Cerrahisi Eğitim ve Araştırma Hastanesi

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2001	2. Resim Şenliği Birincilik Ödülü	Konak 1.Eğitim bölgesi
1997	Yaşar aksoy Özel Ödülü	Hürriyet

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