

CERTAIN CHEMICAL, PHYSICAL AND
MICROBIOLOGICAL PROPERTIES
OF THE FREEZE-DRIED (LYOPHILIZED)
APPLE OF CAIN/CANE APPLE
(*ARBUTUS UNEDO*) FRUIT

by

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ABSTRACT

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The apple of cain (*Arbutus unedo* L.) is an edible fruit which mostly grows in temperate zones of the world and Turkey, common to Black Sea region. The fruit were reported to have medicinal use concerning antidiabetic, diuretic, hypotensive, urinary antiseptic and anti-inflammatory activity. In this study, the fresh and freeze-dried *Arbutus unedo* fruits were investigated by means of certain antimicrobial, chemical and physical properties. It was found that the freeze-dried form of *Arbutus unedo* L. was sensitive to *E.coli*, *S.aureus*, *S.epidermidis* and *Salmonella spp.*, in addition there was no significant difference between freeze-dried and fresh fruits in color, pH, total soluble solids, vitamin C content, ash content, taste panel and SEM analysis.

Keywords: *Arbutus unedo*, Apple of cain, Freeze-drying.

ÖZET

DONDURUP KURUTULMUŞ KOCAYEMİŞ (*ARBUTUS UNEDO*) MEYVESİNİN BELİRLİ KİMYASAL, FİZİKSEL VE MİKROBİYOLOJİK ÖZELLİKLERİ

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Kocayemiş (*Arbutus unedo*), Türkiye'nin Karadeniz bölgesinde ve dünyada daha çok ılıman iklimlerde yetişen yenilebilir bir meyvedir. Bu meyvenin bazı tıbbi özellikleri vardır. Bunlar antidiyabetik, idrar söktürücü, tansiyon düşürücü, idrar yolu temizleyici ve iltihap sökücü gibi özelliklerdir. Bu çalışmada taze ve dondurulup kurutulmuş *Arbutus unedo* meyvesinin belirli fiziksel, kimyasal ve antimikrobiyal özellikleri araştırılmıştır. *Arbutus unedo*'nun dondurup kurutulmuş biçiminin *E.coli*, *S.aureus*, *S.epidermidis* ve *Salmonella spp.* bakterileri üzerinde duyarlı olduğu bulunmuştur. Ek olarak kurutulmuş ve taze meyve arasında pH, renk, toplam çözünmüş katı madde miktarı, vitamin C içeriği, ağırlığı, kül miktarı, tat testi ve SEM analizleri gibi bazı deneylerde çok önemli farklar görülmemiştir.

Anahtar Kelimeler: *Arbutus unedo*, Koca yemiş, Dondurup Kurutma.

Dedicated to My Husband

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List of Abbreviations

EPEC	Enteropathogenic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
EHEC	Enterohemorrhagic <i>E. coli</i>
spp	Species pluralis
pH	Power of Hydrogen
DPPH	2,2-diphenylpicrylhydrazyl
AAPH	2,2'-azobis (2-amidinopropane) hydrochloride
FSP	Freeze-dried Strawberry Powder
<i>CFU</i>	Colony-Forming Units
<i>m</i>	Meter
<i>cm</i>	Centimeter
<i>mm</i>	Millimeter
<i>g</i>	Gram
<i>mg</i>	Milligram
<i>ml</i>	Milliliter
μm	Micrometer
μl	Microliter
μg	Microgram
M	Molar Mass
°C	Centigrade

SEM	Scanning Electron Microscope
CIE	Commission Internationale d'Eclairage
NB	Nutrient Broth
NA	Nutrient Agar
KI	Potassium Iodide
KIO ₃	Potassium Iodate
Au	Gold
Pd	Palladium
h	Hour
bpm	Bit-patterned Media
<i>nm</i>	Nanometer
<i>kV</i>	Kilovolts
<i>mA</i>	Milliampere
Smp	Sample

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Chapter 1

INTRODUCTION

1.1 *Arbutus unedo*

Arbutus unedo is a fruit, which its populations are severely destroyed by deforestation and over-collecting. The species occurs in small scattered populations decaying progressively in size. As well, there is a lack of information relating the amount of genetic diversity and its repartition for this resource [1].

It is native to Greece, Lebanon, Ireland, Southern Europe and Anatolia and termed as a typical Mediterranean climate species [2]. However, the most important species of the *Arbutus* genus are *Arbutus unedo* L. and *Arbutus andrachne* L. In the natural flora of Turkey these two species of *Arbutus* genus are present [3]. *Arbutus* genus is native to the Mediterranean, Black Sea, the Marmara and Aegean coastal zone [4].



Figure 1.1: *Arbutus unedo* fruits.

In Black Sea Region, it is widespread in Sinop, Trabzon, Ordu, Giresun, Bolu, Düzce, Zonguldak and Artvin. In Mediterranean Region, it is present in Mersin, Muğla and Antalya. The other provinces in which *Arbutus* species existed are Balıkesir, Kocaeli, Sakarya, Hatay and Kahramanmaraş [5-7]. It is named belonging to the region: France 'Arbousier commun', Germany 'Erdbeerbaum', Greece 'Koumarja', Italy 'Mardono arboser' and Turkey 'Kocayemiş' [8]. In English, it is called Apple of Cain, Cane Apple or Strawberry Tree.

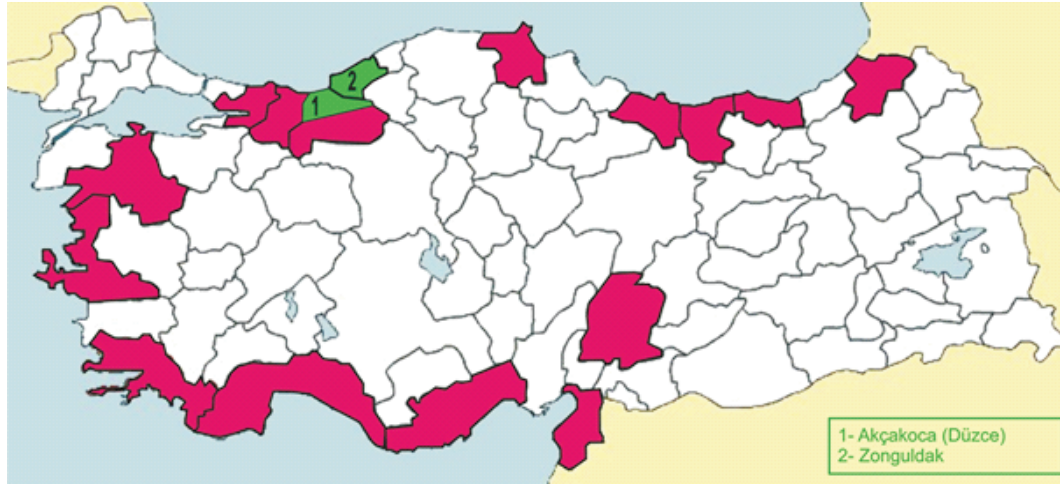


Figure 1.2: The distribution of *Arbutus unedo* in Turkey.

The tree is evergreen shrub or small with erect, branched stem, 5-10 *m* tall, with reddish brown bark becoming flaky with age, young branchlets thinly tomentose. Its leaves are alternate, elliptic, coriaceous, 5-10 *cm* long, 2-3 *cm* wide, with finely serrated margin and glabrous surface. The flowers are bisexual, fragrant, in 10-30 flowered, drooping racemes and sepal is persistent, connate basally, ovate to deltate; petals connate nearly their entire lengths, creamy white, corolla urceolate; stamens and ovary locular; stigma capitate. The fruits are globose to subglobose berries, 2 *cm* diameter, roughened tuberculate, finely

tomentose, green to yellow to bright red when ripe with 5 pyrenes, each with one seed [9].

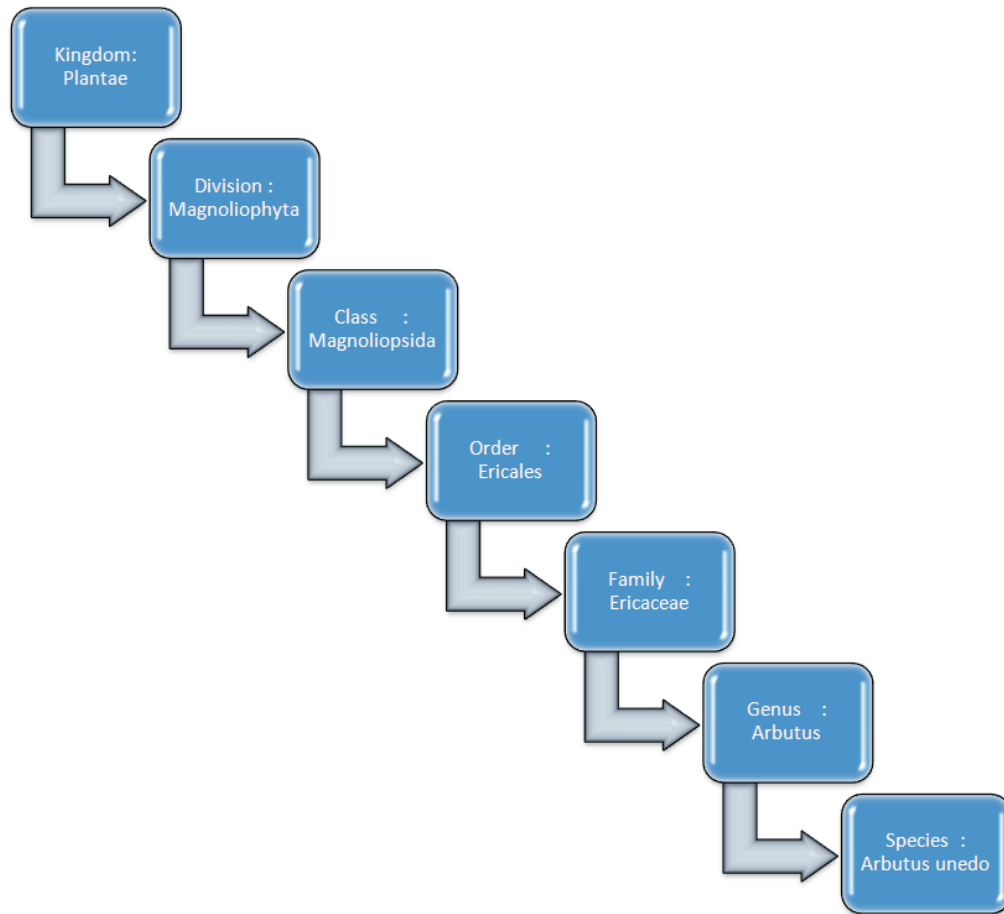


Figure 1.3: Taxonomic classification of *Arbutus unedo*.

The flowers are white, pink or light greenish-end parts. Their form is such as bell or water jug. Flowers are 8-9 *mm* long, aggregated in compound panicle [2, 10], with a length of 6-10 *cm* tall. Flowering period coincides with the period of maturation of the fruits of the previous year. However, the flowers are seen busy in November and December.

Its fruits has an average length of 15-20 *mm*, 4-8 *g* weight. The outer surface of the fruit is rough. Fruit color turns from green to yellow at the end of the summer. In November and December, the fruits become mature and color turns

from yellow to red or orange. Batches of the fruit remain on the tree for a long time and matured ones fall down the tree in time.

Arbutus unedo possesses many medicinal uses; it is used as an antidiabetic, diuretic, hypotensive, urinary antiseptic, astringent, anti-inflammatory and depurative, and it also shows antioxidant activity [12]. Although the fruits of the strawberry tree (*Arbutus unedo* L.) are consumed mainly as processed product, they may be a good source of antioxidants if consumed as fresh [13]. They contain a wide and excellent range of antioxidant components including phenolic compounds (e.g. anthocyanins, gallic acid derivatives, flavonoids and tannins), vitamin C, vitamin E and carotenoids suggesting a potentially high value as a 'health promoting food' [14].

1.2 Antimicrobial Activity

Many different methods have been in use for the treatment of pathogenic diseases. In recent years, infections in humans have been usually treated by commercial (synthetic) antimicrobial drugs but resistance to them have gained importance due to their extensive use. Medicinal plants have been regarded as alternative in fighting with these pathogenic strains [15].

Even before the discovery of the existence of microbes, it was a common belief that certain plants contained potential healing features which are characterized as antimicrobial principles [16]. Although historically plants have provided a good source of anti-infective agents in the fight against microbial infections, by the advent of antibiotics in the 1950's the use of plant derivatives as antimicrobials has been ignored [17, 18]. Several scientific studies have also indicated the importance of new bioactive phytochemicals against multi-drug resistant bacteria.

1.3 Review of the Literature

According to **Pallauf et al. (2007)** the antioxidant components present in apple of cain includes flavonoids, vitamins C and E and the carotenoids. The fruits are a very good source of antioxidants precisely because they have a high flavonoid content (32.37 mg/100 g edible portion) and, within this group of antioxidant compounds, proanthocyanidins are the most abundant, representing more than 80% of the total flavonoid contents [13].

In a study, conducted by **Yurdugul, (2008)** [19] alpine strawberries (*Fragaria vesca*) were compared in fresh and freeze-dried forms, according to their chemical, physical and microbiological parameters. They have been used in fruit juice, marmalade, or jam production and as a result have economical importance in food sector. The fresh alpine strawberries have a tendency to lose their quality in a short period as a result of high water loss and spoilage. The effects of freeze drying process on the characteristics of the alpine strawberries, such as firmness, sugar content, pH, color, weight loss, dissolved solids, anthocyanin and vitamin C content with reference to the fresh, are investigated. Freeze-drying indicated no difference in the characteristics of the alpine strawberries when compared with the fresh. It is found that a slight acid or base addition onto the rehydrated alpine strawberry juice preserved the stability of pigments and the color. In addition, the rehydrated alpine strawberry juice exhibited an antimicrobial activity towards an important foodborne pathogen, *Enterobacter faecium* ATCC 6057.

Ruiz-Rodriquez et al. (2010) studied the nutritional value and the biomass production of wild strawberry-tree fruits, according to the variation of these characteristics in fruits harvested in different years, from two different

Spanish areas [20].

According to **Mendes et al. (2011)** assays on the antioxidant properties of *Arbutus unedo* L. leaf and fruit extracts using different in vitro assays including (i) reducing power, (ii) scavenging effect on DPPH free radicals and (iii) inhibitory effect on AAPH-induced hemolysis and lipid peroxidation in human erythrocytes demonstrated that the antioxidant efficiency for *A. unedo* L. aqueous extracts, were found to be higher in the leaf [21].

Malheiro et al. (2012) studied nineteen *Arbutus unedo* L. leaves genotypes, concerning their bioactive properties. *A. unedo* leaves contain appreciable amounts of total phenols. Extraordinary antioxidant and antimicrobial properties were revealed by the extracts and they were found to be suitable to be used in food and pharmaceutical industry [22].

According to **Basu et al. (2009)** strawberry flavonoids are potent antioxidants and anti-inflammatory agents that have been shown to reduce cardiovascular disease risk factors in prospective cohort studies. The freeze-dried strawberry powder (FSP) will lower fasting lipids and biomarkers of oxidative stress and inflammation at four weeks compared to baseline due to its polyphenolic flavonoids, fiber and phytosterols [23].

Ascorbic acid content per 100 g of edible portion ranged between 0.925 ± 0.018 mg for kiwano fruit and 117 ± 1.64 mg for *Arbutus spp.* by **Costa et al.(2011)** [24].

Alarcaão-E-Silva et al. (2001) studied the determination of some physical and chemical characteristics of fresh strawberry-tree fruit (*Arbutus unedo* L.), a typical Mediterranean climate species, namely of the Portuguese region of Algarve. It intends to be a contribution to its valorization and other applications following research by other scientists and local development associations as far as better quality of alcoholic distillate of arbutus berry is concerned. Arbu-

tus berry appears to be a good source of vitamins, namely niacin, ascorbic acid and β (beta)-carotene (content of 9.1, 346.3 and 70.9 *mg*/100 *g*, respectively), organic acids (nearly 9%), total sugars (c. 42%) and tannins (1.75 *mg*) [25].

Santo et al. (2012) implied that *Arbutus unedo* is an agro-sustainable resource in the Mediterranean region. Yeast succession during the solid-state fermentation of *A. unedo* fruits was studied and found that *Arbutus* fruits are a source of yeasts with potential biotechnological applications. Strains of *S. cerevisiae* tolerant to ethanol and killer toxins were isolated [26].

According to **Demirsoy et al. (2008)** and **Sakaldas (2012)** [27, 28], *Arbutus unedo* L. fruits are native to the vegetation of Black Sea and Çanakkale, respectively.

1.4 Examples of Fruit Based Extracts on Important Pathogenic Strains

1.4.1 *Escherichia coli*

Escherichia coli is a gram-negative, non-sporeforming, rod-shaped, mesophilic and facultative anaerobic, enteric bacterium [29, 30]. *E. coli* is a common part of the normal microflora in the intestinal tract of healthy human and warm-blooded animals [31]. Not all strains of *E. coli* are pathogenic for humans, even harmless strains are normally found in a healthy intestine. Pathogenic strains are split into four main groups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC) based on their virulence mechanism. Besides being acid and low tolerant, it is even able to survive in acidic and semi-dry foods that are considered as safe from food-borne pathogens[32]. However, it does not have a

tolerance to salt concentration higher than 6.5 % [33]. The most well-known member of EHEC group is *E. coli* O157:H7 which was first identified as a food-borne pathogen in 1982 [34]. The major reservoir of *E. coli* O157:H7 is the intestines of cattle but it can also be nestled in sheep, goats, pigs, horses, dogs, poultry and deer. Thus, feces of these animals could pollute the environment easily [35]. *E. coli* O157:H7 could survive in the environment for more than 10 months. Therefore, it most likely contaminates foods which are in contact with the contaminated environment or meat during slaughtering of cattle and other farm animals [36]. Grinding of meat would cause penetration of bacteria into minced meat that would be protected from heat treatment or cooking [37]. In many countries in the world, animal manures, especially bovine are used as pond fertilizer. Thus, pond water contains risk factor of pathogenic *E. coli* strains [38]. The major sources of this pathogen are uncooked and undercooked ground beef, lamb, dry cured salami, pork, poultry, and raw milk, raw vegetables, unpasteurized fruit juices and water. Transmission of this bacterium is primarily through foods and water, and then directly from person to person or animal to person [32, 33]. In a traced outbreak in Washington State and California, the infection dose for this pathogen was determined as fewer than 50 organisms [39]. It firstly invades and colonizes in small intestine. After 3-4 days incubation period, hemorrhagic colitis complaints appear as severe abdominal cramps and watery diarrhea. It can cause mortality in children.

1.4.2 *Salmonella spp*

The genus *Salmonella spp.* is composed of motile bacteria that conform to definition of the family Enterobacteriaceae. *Salmonella spp.* is a mesophilic, gram-negative, motile, rod shaped, non-sporeforming and facultative anaer-

obic bacterium like other closely related enteric bacteria [29, 37]. Optimal growth temperature and pH of *Salmonella spp.* are 35-37°C and 6.5-7.5, respectively. However, it can survive at the temperature range of 5-45°C and at pH 4-9. *Salmonella spp.* cause an intestinal infection (accompanied by diarrhea, fever, abdominal cramps) that often lasts 1 week or longer. *Salmonella typhii*, *Salmonella paratyphii*, *Salmonella enteritidis* are the main representatives of this species. Persons of all ages are affected ; the incidence is highest in infants. *Salmonella spp.* is ubiquitous in animal populations, and human illness is usually linked to foods of animal origin. Salmonellosis also is transmitted by direct contact with animals, non-animal foods, by water, and occasionally, by human contact. *Salmonella spp.* is responsible for 14 % of all salmonellosis cases caused by other *Salmonella serovars*. According to 2003 surveillance data in the US, 4890 cases due to *Salmonella* infection were reported, but the real number was estimated as 185829 [40]. Rodents are the main animal reservoir for *S. enteritidis*; therefore, this bacterium is introduced into poultries from rodents [41]. The organism contaminates eggs internally by transovarian transmission and these pathogens pass into the eggs before the egg shell forms [40-42]. Each year, an estimated 1 million to 4 million cases of illness and 1.000 deaths are caused by nontyphoidal salmonellosis in the United States.

1.4.3 *Staphylococcus aureus*

S. aureus appears as gram-positive cocci in grape-like clusters microscopically. They are non-motile, non-spore forming, spherical cells of 1 μm in diameter. On a nutrient rich medium, the organism forms large yellow colonies, whereas *S. aureus* forms relatively small white colonies. The Staphylococci are facultative anaerobes and they can grow by aerobic respiration or by fermenta-

tion producing lactic acid. The organism is able to grow at a wide temperature range of 15°C to 45°C, a pH range of 4 to 11 and at NaCl concentration as high as 15%. The ability of the organism to grow in such wide temperature, pH, salt concentrations makes it a common foodborne pathogen. The bacteria are catalase-positive and oxidase-negative. It is hemolytic on blood agar and almost all strains of *S. aureus* produce the enzyme, coagulase. The organism ferments glucose and mannitol producing lactic acid.

The *Staphylococci* are common inhabitants of the nasal passage, skin and other anatomical locales on humans and other warm blooded mammals. *S. aureus* is a very significant bacterium as it causes a number of suppurative infections by its toxins in human and animals [43].

1.4.4 *Staphylococcus epidermidis*

S. epidermidis forms white colonies on blood agar. It is catalase positive, oxidase negative and does not ferment mannitol. Tolerates salt, survives drying and is highly antibiotic resistant. It is normal skin commensal. Carriage rate is as high as 100%. This bacterium is transmitted by self-inoculation or by contact with infected patients.

The ability to produce slime is an important virulence factor of the bacterium. *S. epidermidis* causes infection by adhering itself to the surface of the intravenous plastic catheters and prosthetic devices. The adherence is believed to be facilitated by polysaccharide glycocalyx known as slime, produced in large quantities by the bacteria. Slime also inhibits the action of lymphocytes and neutrophils.

1.5 The Aim and Scope of the Study

The apple of cain (*Arbutus unedo*) was believed to cure certain diseases in folk medicine such as diarrhea and constipation in the Black Sea region. This study aims to prolonge the shelf life of this fruit by freeze-drying and to test its effectiveness on certain microorganisms and chemical and physical characteristics by comparing with the fresh. According to its success, it may be involved in different food sectors like fruit juice, pastry, confectionery etc.

Chapter 2

MATERIALS and METHODS

2.1 Materials

2.1.1 *Arbutus unedo*

Arbutus unedo were harvested from Akçakoca county of Düzce, Turkey and the environs of Zonguldak, Turkey in November 2012. The fruits were immediately brought to the laboratory (Abant İzzet Baysal University, Department of Biology, Bolu/Turkey) and the defective ones were eliminated. After washing under tap water, the apple of cain fruits were stored at -80°C for further studies.

2.1.2 Microorganisms

The microorganism used in this study (*Staphylococcus aureus* 25923, *Staphylococcus epidermidis* 12228, *E. coli*, O157:H7 and *Salmonella* spp.) were obtained from the Provincial Directorate of the Agriculture, Bolu and propagated in the relative agar medium.

2.2 Method

2.2.1 Antibacterial Assay

An extract of *Arbutus unedo* was prepared to investigate the antimicrobial activity in the study by direct extraction method. The disc diffusion assay (Kirby-Bauer method) was used to screen the antimicrobial activity [44]. Four bacterial strains were used in the bioassay: *E. coli* (O157:H7), *Salmonella spp.* which are gram-negative and *Staphylococcus aureus* (ATTC 25923) and *Staphylococcus epidermidis* (ATCC 12228), as gram-positive. Each strain was transferred to test tubes containing 2 *ml* of Nutrient Broth (NB) and incubated overnight at 37°C. After incubation, a loop from each broth was streaked on Nutrient Agar (NA) plates and incubated for 2 days at 37°C. After 2 days, a single colony was removed and streaked on a new NA plate for pure culture and incubated 37°C for 2 additional days. After 2 days, 1-2 loops of pure culture were transferred to 10 *ml* of 0.9% saline suspension in a test tube for each bacterial strain. Each suspension is adjusted to obtain turbidity, comparable to that of a 0.5 McFarland standard (1.5×10^8 CFU/*ml*). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly streak the entire surface of a (60 x 15 *mm*) Mueller-Hinton (M-H) agar plate (One test tube was used for three agar plate).

All freeze dried and fresh samples were dissolved in ethanol in order to obtain a final concentration of 101 *mg/ml*. Then, all dissolved extracts were sterilized by filtering through a 0.22 μm filter (Pal-Gelman Laboratory) and sterile filter paper discs (Glass Microfibre filters, Whatman; 6 *mm* in diameter) were impregnated with 13 μl of extract. There were three replicates in each plate and two plates for each extract tested for each bacterium. One antimicrobial

susceptibility test disc was used as positive controls which consist of (Bioanalyse): Erythromycin ($15\ \mu g$) (E-15), Carbenicillin ($100\ \mu g$) (CB-100), antibiotic discs were used for each plate and run in duplicate. Inoculated plates with discs were placed in a 37°C incubator. After 16 to 18 *h* of incubation, the diameters (*mm*) of the inhibition zones were measured. All experiments were repeated in triplicates.

2.2.2 The Physical and Chemical Analysis

2.2.2.1 The Determination of Vitamin C by Iodine Titration

The determination of vitamin C procedure;

- 1% Starch Indicator solution was prepared by adding 0.50 *g* soluble starch to one liter distilled water, which was about to boil, it was mixed well and allowed to cool before use. The iodine solution was prepared by dissolving 5 *g* potassium iodide (KI) and 0.268 *g* potassium iodate (KIO_3) in 200 *mL* of distilled water. To this mixture, 30 *mL* of 3M sulfuric acid was added and diluted to a final volume of 500 *mL* with distilled water by mixing. The solution was mixed and transferred to a 600 *mL* beaker.
- Vitamin C Standard Solution was prepared by dissolving 0.250 *g* vitamin C (ascorbic acid) in 100 *ml* distilled water and it was diluted to 250 *mL* with distilled water in a volumetric flask.
- In order to standardize the solutions, 25 *mL* of vitamin C standard solution was added to a 125 *mL* erlenmeyer flask. 10 drops of 1% starch solution was subsequently mixed and transferred to the buret by rinsing with a small volume of the iodine solution. The initial volume was recorded and the solution was titrated until the endpoint is reached and blue color was

observed. The final volume of iodine solution was recorded. The volume required is the starting volume minus the final volume. The titration was performed in triplicates.

2.2.2.2 The Determination of pH

The pH of the homogenized samples of apple of cain were detected by a pH meter (Jenway Scientific model-3505) supported by a solid electrode, in triplicates.

2.2.2.3 Determination of Color

The color determination was carried out by colorimeter which name is CR-400, according to L*, a*, b* principles. L* represents lighter or darker, a* represents red or green and b* represents yellow or blue.

2.2.2.4 The Weight Determination

The fruits were weighed by a digital balance in triplicates.

2.2.2.5 The Soluble Solid Content (°Brix)

The soluble solid content was measured for fresh (1 mg/100 mL) and the freeze-dried (1 mg/mL) apple of cain fruits by a refractometer in triplicates after homogenization of the samples.

2.2.2.6 Determination of Ash Content

The ash content of the fresh and freeze dried fruits were detected by the gravimetric procedure in triplicates. 1.0 g of *Arbutus unedo* sample was weighed into a porcelain crucible at constant weight. The fruit was first dried in a desiccator for 24 h. The crucible was then placed on a hotplate and the dried

sample was charred at 525°C for 6 *h* in an oven. After cooling the crucible in a desiccator, it was reweighed and % ash was calculated as:

$$\% \text{ ash} = \text{weight of residue} \times 100 / \text{weight of sample (p<0.01)}.$$

2.2.2.7 Taste Panel

Fifteen panelists were evaluated the fresh fruits and freeze-dried samples of apple of cain, according to texture, color, appearance, taste and flavor with a point scale (1 to 5).

2.2.2.8 SEM Analysis

The freeze-dried and fresh samples of apple of cain were studied by using scanning electron microscope (SEM) JEOL 6390-LV, operated at 20 *kV*, with a resolution power of 3 *nm*.

2.2.2.9 Statistical Analysis

Statistical analysis is normally referred to a collection of methods that are used in processing large amount of information or data and also report the overall trends. The experiments started with two species per treatment comprising at least three replicates of fresh fruit and freeze-dried fruits. The statistical analysis was performed by ANOVA (STATISTICA 6) and the significantly different means ($P \leq 0.05$ or $P \leq 0.01$) were identified by using Duncan's test [45].

Chapter 3

RESULTS and DISCUSSION

A wide variability in nutrient composition of strawberry-tree fruits was found in different chemical composition, depending on the year or location of harvest. Results have shown that an individual sample may not be a whole representative of the mentioned variety. The analysis of two different samples from different origins are required to provide average reliable data about the chemical composition of wild fruits.

3.1 The Physical and Chemical Properties

3.1.1 pH Values

The pH of *Arbutus unedo* (100 mL squeezed sample) was found to be ranging between 3.67 – 3.84 for Akçakoca and 2.76 – 2.91 for Zonguldak varieties. Various studies pointed out that 3.21 – 7.66 range was declared for *Arbutus unedo* in different varieties. The pH difference was possibly attributable to climatic conditions and the texture of soil in Figure 3.1 (Appendix A.1) and statistical evaluation of pH (Appendix B.1). The samples were found to be statistically different ($p < 0.05$).

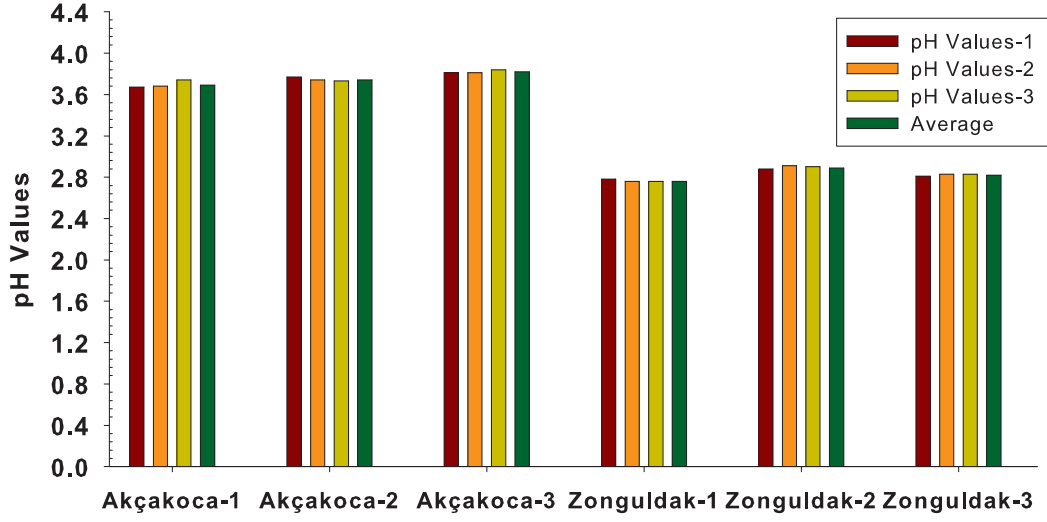


Figure 3.1: The pH values of *Arbutus unedo* fresh fruit samples from Akçakoca and Zonguldak varieties.

3.1.2 Color Measurements

The color of the fresh and freeze dried were measured with a Minolta Chroma Meter CR-400 portable colorimeter (Minolta Corp, Osaka, Japan) and recorded in Commission Internationale d'Eclairage (CIE) color space coordinates (L^* , a^* and b^*). The colorimeter was standardized by using the Minolta calibration plate CR.A43 before each measurement date. Hue angle was calculated as arctangent (b^*/a^*) 57.3 and expressed in degrees.

Both of the fresh and freeze-dried samples of *Arbutus unedo* were measured by chromometer. The L^* value indicates lightness ($L^* = 0$ darkness, $L^* = 100$ lightness); a^* value indicates redness ($+60 = \text{red}$, $60 = \text{green}$) and b^* value indicates yellowness ($+60 = \text{yellow}$, $60 = \text{blue}$). Respectively L^* , a^* , b^* values were found to be 21.93, 1.02 and 1.58 for Akçakoca fresh samples in Table 3.1 and 21.51, 0.81 and 1.80 for Akçakoca freeze-dried samples in Table 3.2. The samples in Zonguldak were measured and L^* , a^* , b^* values 22.69, -0.95, 1.91 for

fresh samples in Table 3.1 and 22.59, -0.95, 1.03 for freeze-dried samples in Table 3.2. According to Ganhao et al. [46], L^* , a^* , b^* values of *Arbutus unedo* were found to be 65.24, 3.90 and 13.04. The statistical evaluation of color was shown in Appendix B.2 and Appendix B.3. The samples were found to be statistically different ($p < 0.05$).

Table 3.1: The color values of *Arbutus unedo* fresh samples from Akçakoca and Zonguldak varieties.

	Akçakoca-1	Akçakoca-2	Zonguldak-1	Zonguldak-2
	Fresh Samples	Fresh Samples	Fresh Samples	Fresh Samples
L^* values	20.66	22.08	21.41	23.14
	20.75	22.28	21.56	23.63
	20.88	22.42	21.61	24.19
a^* values	0.79	1.01	-0.52	-1.17
	0.67	0.91	-0.58	-1.36
	0.59	0.89	-0.58	-1.52
b^* values	1.23	2.27	0.17	1.51
	1.28	2.34	0.26	1.51
	1.31	2.38	0.28	2.05

Table 3.2: The color values of *Arbutus unedo* freeze dried samples from Akçakoca and Zonguldak varieties.

	Akçakoca-1 Freeze-dried Samples	Akçakoca-2 Freeze-dried Samples	Zonguldak-1 Freeze-dried Samples	Zonguldak-2 Freeze-dried Samples
L* values	21.77 21.79 22.17	21.89 21.97 22.01	22.55 22.58 22.63	22.38 22.79 22.81
a* values	1.13 1.03 0.99	1.00 0.96 0.97	-0.79 -0.80 -0.84	-0.99 -1.12 -1.18
b* values	1.44 1.50 1.52	1.67 1.67 1.73	1.66 1.73 1.74	2.11 2.11 2.15

3.1.3 The Weight Determination

The fruits of apple of cain were weighed by a digital balance for six times. The average values were 5.34 and 5.54 for the fresh samples of Akçakoca and 4.88 and 4.73 for fresh samples of Zonguldak varieties. According to Demirsoy et al. the fruits were weighted 11.08, 6.17, 6.30, 6.95 and 8.06 g, respectively [27](Appendix A.2) in Figure 3.2. The groups were found to be statistically different ($p < 0.05$) (Appendix B.4).

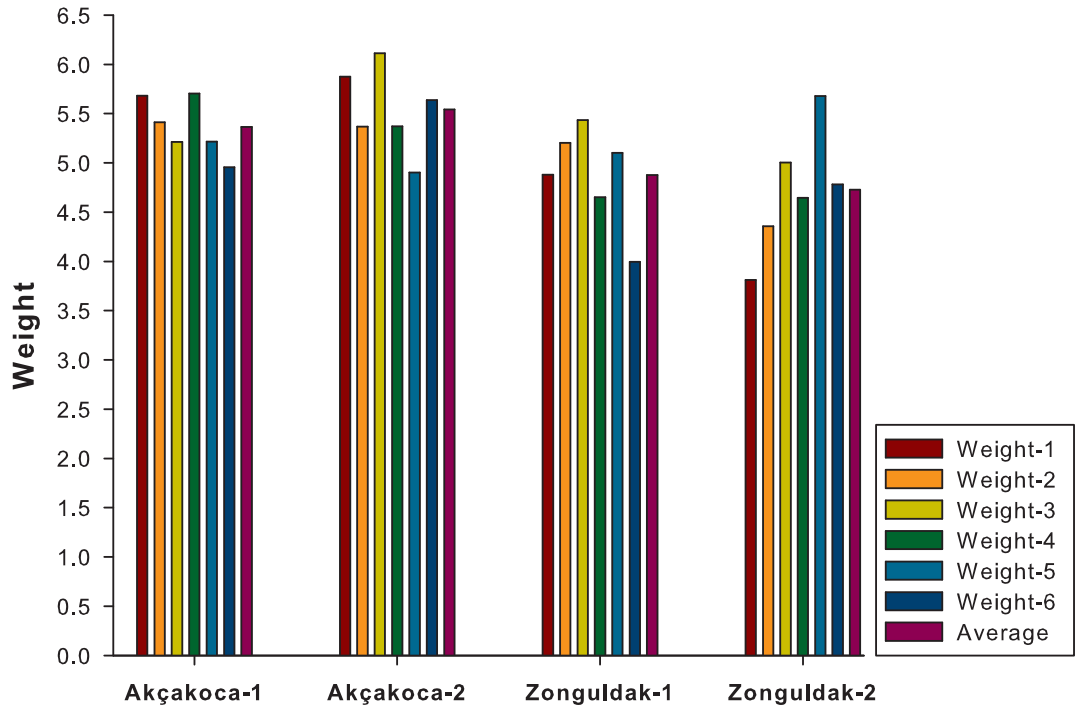


Figure 3.2: The weights of Akçakoca and Zonguldak varieties.

3.1.4 The Soluble Solid Content (°Brix)

The soluble solid content were measured for the fresh (1 mg/100 mL) and freeze dried *Arbutus unedo* by a refractometer. The soluble solid content of fresh fruits were 14.74%, 14.02% in Akçakoca and 13.34%, 13.51% in Zonguldak. The freeze dried samples were found to be 16.74%, 16.77% in Akçakoca and 15.27%, 15.68% in Zonguldak (A1-A2 means of Akçakoca and Z1-Z2 means of Zonguldak) in Figure 3.3. In another study, they were found to be ranging between 15.6% and 24.0% [27](Appendix A.3). The groups were found to be statistically different ($p < 0.05$) (Appendix B.5 and Appendix B.6).

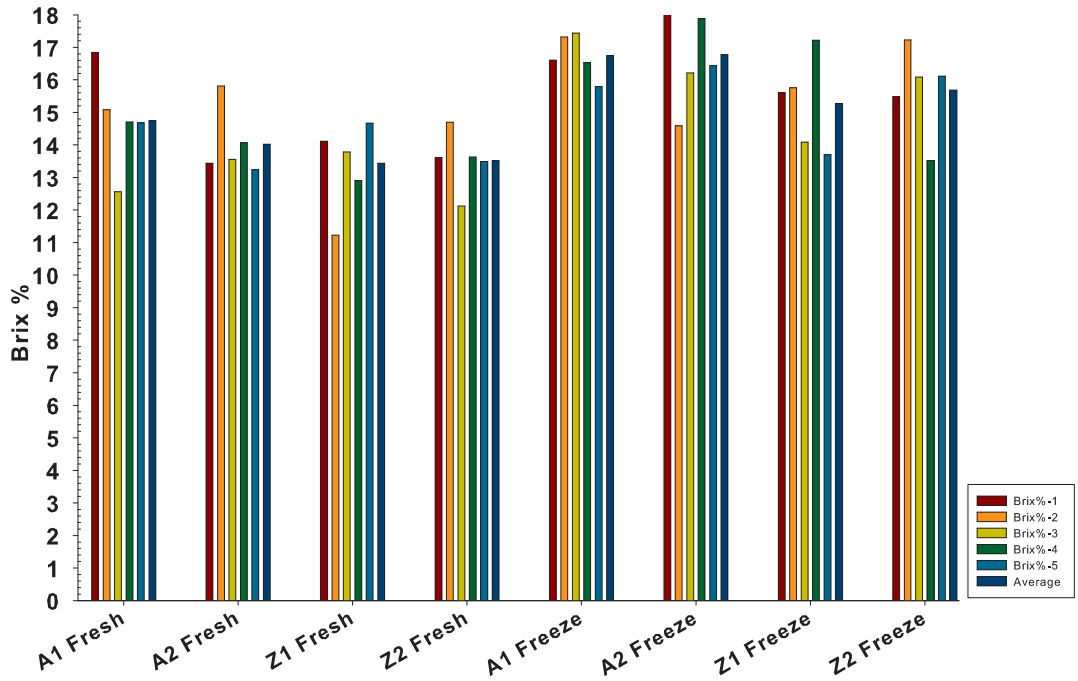


Figure 3.3: The °Brix of Akçakoca and Zonguldak varieties.

3.1.5 Vitamin C Content

The average concentration of vitamin C was found to be 55 *mg/ml* in Akçakoca and 53 *mg/ml* in Zonguldak varieties of freeze dried *Arbutus unedo* (Figure 3.4). Silva et al. reported that the ascorbic acid content of the fresh *Arbutus unedo* was in line with this study [25](Appendix A.4). The samples were found to be statistically different ($p < 0.05$) (Appendix B.7).

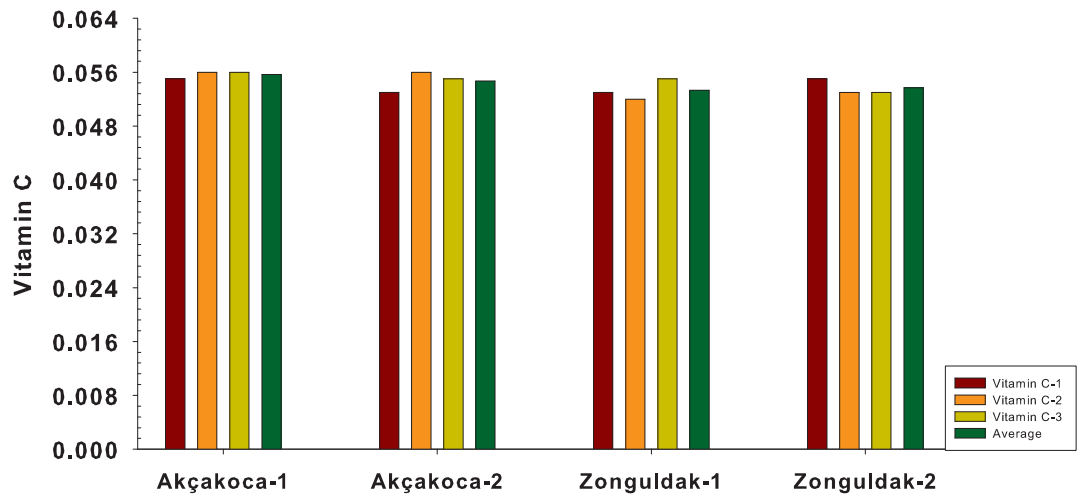


Figure 3.4: Vitamin C contents of *Arbutus unedo*.

3.1.6 Ash Content Values

The ash content of the fresh fruits of *Arbutus unedo* was detected by the gravimetric procedure. A1, A2, A3, A4 represents Akçakoca and Z1, Z2, Z3, Z4 stands for Zonguldak. The ash content was tabulated in Table 3.3 and Table 3.4. The procedure was repeated in quadruples. Other studies had revealed 2.824 [47, 20] and 0.864 [20] grams, therefore these values were found to be slightly greater than ours. The samples were found to be statistically different ($p < 0.05$) (Appendix B.8).

Table 3.3: **The ash content of *Arbutus unedo* (Akçakoca variety).**

Fruit No	Fruit Weight (g)	Tare (g)	Crucible+Fruit (g)	Crucible Results	Results (g)	%
A1	3.0019	17.9465 ± 0.0005	19.6686	17.9776	0.0311	1.036
A2	3.2345	16.7584 ± 0.0005	19.0258	16.7390	0.0194	0.599
A3	2.7652	16.7005 ± 0.0005	18.6431	16.6712	0.0293	1.059
A4	2.6651	18.1210 ± 0.0005	19.4131	18.1447	0.0237	0.889

Table 3.4: **The ash content of *Arbutus unedo* (Zonguldak variety).**

Fruit No	Fruit Weight (g)	Tare (g)	Crucible+Fruit (g)	Crucible Results	Results (g)	%
Z1	2.4982	16.3347 ± 0.0005	18.1355	16.3580	0.0233	0.932
Z2	2.3301	17.4881 ± 0.0005	19.0705	17.5081	0.0200	0.858
Z3	2.7519	16.2925 ± 0.0005	18.3791	16.3220	0.0295	1.071
Z4	2.5528	16.2925 ± 0.0005	18.3791	16.3220	0.0295	1.071

3.1.7 The Taste Panel

Fifteen panelists evaluated the fresh fruits and freeze-dried samples of apple of cain, according to texture, color, appearance, taste and flavor with a point scale (one to five). The flavor of *Arbutus unedo* was the most liked characteristic according to the panelists (Table 3.5). The samples were found to be statistically different ($p < 0.05$) (Appendix B.9).

Table 3.5: *Arbutus unedo* taste panel results.

Point	Texture	Color	Appearance	Taste	Flavour
1	7	-	-	-	-
2	4	-	2	2	-
3	2	3	5	1	-
4	1	5	4	4	1
5	1	7	4	8	14
%	40	85	73	84	98

3.1.8 SEM Analysis

Freeze-dried and fresh samples of *Arbutus unedo* were analyzed by using a scanning electron microscope (SEM) JEOL 6390-LV, operated at 20 *kV*, with a resolution power of 3 *nm*. Sections were obtained from the portions of fruit bowel and surface for fresh fruits. The freeze dried samples were grinded to powder. Both freeze-dried and fresh samples were covered by a carbon sticker. These carbon stickers were coated with gold (Au) and Palladium (Pd) with Ion Coater (KIC-IA Current: 4 *mA*, Time: 200 seconds). After the coating process, samples were placed in SEM. The samples were scanned by different magnification (120bpm, 500bpm, 800bpm, 1000bpm). The freeze dried samples in Akçakoca and Zonguldak were observed as waxy structure under SEM due to their powdered form (Figure 3.5 and Figure 3.6). The bowel fresh samples in Akçakoca were observed like fibrous structure. Filamentous structures were more clearly observed in the bowel part rather than the surface. In all samples the fibers were observed in both of the bowel and surface sections of *Arbutus unedo* (Figure 3.7 and 3.8).

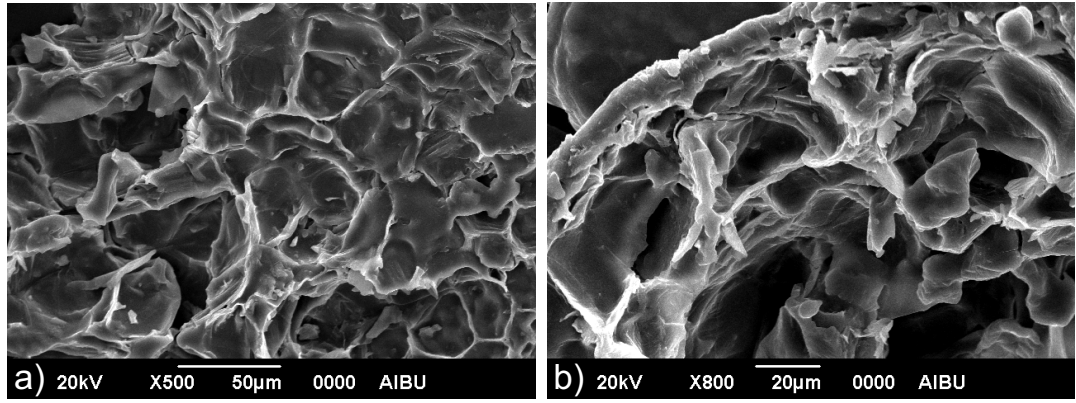


Figure 3.5: a) Freeze-dried samples of Akçakoca variety (500bpm) and b) Freeze-dried samples of Akçakoca variety (800bpm).

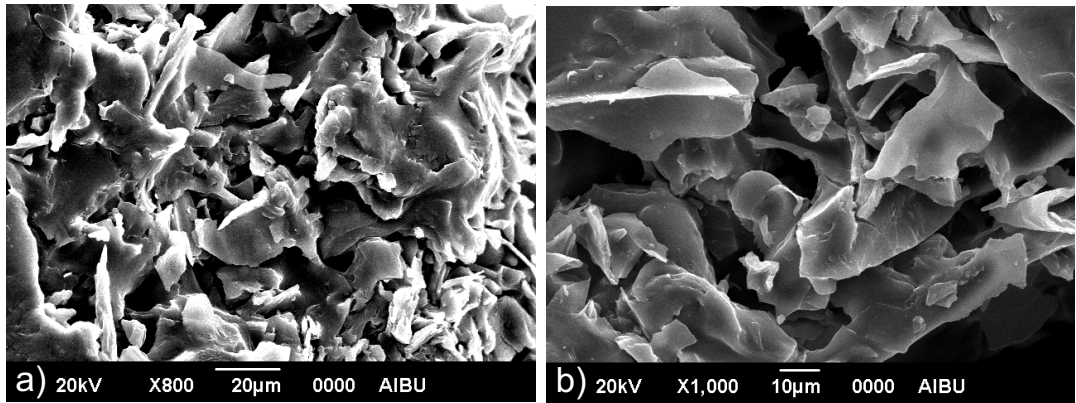


Figure 3.6: a) Freeze-dried samples of Zonguldak variety (800bpm) and b) Freeze-dried samples of Zonguldak variety (1000bpm).

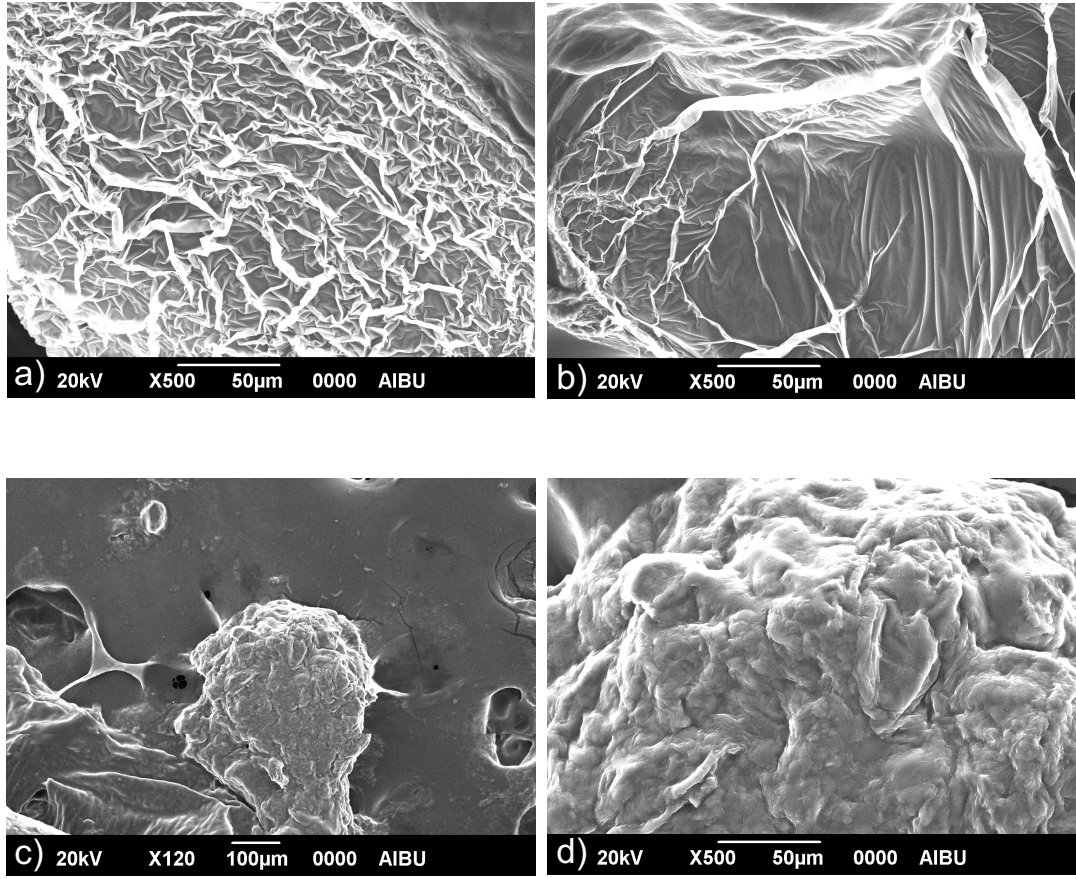


Figure 3.7: a) and b) The bowel side of fresh sample from Akçakoca variety (500bpm), c) The surface side of fresh sample from Akçakoca variety (120bpm) and d) The surface side of fresh sample from Akçakoca variety (500bpm).

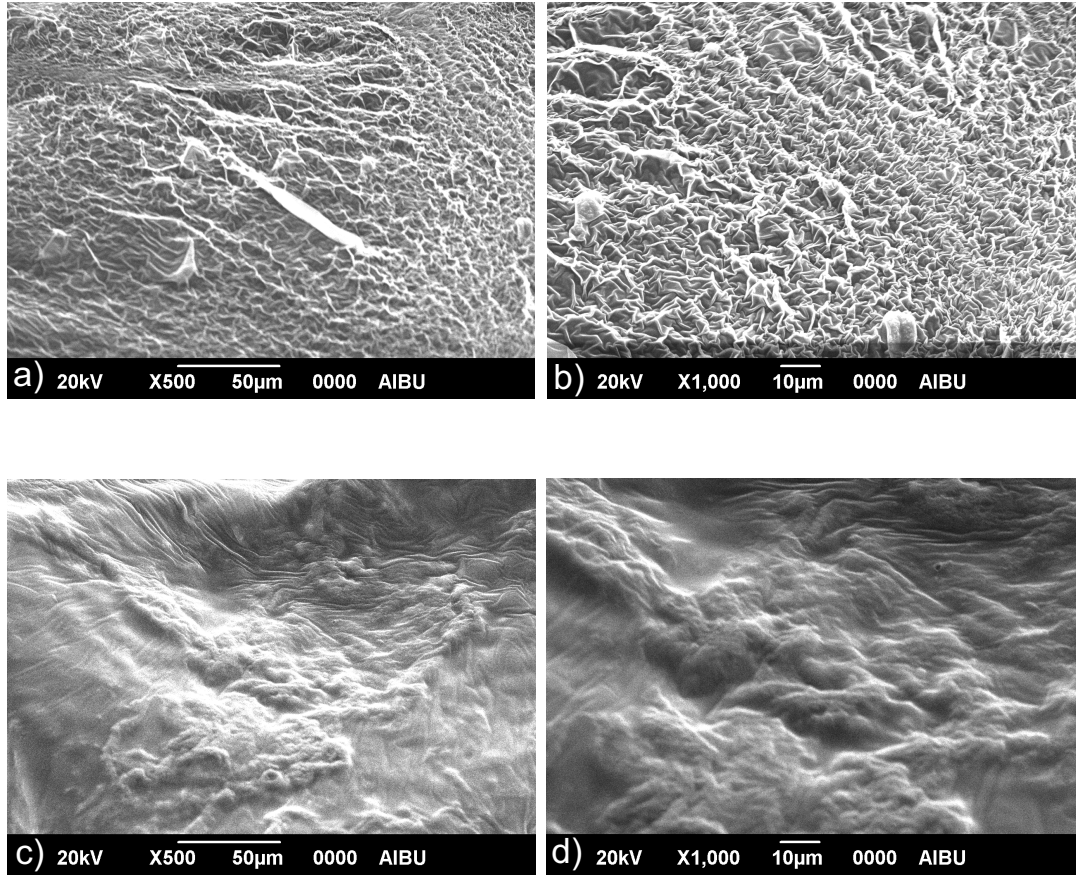


Figure 3.8: a) The bowel side of fresh sample from Zonguldak variety (500bpm), b) The bowel side of fresh sample from Zonguldak variety (1000bpm), c) The surface side of fresh sample from Zonguldak variety (500bpm) and d) The surface side of fresh sample from Zonguldak variety (1000bpm).

3.1.9 Results of Antimicrobial Activity

Effects of the ethanol and distilled water extract of *Arbutus unedo* on gram(+) and gram(-) bacteria were determined with disc diffusion method and zones of inhibition were measured in millimeters. The freeze dried samples were used for preparation of these extract. A negative control was used as an ethanol-impregnated disc on bacterial strains. The positive control was Carbenicillin for gram positive and Erythromycin for gram negative antibiotic-impregnated disc on bacterial strains. In this study, *E.coli*, *S. aureus*, *S. epidermidis* and *Salmonella spp.* were found to be greatly sensitive according to *Arbutus unedo* fruits dissolved in ethanol extract. *E.coli* and *Salmonella spp.* were found to be more resistant to *Arbutus unedo* fruits extract on *S. aureus* and *S. epidermidis*.

In 2011, a study was conducted on the antimicrobial activity of the roots of *Arbutus unedo* on *E.coli*, *P. aeruginosa* and *S. aureus*. The root extracts were prepared by using water and methanol. The inhibition zone of *E.coli* were found to be 30 mm in water and 15 mm in methanol extract. The inhibition zone of *P. aeruginosa* was 9 mm in water but there was no zone in methanol extract. The inhibition zone of *S. aureus* were 12 mm in water and 11 mm in methanol extract (El Amine Dib et al. 2011). The inhibition zones of *Escherichia coli* are 11 mm, 10.5 mm, 9 mm and 9.5 mm in freeze-dried Akçakoca variety. The inhibition zones of *E. coli* were 10.5 mm, 11mm, 12 mm and 8.5 mm in Zonguldak variety. No inhibition zone was observed around the ethanol impregnated disc of *E. coli*, which serves as negative control. 28 mm of inhibition were recorded on the antibiotic impregnated positive control disc on *E. coli*. The inhibition zones of *Staphylococcus aureus* was 14 mm, 13.5 mm, 12 mm and 11.5 mm in freeze-dried Akçakoca variety (Figure 3.9 and 3.10)(Table 3.6). The freeze-dried Zonguldak variety fruits showed 13.5 mm, 14 mm, 12 mm and 11.5 mm inhibition when

their extracts were impregnated on sterile filter paper disc on *S. aureus*. On the ethanol impregnated disc overlaid on the indicator lawn of *S. aureus* no inhibition zone was observed. On the other hand, the positive control which was the antibiotic-impregnated disc showed 36 mm of inhibition zone (Figure 3.11 and 3.12)(Table 3.6). The extract of the freeze-dried Akçakoca variety of *Arbutus unedo* showed 10 mm, 12mm, 9mm and 8.5mm on the indicator lawn of *Staphylococcus epidermidis*, while Zonguldak variety showed 13.5 mm, 14 mm, 12 mm and 11.5 mm on the same strain (Figure 3.13 and 3.14)(Table 3.6). When *Salmonella spp.* are considered the extract of the freeze-dried Akçakoca variety of *Arbutus unedo* showed 10 mm, 12mm, 9mm and 8.5mm, in addition Zonguldak variety indicated 13.5 mm, 14 mm, 12 mm and 11.5 mm (Figure 3.15 and 3.16)(Table 3.6). The groups were found to be statistically different ($p < 0.05$) (Appendix B10.1, B10.2, B10.3 and B10.4).

Table 3.6: **The diameter of inhibition zones on different strains.**

Strains	Akçakoca Extract(<i>mm</i>)	Zonguldak Extract(<i>mm</i>)	Ethanol (<i>mm</i>)	Antibiotic (<i>mm</i>)
<i>Escherichia coli</i>	11.5	10.5	-	28
	10.5	11		
	9	12		
	9.5	8.5		
<i>Staphylococcus aureus</i>	14	13.5	-	36
	13.5	14.5		
	12	12		
	11.5	11.5		
<i>Staphylococcus epidermidis</i>	10	11	-	26
	12	13		
	9	9.5		
	8.5	9		
<i>Salmonella spp.</i>	9	10	-	38
	9	10.5		
	10	9.5		
	9.5	9		

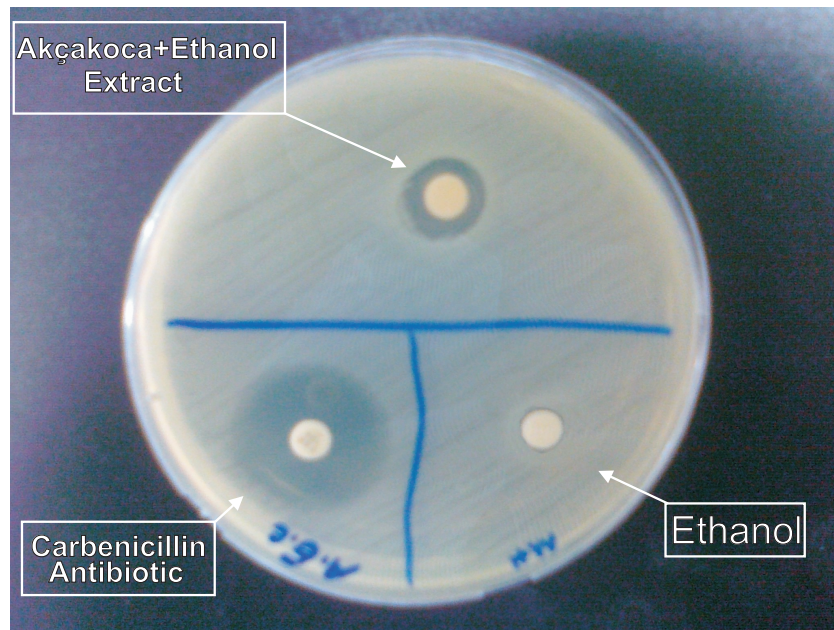


Figure 3.9: The antimicrobial effects of Antibiotic, Ethanol and Akçakoca variety of *Arbutus unedo*+Ethanol extract on *E. coli*.

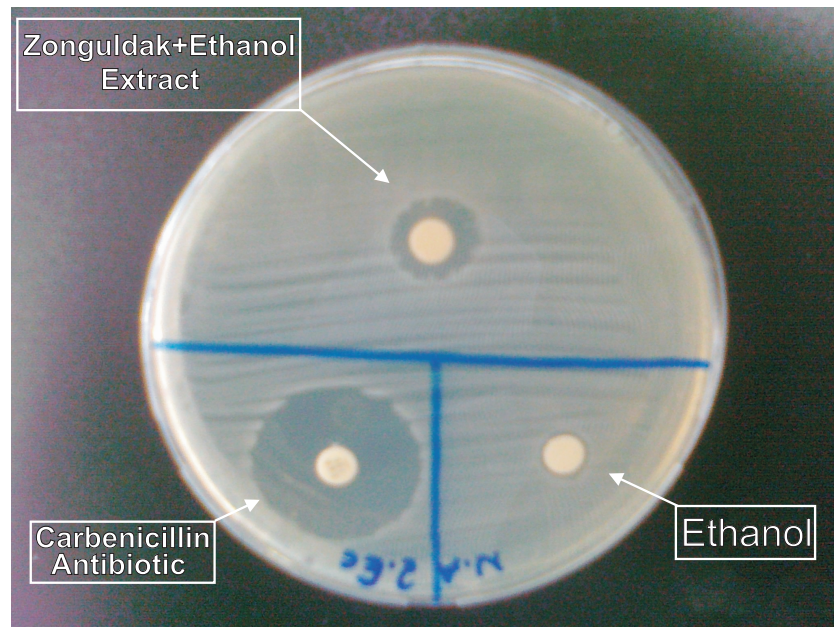


Figure 3.10: The antimicrobial effects of Antibiotic, Ethanol and Zonguldak variety of *Arbutus unedo*+Ethanol extract on *E. coli*.

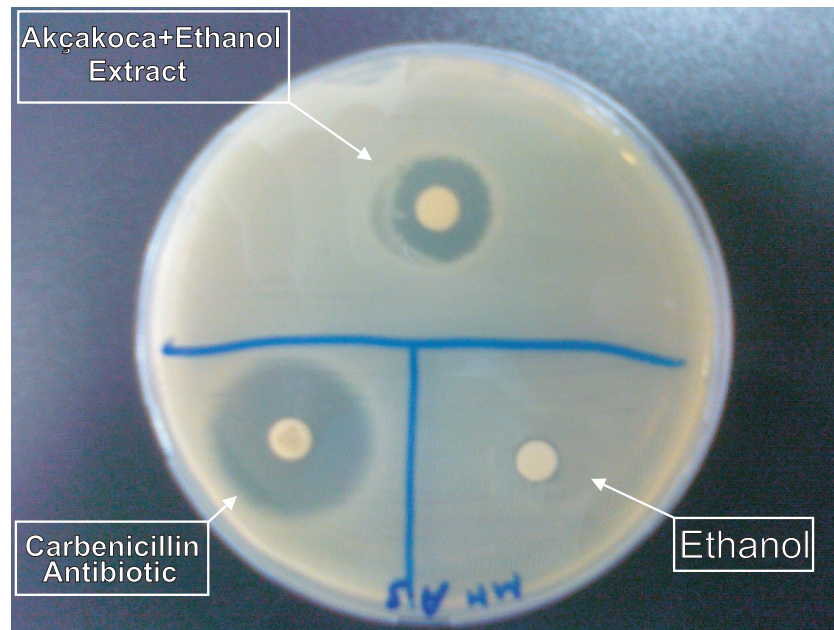


Figure 3.11: The antimicrobial effects of Antibiotic, Ethanol and Akçakoca variety of *Arbutus unedo*+Ethanol extract on *Salmonella spp.*

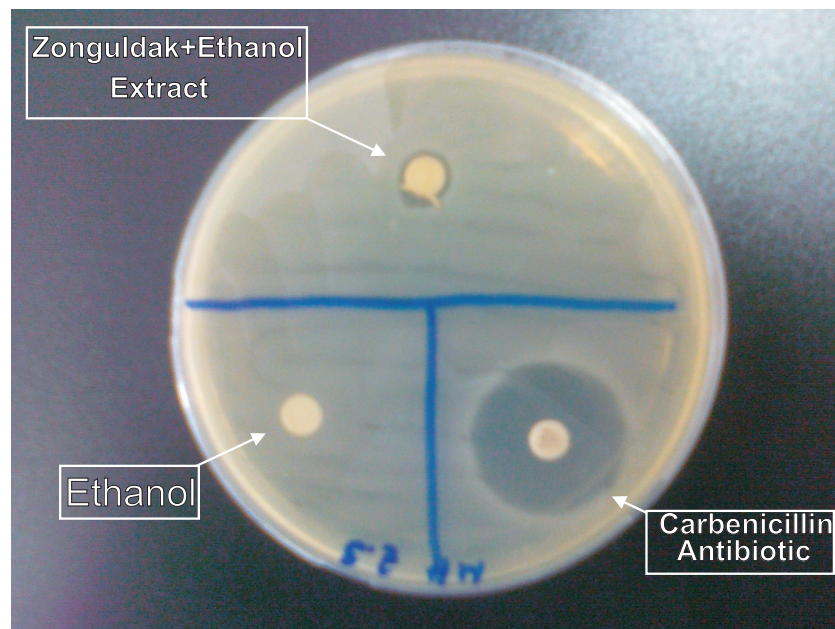


Figure 3.12: The antimicrobial effects of Antibiotic, Ethanol and Zonguldak variety of *Arbutus unedo*+Ethanol extract on *Salmonella spp.*

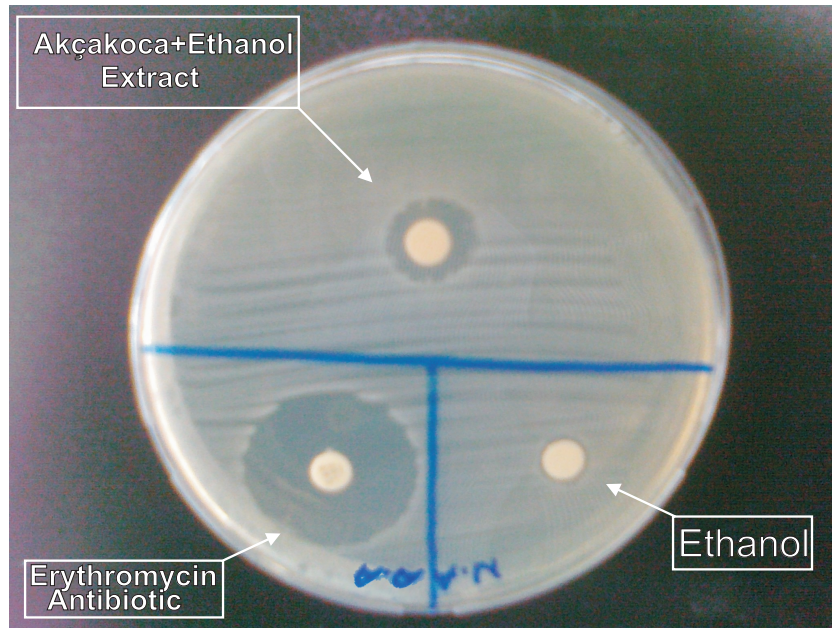


Figure 3.13: The antimicrobial effects of Antibiotic, Ethanol and Akçakoca variety of *Arbutus unedo*+Ethanol extract on *S. aureus*

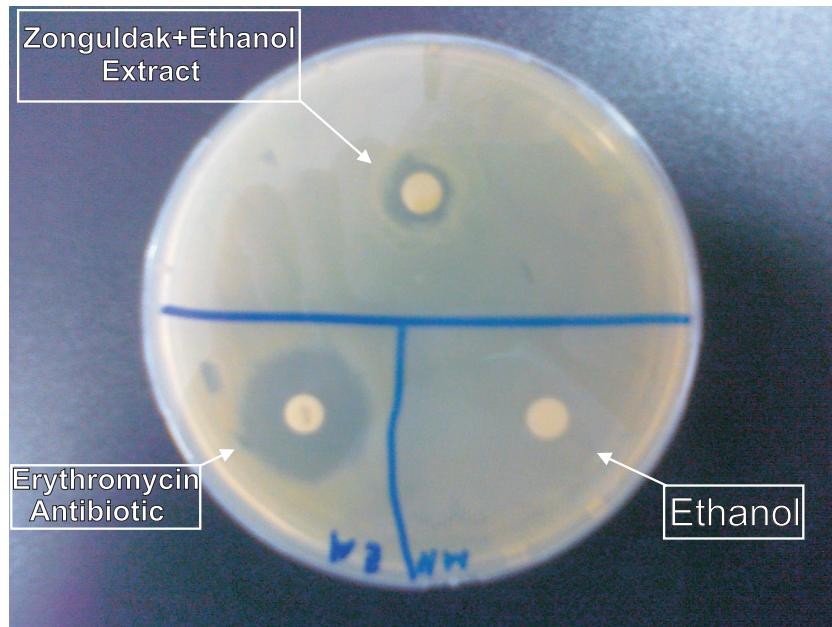


Figure 3.14: The antimicrobial effects of Antibiotic, Ethanol and Zonguldak variety of *Arbutus unedo*+Ethanol extract on *S. aureus*

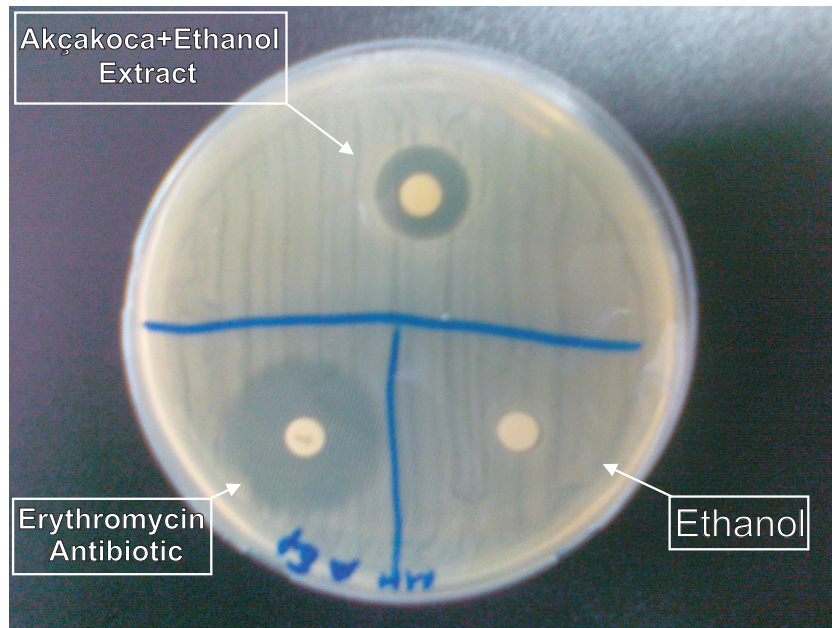


Figure 3.15: The antimicrobial effects of Antibiotic, Ethanol and Akçakoca variety of *Arbutus unedo*+Ethanol extract on *Staphylococcus epidermidis*

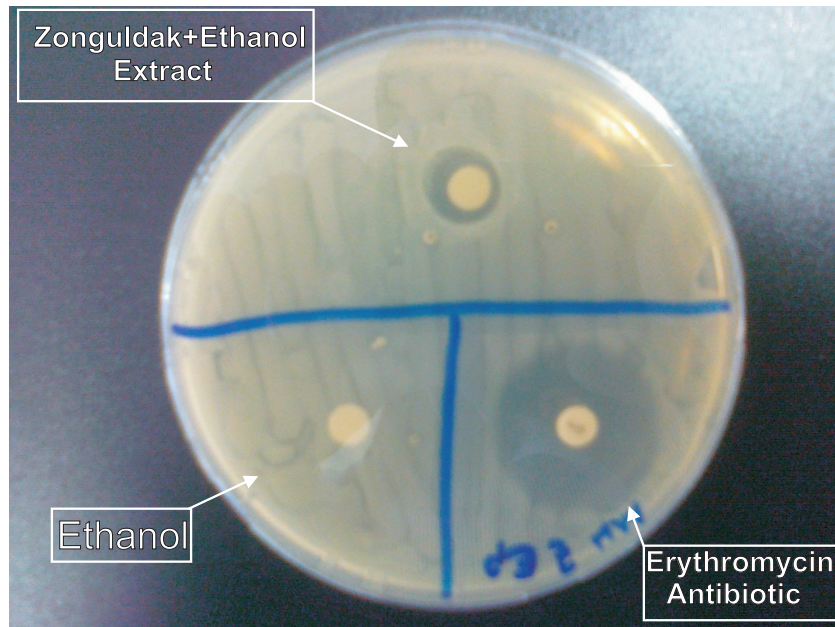


Figure 3.16: The antimicrobial effects of Antibiotic, Ethanol and Zonguldak variety of *Arbutus unedo*+Ethanol extract on *Staphylococcus epidermidis*

Chapter 4

CONCLUSION

In this study, two *Arbutus unedo* species from Akçakoca and Zonguldak region were analyzed in order to determine their antibacterial activities and physical and chemical properties. The extracts of *Arbutus unedo* was prepared by sterile distilled water and ethanol. Extraction yields for each species were obtained after a standardized solvent extraction protocol. After the extraction process the antibacterial activities of these extracts were evaluated by using disc diffusion assays. In the disc diffusion assays *E. coli*, *S. aureus*, *S.epidermidis* and *Salmonella spp.* were used as indicator microorganisms. The disc diffusion test results showed that, both Akçakoca and Zonguldak varieties exhibited antibacterial activity by inhibiting all of these microorganisms mentioned above.

The physical and chemical properties of both Akçakoca and Zonguldak varieties were analyzed. The pH, vitamin C, color, weight, the soluble solid (°Brix), ash, taste panel and SEM analysis was carried out. The samples of *Arbutus unedo* fruits from Akçakoca and Zonguldak have some differences due to morphological and physiological properties. The physical and chemical properties were found to be similar to each other when compared with fresh. Due to the findings in this study, the apple of cain fruits showed a potential to be used as raw materials in different food industries, especially in the freeze-dried form.

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Appendix A

A.1 The pH values of *Arbutus unedo* fresh fruit samples

The pH values of *Arbutus unedo* fresh fruit samples from Akçakoca and Zonguldak.

pH Values	Akçakoca-1 fresh sample	Akçakoca-2 fresh sample	Akçakoca-3 fresh sample	Zonguldak-1 fresh sample	Zonguldak-2 fresh sample	Zonguldak-3 fresh sample
1	3.67	3.77	3.81	2.78	2.88	2.81
2	3.68	3.74	3.81	2.76	2.91	2.83
3	3.74	3.73	3.84	2.76	2.90	2.83
Average	3.696	3.746	3.820	2.766	2.896	2.823

A.2 *Arbutus unedo* weights

Weights of *Arbutus unedo* from Akçakoca and Zonguldak varieties.

Weight (<i>g</i>)	Akçakoca-1	Akçakoca-2	Zonguldak-1	Zonguldak-2
	Fresh	Fresh	Fresh	Fresh
	Sample	Sample	Sample	Sample
1	5.681	5.877	4.881	3.810
2	5.414	5.367	5.202	4.358
3	5.214	6.112	5.435	5.003
4	5.705	5.371	4.651	4.644
5	5.216	4.902	5.102	5.680
6	4.955	5.638	3.997	4.781
Average	5.364	5.544	4.878	4.727

A.3 The Brix% values of *Arbutus unedo*

Brix% values of *Arbutus unedo* from Akçakoca and Zonguldak varieties.

Brix %	A1	A2	Z1	Z2	A1	A2	Z1	Z2
	Fresh	Fresh	Fresh	Fresh	Freeze	Freeze	Freeze	Freeze
	Sample	Sample	Sample	Sample	Dried	Dried	Dried	Dried
1	16.84	13.44	14.11	13.61	16.61	18.75	15.60	15.48
2	15.08	15.81	11.23	14.70	17.32	14.59	15.76	17.23
3	12.56	13.56	13.79	12.12	17.44	16.21	14.09	16.08
4	14.71	14.07	12.91	13.63	16.53	17.88	17.22	13.52
5	14.68	13.24	14.67	13.49	15.79	16.43	13.70	16.11
Average	14.74	14.02	13.34	13.51	16.74	16.77	15.27	15.68

A.4 Vitamin C contents of *Arbutus unedo*

Vitamin C contents of *Arbutus unedo* from Akçakoca and Zonguldak varieties.

Vitamin C <i>mg/ml</i>	Akçakoca-1 Freeze-Dried	Akçakoca-2 Freeze-Dried	Zonguldak-1 Freeze-Dried	Zonguldak-2 Freeze-Dried
1	0.055	0.053	0.053	0.055
2	0.056	0.056	0.052	0.053
3	0.056	0.055	0.055	0.053
Average	0.0556	0.0546	0.0533	0.0536

Appendix B

B.1 The statistical evaluation of pH

The pH values of *Arbutus unedo* fresh fruit samples from Akçakoca and Zonguldak in statistical evaluation.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Fresh Smp.	3.6967 ^c	.03786	.02186	3.6026 3.7907
Akçakoca-2 Fresh Smp.	3.7467 ^b	.02082	.01202	3.6950 3.7984
Akçakoca-3 Fresh Smp.	3.8200 ^a	.01732	.01000	3.7770 3.8630
Zonguldak-1 Fresh Smp.	2.7667 ^f	.01155	.00667	2.7380 2.7954
Zonguldak-2 Fresh Smp.	2.8967 ^d	.01528	.00882	2.8587 2.9346
Zonguldak-3 Fresh Smp.	2.8233 ^e	.01155	.00667	2.7946 2.8520
Total	3.2917	.47951	.11302	3.0532 3.5301

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.904	5	.781	1756.582	.000
Within Groups	.005	12	.000		
Total	3.909	17			

B.2 The statistical evaluation of the color values of *Arbutus unedo* fresh samples

- The statistical evaluation of the color values of *Arbutus unedo* fresh samples for L* values.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Fresh Smp.	20.7633 ^d	.11060	.06386	20.4886 21.0381
Akçakoca-2 Fresh Smp.	22.2600 ^b	.17088	.09866	21.8355 22.6845
Zonguldak-1 Fresh Smp.	21.5267 ^c	.10408	.06009	21.2681 21.7852
Zonguldak-2 Fresh Smp.	23.6533 ^a	.52539	.30333	22.3482 24.9585
Total	22.0508	.38961	.32901	21.3267 21.3267

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.632	3	4.544	55.366	.000
Within Groups	.657	8	.082		
Total	14.289	11			

- The statistical evaluation of the color values of *Arbutus unedo* fresh samples for a* values.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Fresh Smp.	.6833 ^b	.1006645	.0581187	.433269 .933398
Akçakoca-2 Fresh Smp.	.9366 ^a	.0642910	.0371184	.776959 1.09637
Zonguldak-1 Fresh Smp.	-.5600 ^c	.0346410	.0200000	-.646053 -.473947
Zonguldak-2 Fresh Smp.	-1.3500 ^d	.1752142	.1011599	-1.78525 -.914744
Total	-.0725	.9756315	.2816406	-.692387 .547387

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.378	3	3.459	299.728	.000
Within Groups	.092	8	.012		
Total	10.470	11			

- The statistical evaluation of the color values of *Arbutus unedo* fresh samples for b* values.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Fresh Smp.	1.2733 ^c	.0404145	.0233333	1.172938 1.373729
Akçakoca-2 Fresh Smp.	2.3300 ^a	.0556776	.0321455	2.191689 2.468311
Zonguldak-1 Fresh Smp.	.2366 ^d	.0585947	.0338296	.091109 .382224
Zonguldak-2 Fresh Smp.	1.6900 ^b	.3117691	.1800000	.915523 2.46447
Total	1.3825	.8069147	.2329362	.869811 1.895189

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.951	3	2.317	87.966	.000
Within Groups	.211	8	.026		
Total	7.162	11			

B.3 The statistical evaluation of the color values of *Arbutus unedo* freeze dried samples

- The statistical evaluation of the color values of *Arbutus unedo* freeze dried samples for L* values.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Freeze Smp.	21.9100 ^d	.22539	.13013	21.3501 22.4699
Akçakoca-2 Freeze Smp.	21.9567 ^c	.06110	.03528	21.8049 22.1084
Zonguldak-1 Freeze Smp.	22.5867 ^b	.04041	.02333	22.4863 22.6871
Zonguldak-2 Freeze Smp.	22.6600 ^a	.24269	.14012	22.0571 23.2629
Total	22.2783	.38961	.11247	22.0308 22.5259

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.440	3	.480	16.682	.001
Within Groups	.230	8	.029		
Total	1.670	11			

- The statistical evaluation of the color values of *Arbutus unedo* freeze dried samples for a^* values.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Freeze Smp.	1.0500 ^a	.0721110	.0416333	.870866 1.229134
Akçakoca-2 Freeze Smp.	.9766 ^b	.0208167	.0120185	.924955 1.028378
Zonguldak-1 Freeze Smp.	-.8100 ^c	.0264575	.0152753	-.875724 -.744276
Zonguldak-2 Freeze Smp.	-1.0966 ^d	.0971253	.0560753	-1.33793 -.855394
Total	.0300	1.0342410	.2985597	-.627125 .687125

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.735	3	3.912	992.361	.000
Within Groups	.032	8	.004		
Total	11.766	11			

- The statistical evaluation of the color values of *Arbutus unedo* freeze dried samples for b* values.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Freeze Smp.	1.4867 ^d	.04163	.02404	1.3832 1.5901
Akçakoca-2 Freeze Smp.	1.6900 ^c	.03464	.02000	1.6039 1.7761
Zonguldak-1 Freeze Smp.	1.7100 ^b	.03464	.02517	1.6017 1.8183
Zonguldak-2 Freeze Smp.	2.1233 ^a	.02309	.01333	2.0660 2.1807
Total	1.7525	.24354	.07030	1.5978 1.9072

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.642	3	.214	159.427	.000
Within Groups	.011	8	.001		
Total	.652	11			

B.4 The statistical evaluation of the *Arbutus unedo* weights

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Fresh Smp.	5.3641 ^b	.2935830	.1198548	5.056070 5.672263
Akçakoca-2 Fresh Smp.	5.5445 ^a	.4279648	.1747159	5.095378 5.993622
Zonguldak-1 Fresh Smp.	4.8780 ^c	.5085469	.2076134	4.344313 5.411687
Zonguldak-2 Fresh Smp.	4.7126 ^d	.6276954	.2562556	4.053941 5.371393
Total	5.1248	.5670321	.1157449	4.885397 5.364270

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.785	3	.928	4.028	.022
Within Groups	4.610	20	.230		
Total	7.395	23			

B.5 The statistical evaluation of the Brix% for *Arbutus unedo* fresh samples

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Fresh Smp.	14.7740 ^a	1.52289	.68106	12.8831 16.6649
Akçakoca-2 Fresh Smp.	14.0240 ^b	1.04438	.46706	12.7272 15.3208
Zonguldak-1 Fresh Smp.	13.3420 ^d	1.34176	.60005	11.6760 15.0080
Zonguldak-2 Fresh Smp.	13.5100 ^c	.91829	.41067	12.3698 14.6502
Total	13.9125	1.26542	.28296	13.3203 14.5047

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.210	3	2.070	1.368	.288
Within Groups	24.214	16	1.513		
Total	30.424	19			

B.6 The statistical evaluation of the Brix% for *Arbutus unedo* freeze-dried samples

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Freeze Smp.	16.7380 ^b	.6689320	.2991555	15.9074 17.5685
Akçakoca-2 Freeze Smp.	16.7720 ^a	1.607333	.7188213	14.7762 18.7677
Zonguldak-1 Freeze Smp.	15.2740 ^d	1.414984	.6328001	13.5170 17.0309
Zonguldak-2 Freeze Smp.	15.6840 ^c	1.365001	.6104474	13.9891 17.3788
Total	16.1170	1.379351	.3084325	15.4714 16.7625

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.564	3	2.855	1.656	.216
Within Groups	27.586	16	1.724		
Total	36.150	19			

B.7 The statistical evaluation of Vitamin C contents of *Arbutus unedo*

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca-1 Fresh Smp.	.055667 ^{a,b}	.0005774	.0003333	.054232 .057101
Akçakoca-2 Fresh Smp.	.055667 ^{a,b}	.0015275	.0008819	.050872 .058461
Zonguldak-1 Fresh Smp.	.053333 ^d	.0015275	.0008819	.049539 .057128
Zonguldak-2 Fresh Smp.	.053667 ^c	.0011547	.0006667	.050798 .056535
Total	.054333	.0014355	.0004144	.053421 .055245

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	2.105	.178
Within Groups	.000	8	.000		
Total	.000	11			

B.8 The statistical evaluation of Ash contents of *Arbutus unedo*

- The statistical evaluation of the Ash contents of *Arbutus unedo* fresh samples for weight values.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca Fresh Smp.	2.9166 ^a	.254630	.127315	2.6651 3.2345
Zonguldak Fresh Smp.	2.5332 ^b	.254630	.086931	2.3301 2.7519
Total	2.7249	.287650	.101702	2.3301 2.7519

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.294	1	.294	6.186	.047
Within Groups	.285	6	.048		
Total	.579	7			

- The statistical evaluation of the Ash contents of *Arbutus unedo* fresh samples.

Duncan^a

Descriptives				
Samples Name	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca Fresh Smp.	.025875 ^a	.005344	.002672	0.0194 0.0311
Zonguldak Fresh Smp.	.025575 ^b	.004728	.002364	0.0200 0.0295
Total	.025725	.004674	.001652	0.0194 0.0311

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	1	.000	.007	.936
Within Groups	.000	6	.000		
Total	.000	7			

B.9 The statistical evaluation of taste panel.

One-Sample Statistics				
	N	Mean	Standard Deviation	Standard Error
Texture	15	2.00	1.2535	.3236
Color	15	4.27	.79881	.2063
Appearance	15	3.40	.73701	.1904
Taste	15	4.20	1.0823	.2791
Flavour	15	4.93	.25830	.0673

B.10 The statistical evaluation of the diameter of inhibition zones on different strains.

B.10.1 The statistical evaluation of diameter of inhibition zones on *Escherichia coli*.

Duncan^a

Descriptives				
Strains	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca Extract	10.125 ^c	1.1087	.5543	8.361 11.889
Zonguldak Extract	10.500 ^b	1.4720	.7360	8.158 12.842
Ethanol	.000 ^d	.0000	.0000	.0000 .0000
Antibiotic	28.000 ^a	.0000	.0000	28.000 28.000
Total	12.156	10.4335	2.6084	6.597 17.716

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1622.672	3	540.891	637.123	.000
Within Groups	10.188	12	.849		
Total	1632.859	15			

B.10.2 The statistical evaluation of diameter of inhibition zones on *Staphylococcus aureus*.

Duncan^a

Descriptives				
Strains	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca Extract	12.750 ^c	1.1902	.5951	10.856 14.644
Zonguldak Extract	12.875 ^b	1.3769	.6884	10.684 15.066
Ethanol	.000 ^d	.0000	.0000	.0000 .0000
Antibiotic	36.000 ^a	.0000	.0000	36.000 36.000
Total	15.406	13.4403	3.3601	8.244 22.568

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2699.672	3	899.891	1086.660	.000
Within Groups	9.938	12	.828		
Total	2709.609	15			

B.10.3 The statistical evaluation of diameter of inhibition zones on *Staphylococcus epidermidis*.

Duncan^a

Descriptives				
Strains	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca Extract	9.875 ^c	1.5478	.7739	7.412 12.338
Zonguldak Extract	10.625 ^b	1.7970	.8985	7.766 13.484
Ethanol	.000 ^d	.0000	.0000	.0000 .0000
Antibiotic	26.000 ^a	.0000	.0000	26.000 26.000
Total	11.625	9.6618	2.4154	6.477 16.773

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1383.375	3	461.125	327.911	.000
Within Groups	16.875	12	1.406		
Total	1400.250	15			

B.10.4 The statistical evaluation of diameter of inhibition zones on *Salmonella spp.*

Duncan^a

Descriptives				
Strains	Mean	Standard Deviation	Standard Error	Confidence Interval for Mean Lower Bound Upper Bound
Akçakoca Extract	9.375 ^c	.4787	.2394	8.613 10.137
Zonguldak Extract	9.750 ^b	.6455	.3227	8.723 10.777
Ethanol	.000 ^d	.0000	.0000	.0000 .0000
Antibiotic	38.000 ^a	.0000	.0000	38.000 38.000
Total	14.281	14.7116	3.6779	6.442 22.121

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3244.547	3	1081.516	6698.419	.000
Within Groups	1.937	12	1081.516		
Total	3246.484	15			