International Journal of Environmental Trends (IJENT)
http://dergipark.gov.tr/ijent
International Journal of Environmental Trends (IJENT)
2019; 3 (2),175-184
ISSN: 2602-4160
Turkish JournalPark ACADEMIC

Research Article

Production of Hand Sterilization Fluid of Herbal Origin

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Abstract

When the body is exposed to microorganisms of daily life, their negative effects on human health will be observed. Industrial disinfection gels especially used for effective and rapid hand disinfection, contain ethyl or isopropyl alcohol. Due to the presence of alcohol, they cause some allergic reactions on the skin. The aim of this study was to investigate the usability of flavonoids obtained from horse chestnut shells as hand disinfection gel material. Single and multi-parameter optimizations were applied to obtain the maximum amount of flavonoids from the material by ultrasonic extraction. In single optimization, it was determined that the application of 50^{o}C, 2g-plant/100ml-water, 10 min-continuous sound waves increased the extraction efficiency. To determine the optimum values of parameters in multiple optimization, the Response-Surface Method including Box-Benkhen design was used via the Design-Expert program. As a result of the numerical solution, the application of ultrasonic extraction to 2.44g of horse chestnut and 100ml of water at 40.1^{o}C for 14.89 minutes produced the highest flavonoid content. The amount of antioxidant in extracts was analyzed spectrophotometrically by DPPH method. The Addition of 9.6ml of the extract to mixed microorganism culture media resulted in successful inhibition. Thus, it was concluded that the disinfection liquid containing the same concentration produced by thickening it with sodium alginate was found as an alternative to the existing product containing synthetic chemicals.

Received
4 October 2019

Accepted
13 December 2019

Keywords
Aesculus hippocastanum
Ultrasonic extraction
Disinfection
DPPH

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INTRODUCTION

It is a known fact that various microorganisms on the materials used in daily life cause serious diseases, especially infectious diseases, which may adversely affect human health, when entered into the human body [1]. Therefore, it is necessary to pay attention to environmental cleanliness and individual sterilization. In order to provide a faster and exact solution to disinfection of microorganisms, hand disinfectant gels contain 45-95% of ethyl alcohol or isopropyl alcohol [2]. Alcohol content of it is one of the main disadvantages of the product that causes some allergic reactions. Infection-related diseases and deaths, which are common problems from past to present, are increasing day by day. New methods have been tried to develop every day to prevent and/or treat them. Therefore, it has been suggested that in the field of pharmacology, the products obtained from antimicrobial plants may provide an advantage to human health compared to the products containing alcohol.

Basic compounds having antimicrobial effect in various plants and spices are declared as phenolics, phenolic acids, flavonoids, tannins, alkaloids and coumarins [3]. The sources of antimicrobial effect of plants are essential oils and extracts obtained from their seeds, leaves, flowers etc. The extracts contain alcohols, phenols, acids, aldehydes and esters and the combinations of these molecules. Due to their anticancer, antiseptic, antiallergenic and antimicrobial properties, they have been used as functional compounds in many drugs [4, 5]. Flavonoids are defined as a group of polyphenolic compounds and are known to decrease free radical formation [6]. The high efficiency of these compounds depends on the determination of the optimum conditions of the parameters such as solvent, solid-to-liquid ratio, time and temperature to be used during extraction [7]. Phenolic compounds are known to dissolve better in alcohol due to their hydrophilic nature. Thus, extraction of these compounds has been carried out with methanol, ethanol, water, acetone or combinations [8, 9]. In some academic researches, different types of essential oils (pinene, tuyil alcohol, cineol flask and geraniol) have been extracted from the plants, and these essential oils have been declared to have antimicrobial properties against many bacteria and fungi [10]. In addition the usage of plants for medicinal purposes is not new. Positive effects of *Aesculus hippocastanum* plant and *Bunium paucifoliu* on urinary tract inflammation, *Linum nodiflorum* on ulcers, *Centauria kurdica* on neural removal, *Echium italicum* on edema removal, *Salvia verticillata* on cell regeneration, *Ranunculus constantinopolitanus* on rheumatoid and ulcers have been announced in literature studies [11-14]. Erkac and Yıgıtarslan obtained an extract from horse chestnut shells by using various solvents. They showed that this extract could prevent the growth of undesirable microorganisms during fermentation of olives in the table olive production [15]. *Aesculus hippocastanum L.* (*Hippocastanaceae*), also known as horse chestnut, grows on trees at temperate climates. From the past to the present, the extract of horse chestnut bark, flowers and seeds has been used on venous insufficiency diseases, varicose veins, hemorrhoids, phlebitis (inflammation of the veins), skin diseases, and for the treatment of body pain [16,17] . Application of horse chestnuts to cosmetic products such as scalp, oral care, facial, body hygiene started in 1980s. Recently, horse chestnut extracts have been added to enhance the health effects of many commercial products like body lotions, hair shampoos, creams and the like [16].

The aim of this study was to investigate the use of flavonoids derived from horse chestnut (*Aesculus hippocastanum*) shells as hand disinfection gel raw material. To achieve this, the extraction conditions of the horse chestnut were optimized by using response surface methodology and the efficiency of the extracts obtained under optimum conditions on microbial growth was investigated by analyzing the growth of specific microorganisms chosen.

MATERIAL AND METHOD

In this study, ultrasonic extraction method was used to obtain phenolic compounds of horse chestnut shells. This method was preferred because of performing both a faster and more efficient extraction process based on the observance of particles breakage to be extracted by mechanical shaking and the cavitation effect provided by the ultrasonic energy by applying acoustic vibrations at certain frequencies to the material that creates bubbles [18]. In the extraction experiments of the powdered horse chestnut shells, the single and double effects of the parameters such as temperature, solid-to-liquid ratio, time, wavelength, solvent type were optimized. In order to determine the optimum values of the parameters with the minimum number of experiments, the procedure in Balcı and Yigitarslan [19], in which flavonoid extraction by the ultrasonic extraction method from *Cinnamonum zeylaniccum* was optimized, were applied in parallel. Box-Behnken experimental design (Table 1.) containing the response surface method was used with Design -Expert program to express surfaces representing the extraction process.

Table 1. Box-Behnken design parameters used in the study

Parameter Code	-1	0	+1
x_1: Temperature (°C)	40	50	60
x_2: Solid/Liquid (g/ml)	2/100	5/100	8/100
x_3: Time (min.)	5	10	15

The Total amount of antioxidant in the extracts was determined by DPPH (1,1-diphenyl-2-picryl-hydrylase) method. It is based on the reduction reaction between the antioxidant and the oxidant materials. The degree of the color change in the oxidizing agent (DPPH) depends on the concentration of antioxidants in the sample [20-22]. In this study, 1.4 ml phosphate buffer (pH 7.4), 100 µl sample and 1ml DPPH solution were mixed for 30 minutes to perform the reaction. The total amount of antioxidant is expressed by the percent of absorbance values calculated by the following equation due to the linear relationship between them.

$$\%ABS = \frac{ABS\ Blank - ABS\ solution}{ABS\ blank} x100$$

In the experiments which examined the efficacy of the extracts on microbial growth, horse chestnut extracts were prepared at optimum conditions determined by the Response Surface Method. Three different microorganisms and/or microorganism combinations were studied (No 1: *Saccharomyces boulardii*; No 2: *Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium animals ssp lactis* B94; and No 3: *Lactobacillus acidophilus, Lactobacillus rhamnosus*). Samples were prepared in the laminar cabinet (ESCO), by addition of the inoculated microorganisms and aqueous solution of hand samples taken from the volunteers (500µl) into a sterilized MRS broth (100 ml), followed by addition of the extracts (9 ml) when the microbial growth in the medium reached to the log phase. Samples without extract were used as control groups for each microorganism colonies. Microbial growth in control groups and samples was analyzed spectrophotometrically at 600nm.

RESULT AND DISCUSSION

In this study, in order to determine the multiple optimization parameters of the ultrasonic extraction process, single optimization was applied by working with parameters including

temperature, solid per liquid ratio, duration and type of wave. In this process, the optimum values of each parameter were determined as 50^{o}C, 2gr/100ml, 10 minutes and continuous sound waves, respectively. 15 experiments were carried out in accordance with the extraction parameters given in (Table 1), which were found to be the most effective ones on extraction efficiency, and antioxidant contents of extracts at those conditions were analyzed by DPPH method (Table 2). The proposed functions were analyzed statistically by using Design Expert program. The criteria used to determine the best function were as follows: the compatibility of the function proposed by the program must be "significant", the difference between predicted R^2 and adjusted R^2 values must be less than 0.2, and the regression values must be close to 1.0 and close to each other. In the functions proposed by the computer program, the function providing these conditions was determined to be a reduced cubic model (Table 3). P values less than 0,0500 indicate that the model terms are significant. Values greater than 0.1000 indicate that the model terms are not meaningful. The parameter having the highest F value and the lowest p-value is the most effective parameter of the process. When the reduced cubic model data were examined, it was decided that the most important model terms were A, B, BC, C^2 and the most effective parameter was A (Table 3). As a result of multiple optimization, it was determined that the extract with the highest antioxidant content would be obtained by extraction of 2.44 g horse chestnut by using 100 ml of water, and at 40.1^{o}C, and with continuous ultrasonic wave application during 14.89 minutes.

In the literature studies, different alcohol or alcohol combinations have been used as a solvent in the extraction processes [15, 23]. In terms of the intended material, the usage of alcohol was found to increase the extraction efficiency [15]. In this study, water was used as a solvent, because aiming to determine the antimicrobial properties of the *Aesculus hippocastanum* shell were resulted from the antioxidant components of the extract, not from the alcohol; and because of intending production of a non-alcoholic and thus not allergenic product to the human body.

Table 2. Radical scavenging activity of the extracts at conditions determined by Design Expert Program

Experiment No	x_1	x_2	x_3	ABS %
1	-1	-1	0	92.71
2	+1	-1	0	84.24
3	-1	+1	0	92.23
4	+1	+1	0	80.44
5	-1	0	-1	93.69
6	+1	0	-1	88.99
7	-1	0	+1	92.79
8	+1	0	+1	84.90
9	0	-1	-1	91.13
10	0	+1	-1	91.75
11	0	-1	+1	94.50
12	0	+1	+1	84.19
13	0	0	0	86.81
14	0	0	0	89.01
15	0	0	0	87.52

Table 3. ANOVA for Reduced Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	226,44	5	45,29	21,08	0,0001	significant
A-Temperature	134,89	1	134,89	62,79	< 0.0001	
B-Solid/Liquid	24,40	1	24,40	11,36	0,0083	
C-time	10,53	1	10,53	4,90	0,0541	
BC	29,87	1	29,87	13,90	0,0047	
C^2	26,75	1	26,75	12,45	0,0064	
Residual	19,33	9	2,15			
Lack of Fit	16,81	7	2,40	1,91	0,3868	not significant
Pure Error	2,52	2	1,26			
Cor Total	245,77	14				

R^2=0.9213; predicted-R^2=0.7701; adjusted R^2=0.8776; standart deviation=1.47; CV=1.65%

Considering the studies in the literature, it is seen that even if the desired component and extraction parameters are the same, different optimum values are obtained for different plants [15, 18, 23]. For example, in the study conducted by Wang et al., the optimum time for the extraction was determined as 30 minutes, whereas in the study of Stanisavljević et al. 40 minutes was found, and in this study, 14.89 minutes were determined to reach maximum yield. Although both studies used ultrasonic methods and in both phenolic components were extracted, the optimum values of extraction parameters, and thus the extraction yields, in the plants of *Inula helenium* and *Echinacea purpurea L*. were quite different from each other [18, 23]. Therefore, as the extraction conditions and yields are highly dependent on both the plant and the method, comparison of the study findings with the literature data is not possible because there is no match.

In this study, it was found that the extract components obtained at determined optimum conditions inhibited the growth of whole microorganisms (Figures 1, 2 and 3), when they were added to the media prepared with mixed microorganism cultures and palm microflora. Approximately 70% of inhibition in the pathogenic microorganism-predominant medium in 780-1260 minutes, approximately 84% in non-pathogenic microorganism medium between 600-900 minutes, and 55% in probiotic microorganism medium in 900-1140 minutes were determined. Finally, in the study, extracts with the same concentration, hand disinfection gel produced by thickening with sodium alginate were produced.

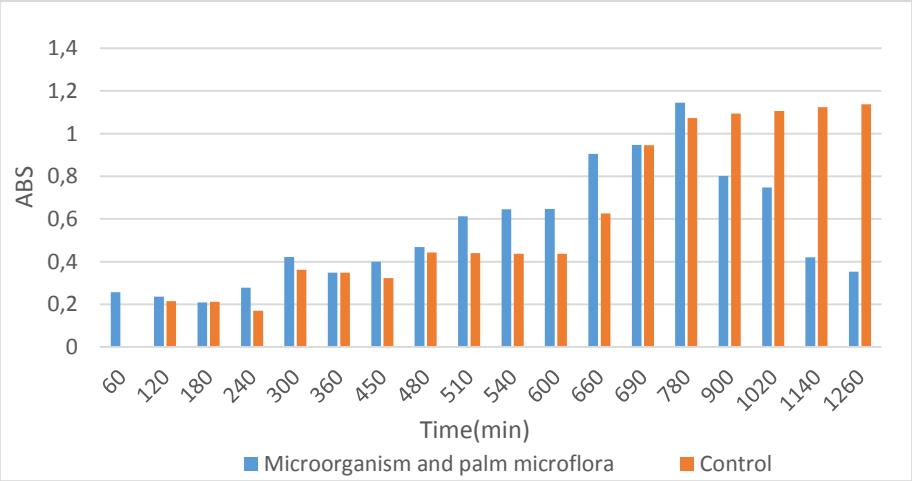

Figure 1. The effect of extracts on the growth of *Saccharomyces boulardii*-palm microflora

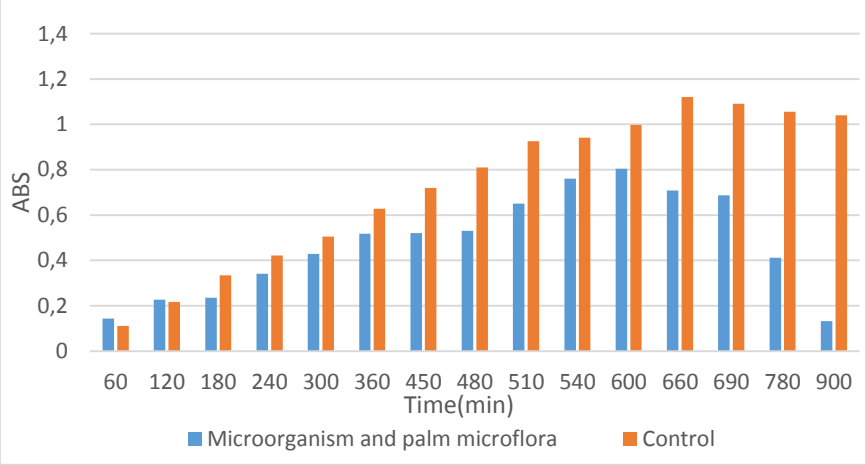

Figure 2. The effect of extracts on the growth of *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium animals ssp lactis* B94 –palm microflora

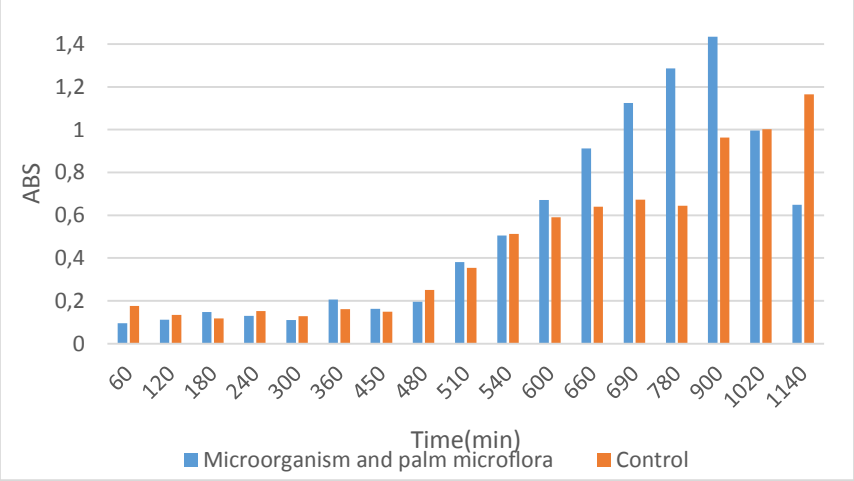

Figure 3. The effect of extracts on the growth of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* – palm microflora

In the study, when multiple optimization techniques were applied to the parameters selected in the ultrasonic extraction method of horse chestnut shells, the best model expressing the

extraction surface was determined as a reduced cubic model. The industrial expression of this process is as follows:

$$\%ABS = +87.56571 - 4.10625.\,\text{temperature} - 1.74625.\frac{\text{solid}}{\text{liquid}} - 1.14750.\,\text{time} - 2.73250.\frac{solid}{liquid}.\,\text{time} + 2.67679.\,\text{time}^2$$

The red regions on the three-dimensional ultrasonic extraction surfaces created using the Design- Expert program refer to the regions with the highest extraction efficiency (Figure 4, 5 and 6). Accordingly, it was found that in order to achieve high efficiency, low solid-liquid ratios required lesser time and high solid-liquid ratios required more time (Figure 4). This may be due to the fact that the antioxidants extracted from the material are not dispersed on the surface, but on the whole. When the other parameters were taken into consideration, it was seen that a better efficiency could be obtained when the temperature and solid/liquid ratio was reduced (Figure 4 and 5). The low temperature indicated that this extraction conditions could be preferred both in terms of low cost and high content of non-degraded antioxidant components.

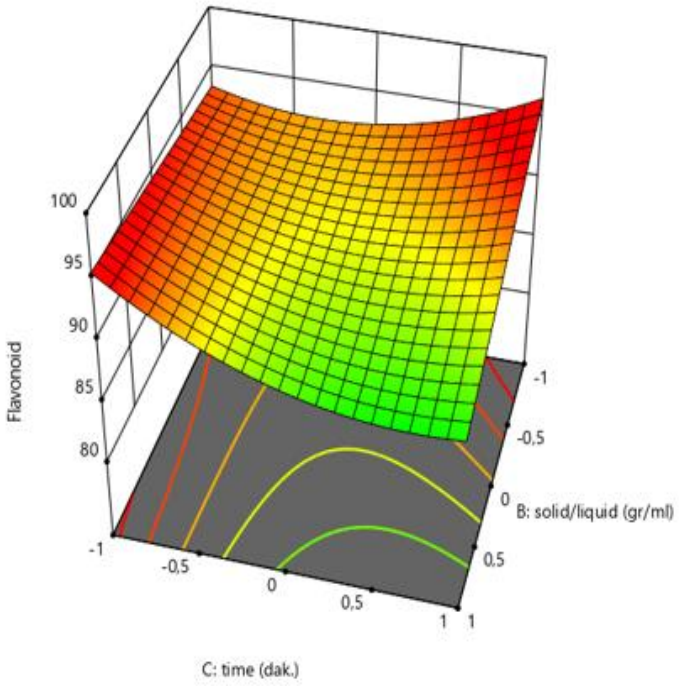

Figure 4. The effect of solid-liquid ratio and time on yield of extraction

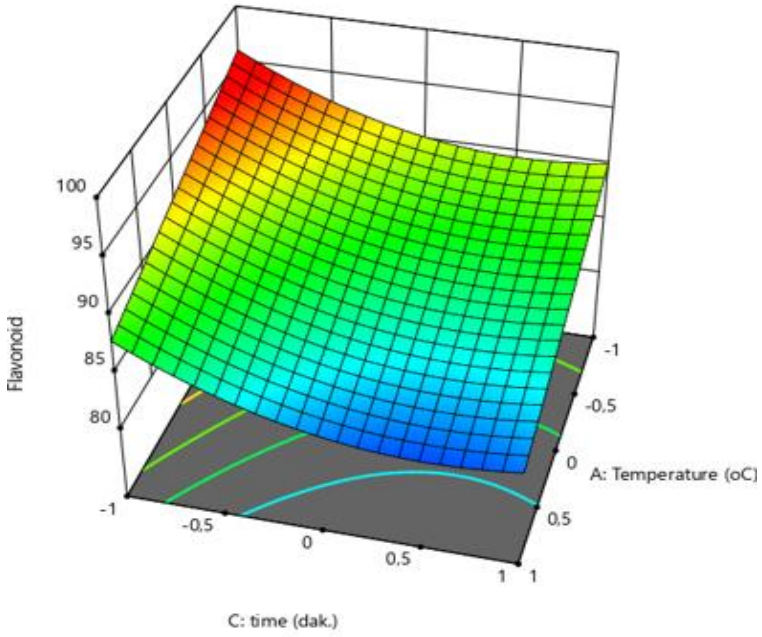

Figure 5. The effect of temperature and time on yield of extraction

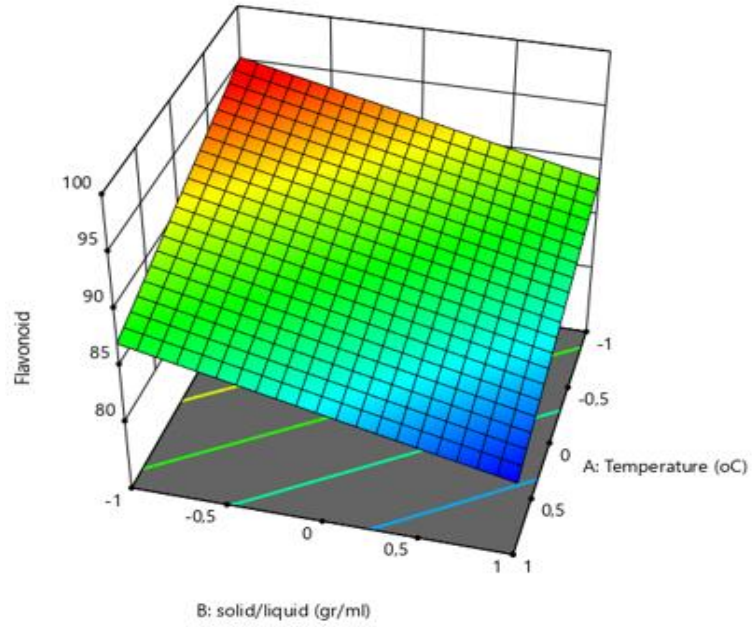

Figure 6. The effect of temperature and solid-liquid ratio on yield of extraction

CONCLUSION

In this study, it was demonstrated that the hand sterilization fluid without alcohol can be produced with extraction of horse chestnut shells, as an alternative to industrial hand disinfection liquid. This will eliminate the disadvantages of alcohol-containing hand disinfectant fluids when used for sterilization. Since horse chestnuts can grow in many geographical areas, spills occur during seasonal changes. Up to now, these materials

considered as waste and disposed of in the trash. Instead of doing this, according to the results of the study, the person appointed by the municipalities should collect the horse chestnuts and forward them to the authorized institutions to turn into useful products. By the way, the cost reduction in the production of sterilization fluids and may be cosmetics also be achieved. In future studies, the effects of the gel on other pathogenic microorganisms, and the usage of other plant sources instead of horse chestnut as antimicrobial materials should be examined and their mechanism of action should be determined. The use of plants in the field of pharmacology, which is increasing in popularity today, may lead to different new studies that may benefit to human health.

REFERENCES

[1] Erdogan, A.E., Everest, A., 2013. Antimikrobiyal ajan olarak bitki bileşenleri, *Türk Bilimsel Derlemeler Dergisi*, (2), 27-32.

[2] Ozyurt, M., 2005. Aldehid, Peroksijen ve Perasetik Asit ile Klor Verici Ajan İçermeyen ve Alet Dezenfektanı Olarak Önerilen Diğer Dezenfektanlar, Genel Kullanım Alanları ve Antimikrobiyal Etkinlikleri, 4. Ulusal Sterilizasyon Dezenfeksiyon Kongresi, 180-199.

[3] Gyawali, R., Ibrahim, S.A., 2014. Natural products as antimicrobial agents. *Food Control*, (46), 412-429.

[4] Çoban, Ö. E., Patır, B., 2010. Antioksidan etkili bazı bitki ve baharatların gıdalarda kullanımı, *Gıda Teknolojileri Elektronik Dergisi*, 5(2), 7-19.

[5] Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94(3), 223-253.

[6] Farkas, O., Jakus, J., Héberger, K., 2004. Quantitative structure–antioxidant activity relationships of flavonoid compounds. *Molecules*, *9*(12), 1079-1088.

[7] Büyüktuncel, E., 2012. Gelişmiş ekstraksiyon teknikleri, I. *Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi*, 32(2), 209-242.

[8] Skrede, G., Wrolstad, R. E., 2002. Flavonoids from berries and grapes. *Functional Foods: Biochemical and Processing Aspects*, *2*, 71-133.

[9] Naczk, M., Shahidi, F., 2004. Extraction and analysis of phenolics in food. *Journal of chromatography A*, *1054*(1-2), 95-111.

[10] Dülger, B., Ceylan, M., Alitsaous, M., Uğurlu, E., 1999. Artemisia absinthium L.(Pelin)'un antimikrobiyal aktivitesi. *Turk. J. Biol*, *23*(3), 377-384.

[11] Kirbağ, S., Zengin, F., 2006. Elazığ yöresindeki bazı tıbbi bitkilerin antimikrobiyal aktiviteleri. *Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Tarım Bilimleri Dergisi*, *16*(2), 77-80.

[12] Kırbağ, S., 1999. Hypericum perforatum L.'un değişik ekstraktlarının antimikrobiyal aktivitesi, *Journal of Quafqaz University*, V:II,N:1 102-108.

[13] Kırbağ, S., Bağcı, E., 2000. Piceae abies (L.) karst. ve Picea orientalis (L.) link uçucu yağlarının antimikrobiyal aktivitesi üzerine bir araştırma" *Journal of Quafqaz University*, V:III,N:1 183-1882.

[14] Matthews, P., Haas, J.G., 1993. Antimicrobial activity of some edible plants: lotus, coffee and others." *J.of Food Projection* V:56 N:1 66-68.

[15] Erkaç, G., Yiğitarslan, S., 2018. Application of Gallic Acid Produced from Horse Chestnut (*Aesculus hippocastanum*) Shell in Table Olive Maturation. *Eurasian Journal of Food Science and Technology*, 44-52.

[16] Wilkinson, J. A., Brown, A. M. G., 1999. Horse Chestnut–Aesculus Hippocastanum: Potential Applications in Cosmetic Skin- care Products. *International journal of cosmetic science*, *21*(6), 437-447.

[17] Dudek, M.M., Matławska, I., 2011. Flavonoids from the flowers of Aesculus hippocastanum. *Acta Pol Pharm*, *68*(3), 403-8.

[18] Stanisavljević, I., Stojičević, S., Veličković, D., Veljković, V., Lazić, M., 2009. Antioxidant and antimicrobial activities of Echinacea (Echinacea purpurea L.) extracts obtained by classical and ultrasound extraction. *Chinese Journal of Chemical Engineering*, *17*(3), 478-483.

[19] Balcı, S., Yigitarslan, S., 2017. Optimization of Ultrasonic Extraction of Total Flavonoids from Cinnamonum zeylaniccum. *International Journal of Secondary Metabolite, 4.*(3, Special Issue 1), 108-116.

[20] Ardağ, A., 2008. *Antioksidan kapasite tayin yöntemlerinin analitik açıdan karşılaştırılması (Master's thesis, Adnan Menderes Üniversitesi, Fen Bilimleri Enstitüsü).*

[21] Wicks, M.D., Wood, L. K., Garg, L. G., 2006. Methodology for the determination of biological antioxidant capacity in vitro: a review. *Journal of the Science of Food and Agriculture*, *86*(13), 2046-2056

[22] Shirazi, O. U., Khattak, M. A. K., Shukri, N. A. M., Nasyriq, M. N., 2014. Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. *Journal of Pharmacognosy and Phytochemistry*, *3*(3), 104-108.

[23] Wang, J., Zhao, Y. M., Tian, Y. T., Yan, C. L., Guo, C. Y., 2013. Ultrasound-assisted extraction of total phenolic compounds from *Inula helenium*. *The Scientific World Journal*, *2013*.