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Antimicrobial Protective Effects of Essential Oil of Pickling Herb in Creams

Kremlerde Çörtük Uçucu Yağının Anitimikrobiyal Koruyucu Etkisi

Aslıhan CESUR TURGUT [1]*, Şevkinaz DOĞAN[2]

[1]Burdur Mehmet Akif Esoy University, Burdur Vocational School of Food, Agriculture and Livestock, Department of Plant and Animal Production, Burdur, Türkiye
[2]Burdur Mehmet Akif Esoy University, Faculty of Health Sciences, Department of Fundamentals of Nursing, Burdur, Türkiye

Abstract: Finding and choosing alternative herbs with strong aroma and antimicrobial properties that grow naturally in Turkey -our country- is especially important for cosmetic products. In this study, the pickling herb (*Echinophora sibthorpiana* Guss.) also known as "çörtük", "tarhana grass" among people was used. Essential oil in plants collected from their natural environment was obtained by distillation and the analysis of volatile components was performed. Then the microbial protection of essential oil in the creams was performed. The pH and viscosity measurements and rapid microbiological analyzes were made in all creams. The results revealed that it was found that no microbial activity was observed in the creams and their pH was compatible with the skin pH.

Keywords: Pickling Herb, Alpha Phellandrene, Methyl Eugenol, Essential Oil, Creams.

Öz: Ülkemizde -Türkiye'de- doğal olarak yetişen, güçlü aroma ve antimikrobiyal özelliğe sahip olan alternatif bitkilerin bulunması ve seçilmesi özellikle kozmetik ürünler açısından oldukça önemlidir. Bu çalışmada halk arasında "çörtük", "tarhana otu" olarak da bilinen *Echinophora sibthorpiana* Guss. kullanılmıştır. Doğal ortamlarından toplanan bitkilerde bulunan uçucu yağlar damıtma yoluyla elde edilmiş ve uçucu bileşenlerin analizi yapılmıştır. Daha sonra uçucu yağın kremdeki mikrobiyal koruyuculuğu çalışılmıştır. Tüm kremlerde pH ve viskozite ölçümleri ile hızlı mikrobiyolojik analizler yapılmıştır. Sonuçlar, kremlerde mikrobiyal aktivitenin gözlenmediğini ve pH'larının cilt pH'ı ile uyumlu olduğunu ortaya koymuştur

Anahtar Kelimeler: Çörtük, Alfa Phellandrene, Metil Öjenol, Uçucu Yağ, Krem.

*Corresponding author : Aslıhan CESUR TURGUT e-mail : acesur@mehmetakif.edu.tr
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Introduction

The pickling herb (*Echinophora sibthorpiana* Guss.) is a member of the *Apiaceae* family. It is a 20-50 cm tall bush like green wild plant with yellow flowers, hairy leaves that can grow easily (Hosseini et al., 2017). This plant, which still waiting to be explored in every aspect, has attracted much attention due to its pharmacological properties.

The pickling herb -a plant with sharp aroma that can be noticed even from a distance and with beautiful appearance due to its yellow flowers- is highly preferred in complementary medicine since its flowers and leaves can be used separately. Its roots, leaves and stem can ve used as drug. While its essential oil (EO) is obtained by steam distillation, in dry form it is consumed as tea and spice in tarhana and pickles. Its most important pharmacological properties are antimicrobial and antifungal activity. It is reported in the literature that the pickling herb contains 0.20-0.95% or 1.3% essential oil (Özcan and Akgül, 2003). Alpha-phellandrene and methyl eugenol are defined as the major compounds in investigated essential oil (Georgiou et al., 2010; Gokbulut et al., 2013). Another important benefit it offers is that it enables those, who suffer from kidney stones or

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renal calculi, are relieved from their problems painlessly. Moreover, in food industry it is used as both as preservative (i.e., in meat) and as flavor (Sengun et al., 2018). It has good antioxidant properties and is generally said to prevent formation of painful grips in women, relaxes the muscles, if any, and relieves them (Cakilcioglu and Turkoglu, 2010). It is known as wound healer in alternative medicine and it is frequently preferred in treatment of dermal diseases such as eczema (Moghaddam et al., 2019). Its content is effective on many disorders such as respiratory and digestive disorders. Therefore, it is known as the healing herb. Its tea is a cure for asthma and bronchitis and facilitates breathing. It prevents accumulation of the infected cells that may come from the respiratory tract to the bronchi and clears the throat from infection (Ozturk et al., 2017).

The major components of the pickling herb essential oil are alpha-phellandrene and methyl eugenol. Alpha-phellandrene, a monoterpene found in the EO of various plants with analgesic and inflammatory effects and is found in high ratio in the pickling herb. Much effort is devoted to exploring its potential to be an analgesic drug (Lima et al., 2012). Moreover, it is thought that it can be used in wound healing as it reduces inflammation and oxidative stress (de Cristo Scherer et al., 2019). It has been observed that this cyclic monoterpene found in plants causes DNA damage that leads to formation of cancer cells (Lin et al., 2015). It was shown to promote natural killer cell activity as well as immunity in normal BALB/c mice (Lin et al., 2014). Methyl eugenol, is a monoterpene with sedative effect on the nervous system and is used in the perfumery and food industry. It is also known that they create a strong antimicrobial effect (Coşkun, 2006).

Humans are dynamic by nature and this pushes them to wear out with aging over time. This situation creates a motivational need for self-renewal and self-care. In modern societies, with the creation of a perception of beauty regardless of gender, cosmetics offers people the solution to this need –the healing from nature- on a gold platter. Cosmetic derives from the Latin word "cosmos" meaning beauty. In the 4th article of the Cosmetic Regulation, cosmetics is defined as "to the outer parts of the human body; All substance prepared to be applied to epiderma, nails, hair, hair, lips and external genitalia or teeth and oral mucosa, whose sole or main purpose is to clean these parts, to smell, to change their appearance, to protect them, to keep them in good condition or to correct body odors or mixtures" (The Ministry of Health, 2005). Human beings have used unique cosmetic products since their existence, and over time this has led to the rise of a new sector. Cosmetics have determined its place in the world economy depending on the demands with technological developments.

Cosmetics are a wide range of products that include many and various products used from health to hygiene, care to treatment. The content of the products has diversified according to the supply-demand situation between the consumer and the producer. Therefore, the frequency of use and the continuous opening during its consumption makes it prone to contamination. In order to prevent the growth of microorganisms that threaten the consumer health addition of protective chemicals to the products in certain proportions is standardized.

Before the chemical and microbiological standards of the product ingredients were regulated by the cosmetic law, consumers were unaware of the harms of the product they used. Romans just as the Egyptians and Romans used mercury and lead without knowing its toxic effects (İlter, 2011). Aside from the negative effects of harmful chemicals contained in the products, it is highly likely that product used would be contaminated and thus use of this would cause serious health problems later (Dao et al., 2018).

In 1946, infant deaths in New Zealand due to the use of talcum powder contaminated with *Clostridium tetani* showed how dangerous the use of contaminated cosmetic products can be (Tan and Tüysüz, 2013). Due to contamination can occur during production or use of the product, it can

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cause visible deterioration in the product. This can cause skin irritation, infection, allergies, or even permanent damage. In medical literature some cases that can lead to blindness due to bad shampoos are reported. Preservatives are chemicals added to the content of the product at sufficient levels for to prevent similar situations, to extend the shelf life of the product, to prevent the growth of microorganisms in cosmetics, and to protect the chemical structure of the product. There are limits for use of preservatives -chemicals that cause biological effects on consumers- have been legalized (The Ministry of Health, 2005). Protecting the product from contamination is of great importance. Hence, even in organic products preservatives are used at certain amounts.

Although preservatives are known to protect the product against microorganisms, it should not be ignored that these chemicals may also have negative effects. For example, paraben, which is frequently preferred and used in cosmetics, food and medicine, passes through the placenta and increases lipophilic character with its estrogenic effect and causes accumulation in adipose tissue (Sinan, 2018) and especially because of this it is thought to be associated with breast cancer.

Today, in order to prevent the above-mentioned health problem's reliability tests are applied to the products. The tests applied measure the degree of antimicrobial activity of the preservatives added to the produced preparations, their durability throughout the shelf life and production starts according to the validity status. Since the consumers are concerned about the use of preservatives used in cosmetics due to their negative health effects, consumers favor medicinal and aromatic herbs that act as natural protective shields. In this study; firstly, the pickling herb was collected from Isparta, then essential oil from the pickling herb was obtained via distilling and volatile components analysis was performed. Then, three cream samples (Control, Protective and PH EO) formulated in different ways were compared in terms of various parameters (pH, viscosity and microbiological activity).

Materials and Method

Collecting the pickling herb for distillation and obtaining essential oil

The pickling herb (*Echinophora sibthorpiana* Guss.) used in the study were collected from Isparta/Turkey. The plants were washed and cleaned. The samples were then quickly transferred to the Clevenger apparatus for to obtain EO via water-steam distillation for 2 hours. The essential oil obtained after distillation was analyzed in Gas Chromatography (GC). The temperature program for the 286 GC was as follows; 60 °C initial temperature, after waiting for 2 minutes at 60 °C to increase 220 °C with 2 °C /min, after reaching this temperature the temperature was kept constant for 20 minutes. The injection was performed in the split mode (20:1). The injection volume was 1 µL. Injector temperature was 240 °C. The GC-MS (Gas Chromatography–Mass Spectrometry) interface was heated at 240°C. MS ion source temperature was 230°C and MS-quadrupole was 150 °C. The electron impact energy was set at 70 eV, and data were collected in the range of 30500 atomic mass units (amu). Compounds' identification was based on mass spectra by comparison with MS spectral database from Wiley. The integrations were performed with MSDCHEM software (Baydar et al., 2013).

Preparation of creams

In the cream production process, which is carried out in accordance with the cosmetics legislation, thickeners, oils, emulsifiers, moisturizers and preservatives are included in the formulation together with essential oil. The oil phase and water phase of the creams were first mixed and heated up to 80 °C, then cooled to 40 °C and mixed for another 15 minutes (Şenses, 2007). Afterwards, they were passed through a homogenizer and the creams took their final shape.

Measurements/analysis of the creams

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In the study, three different creams were created. The first sample was the control and does not contain protective chemicals/PH EO, the second sample was the preservative cream and contains protective chemicals and the third sample was formulated with the EO obtained from the pickling herb and was named the PH EO. The oil phase -a cream forming phase- was obtained by incorporating the EO obtained from the pickling herb in the formulation and to form a new moisturizing cream. Three repetitive (one-month interval) measurements and analysis studies were carried out with in all creams. The pH of the creams was measured by Mettler Toledo brand and S20K KIT model pH meter. Viscosity studies were performed with Brookfield brand, RVDV-11 + PX model viscometer (Erbil, 2000). Microbiological analyses were performed with Biomerux brand Tempo. All analyzes and measurements were carried out at the Burdur Mehmet Akif Ersoy University Application and Research Center.

Statistical analysis

Each experiment was repeated at least three times per sample. Values are expressed as the means ± SD. For all experiments, the overall data were statistically analyzed in SPSS version 25.0 (IBM-SPSS Inc. USA). Duncan's multiple range tests were used ($p < 0.05$).

Results

The results of the measurements and analysis of cream samples were presented below.

Volatile components analysis in GC-MS

The chemical composition of the pickling herb essential oil was summarized in Table 1. Fifteen components were characterized representing 99.9% of the total oil. The oil was characterized by the occurrence of monoterpenes with alpha-phellandrene (28.8%) being the dominant component (Georgiou et al., 2010). The phenylpropanoid derivative methyl eugenol was also found in the oil with a high percentage (24%).

Table 1. Chemical Composition of the Essential Oil of the Pickling Herb

The Components	Retention Time (RT)	Rate %
alpha-thujone	5.08	1.0
alpha pinene	5.2	1.7
camphene	5.4	0.1
sabinene	5.8	0.5
2-beta pinene	5.9	0.3
beta myrcene	6.1	2.1
alpha-phellandrene	6.4	28.8
delta 3-carene	6.5	19.5
gamma terpinene	6.6	2.7
p-cymene	6.7	3.3
beta-phellandrene	6.8	12.9
gamma terpinene	7.3	1.5
alpha-terpinolene	7.8	1.4
1,5,8-p-menthatriene	8.2	0.1
methyl eugenol	12.7	24.0
TOTAL		**99,90**

pH Measurement

The pH values of the creams were measured at 1-month intervals (30th, 60th and 90th days) from their production. The statistically evaluated results are given in the table (Table 2).

Viscosity measurements

Viscosity - a measure of the resistance of a fluid to deformation under surface tension- can be defined as the internal resistance of the fluid against flow. The viscosity results show that while the viscosity results of the first and second sample do not differ greatly, the viscosity was observed to decrease in the third sample -the cream formulated with the EO obtained from the pickling herb. Viscosity measurements were carried out at different rpm (revolution per minute) values (10-100 rpm) and the results are given in Table 3. The viscosity results of the prepared creams are given in Table 3. The results revealed that control sample was determined as 32200-3120 mPa.s. Protective sample and PH EO were found as 31150-2900 mPa.s and 28634-2160 mPa.s, respectively.

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Table 2. The pH Measurements of Creams

Creams	1.pH (30th day)	2. pH (60th day)	3.pH (90th day)
Control	*5.23 ± 0.02[a]	5.36 ± 0.03[c]	5.40 ± 0.01[a]
Protective	5.52 ± 0.01[b]	5.43 ± 0.02[b]	5.49 ± 0.02[b]
PH EO	5.56 ± 0.01[c]	5.30 ± 0.02[a]	5.37 ± 0.02[a]

*Shows values with insignificant difference ($p<0.05$) for each column shown with same letters (± standart deviation)

Table 3. The Viscosity Measurements of Creams

Creams	Viscosity (mPa.s)									
	10 (rpm)	20 (rpm)	30 (rpm)	40 (rpm)	50 (rpm)	60 (rpm)	70 (rpm)	80 (rpm)	90 (rpm)	100 (rpm)
Control	*32200±275[b]	16200±494[b]	11800±659[c]	8500±459[b]	6730±260[b]	5650±258[b]	4750±245[b]	4250±490[b]	3555±430[b]	3120±258[b]
Protective	31150±752[b]	15800±353[b]	10089±549[b]	7845±755[b]	6633±624[b]	5245±430[b]	4605±310[b]	4100±304[b]	3250±354[ab]	2900±324[b]
PH EO	28634±760[a]	12264±370[a]	8200±195[a]	6500±225[a]	4980±180[a]	3544±145[a]	3150±359[a]	2879±230[a]	2656±220[a]	2160±157[a]

*Shows values with insignificant difference ($p<0.05$) for each column shown with same letters (± standart deviation)

Table 4. The Results of the Microbiological Studies

Creams	Total Mesophilic Aerobic Bacteria Count	*E. coli*	Analysis Method
Control	<10 CFB/g	<10 KOB/g	TEMPO, Instrument procedural handbook
Protective	<10 CFB/g	<10 KOB/g	TEMPO, Instrument procedural handbook
PH EO	<10 CFB/g	<10 KOB/g	TEMPO, Instrument procedural handbook

Microbiological studies

The results of the rapid microbiological analysis performed in three replicates with a one-month intervals were presented below. At the end of the 90 days period, no contamination (Total Mesophilic Aerob Bacteria and *E. coli*) above the limit value was observed in any of the creams.

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Discussions

In this study, firstly the pickling herb was collected and distilled via hydrodistilled. Then the volatile components analysis was performed. The creams were formulated with pickling herb essential oil, thickeners, oils, emulsifiers, moisturizers and preservatives. pH, viscosity measurements were made for 90 days (three repetitive and one-month intervals). The protective effect of pickling herb essential oil in creams was studied by rapid microbial tests.

Georgiou et al. (2010) reported that, alpha-phellandrene (43.8%) and methyl eujanol (28.6%) were the major components in pickling herb's essential oil. This study supports our findings. However, the percentage rates were found to be lower than results of Georgiou and colleagues. It is thought that this is related to the location where the plant was collected and the harvesting period of the plant, or it may be due to a different chemotype.

The pH of the skin is acidic (pH=4-6) (Proksch, 2018). Therefore, all cream samples were in the desired pH range. The results of a 90-day this study showed that all three cream samples were found to be derma-friendly creams that comply with the standards.

The Brookfield viscometer has limited sensitivity. Measurements should be made with the same spindle at constant temperature and constant rotation speed (Bovey, 1965; Erbil, 2000). The measurements were carried out with the same spindle at constant temperature in this study. The viscosity of the *control* (32200-3120 mPa.s) and *protective* (31150-2900 mPa.s) sample were similar to each other, while the viscosity of *PH EO* cream was measured lower as 28634-2160 mPa.s. It is estimated that the viscosity decreases due to the fluidity of the essential oil (Kwak, 2015). Consequently, 90 days viscosity values show that all three creams are in spreadable fluidity

As a result of the rapid microbiological analysis, no contamination above the limit value (Total Mesophilic Aerobic Bacteria and *E. coli*) was found in any of the creams. 90 days may not have been enough to detect microbial contamination.

Conclusion

These results show that all creams, including the control, were safe at the end of 90 days, a spreadable consistency and compatible with the skin. However, 90 days dose not enough time to understand product safety. According to the legislation, longer-term stability and challenge tests are needed. It was expected that this study will be informative for future studies and contribute to the literature.

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Ethics Approval

Ethical approval is not required.

Conflict of Interest

The author declare that they have no conflict of interests.

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