2. INTERNATIONAL CONGRESS ON ENVIRONMENT, DISASTER AND FOREST

December 02-03, 2022 / Adana, TÜRKİYE

EDITOR
Asst. Prof. Dr. Simge KOÇ

ISBN: 978-625-7720-87-8

www.ispecongress.org/cevrebilimleriveorman

2. INTERNATIONAL CONGRESS ON ENVIRONMENT, DISASTER AND FOREST

December 02-03, 2022 / Adana, TÜRKİYE

EDITOR

Asst. Prof. Dr. Simge KOÇ

Issued: 15.12.2022
ISBN: 978-625-7720-87-8

Yayın Tarihi: 15.12.2022
ISBN: 978-625-7720-87-8

CONGRESS ID

CONGRESS TITLE

2. INTERNATIONAL CONGRESS ON
ENVIRONMENT, DISASTER AND FOREST

DATE and PLACE

December 2-3, 2022 / Adana, Türkiye

ORGANIZATION

ISPEC INSTITUTE

COORDINATOR

Gönül EDEŞLER
Neslihan BALCI

NUMBER of ACCEPTED PAPERS - 32

NUMBER of REJECTED PAPERS - 10

PARTICIPANTS COUNTRY

Türkiye-15, Coimbatore-1, Indonesia-1, India-1, Nigeria-2, Pakistan-1, Morocco-2, Azerbaijan-2, Tunisia-1, Algeria-1, Iran-1, Romania-4

*Double blinded evaluation proces

ORGANIZING COMMITTEE

Prof. Dr. Hülya ÇİÇEK- Gaziantep University
Prof. Dr. Kağan KÖKTEN- Bingol University
Prof. Dr. Mahmut ERDEMOĞLU- Siirt University
Assoc. Prof. Dr. Abdullah EREN- Mardin Artuklu University
Assoc. Prof. Dr. Beyhan KOCADAĞİSTAN- Ataturk University
Assoc. Prof. Dr. Gökhan ÖMEROĞLU- Ataturk University
Assoc. Prof. Dr. M. Fırat BARAN Siirt University
Assoc. Prof. Dr. Mevlüt ALBAYRAK- Ataturk University
Assoc. Prof. Dr. Mustafa OKANT Harran University
Assoc. Prof. Dr. Özlem TONÇER- Dicle University
Assist. Prof. Dr. Mahire ÖZÇALIK- Kirikkale University

SCIENTIFIC COMMITTEE

Prof. Dr. İbrahim BEKTAŞ- Kahramanmaras Sutcu Imam University
Assoc. Prof. Dr. Abdullah EREN- Mardin Artuklu University
Assoc. Prof. Dr. Beyhan KOCADAĞİSTAN- Ataturk University
Assoc. Prof. Dr. Gökhan ÖMEROĞLU- Ataturk University
Assoc. Prof. Dr. Gülcan DEMİROĞLU TOPÇU- Ege University
Assoc. Prof. Dr. Gülşah BENGİSU- Harran University
Assoc. Prof. Dr. Hamdi KARAKAŞ- Sivas Cumhuriyet University
Assoc. Prof. Dr. M. Fırat BARAN- Siirt University
Assoc. Prof. Dr. Mevlüt ALBAYRAK- Ataturk University
Assoc. Prof. Dr. Murat EYVAZ- Gebze Technical University
Assoc. Prof. Dr. Vedat ÇAVUŞ- Izmir Katip Celebi University
Assoc. Prof. Dr. Veeranoot Nissapatorn- Walailak University
Assist. Prof. Dr. Hale UYAR HAZAR- Bitlis Eren University
Assist. Prof. Dr. Mahire ÖZÇALIK- Kirikkale University
Assist. Prof. Dr. Nizamettin TURAN- Siirt University
Assist. Prof. Dr. Louay S. Yousuf- San Diego State University
Dr. Erdal KARADENİZ- Mardin Artuklu University
Dr. Muhammad Javid Iqbal- COMSATS University Islamabad
Dr. Hassan Hosseinzadeh- University of Wollongong

CONTENTS

Epilobium angustifolium FENOLİK EKSTRAKTININ MANTAR YÜZEYİNDE KORUYUCULUĞUNUN ARAŞTIRILMASI

Fatma ALACA
Mersin University, Faculty of Science, Department of Biology
ORCID: 0000-0002-6209-7018

Ayşe EVEREST
Mersin University, Faculty of Science, Department of Biology
ORCID: 0000-0002-0775-2614

Elif Ayşe ERDOĞAN ELİUZ
Mersin University, Vocational School of Technical Sciences, Department of Food Technology
ORCID: 0000-0003-4317-3000

Arzucan YALÇIN
Mersin University, Vocational School of Technical Sciences, Department of Food Technology
ORCID: 0000-0001-7936-658X

Abstract
Foodborne infections cause diseases that can be fatal to human health. For this reason, food additives or preservatives are used in the food industry to reduce the risk of transmission of pathogens. Today, natural antimicrobial agents are more preferred instead of many synthetic food additives. For this reason, plants are used as natural antimicrobial agents. Among these plants, *Epilobium angustifolium* is in the Onagraceae family and can be found in almost all continents of the world. It grows in streams and lakes, rocky places, meadows, forests, and marshes in our country. In this study, the antimicrobial effect of the extract obtained from the *E. angustifolium* plant against *Escherichia coli*, *Staphylococcus aureus, Acinetobacter baumannii*, and *Candida albicans* microorganisms was investigated by Agar Well Diffusion method. When pathogens are exposed to *E. angustifolium* extract, whether their sensitivity is permanent or temporary (tolerant) was tested by the TDtest (Tolerance Disc Test) method. Accordingly, the sensitivity of the microorganisms continued in the presence of *E. angustifolium* extract and even at the end of the incubation period of 48 hours. No resistance was detected against *E. angustifolium* extract in *S. aureus*, *E. coli*, *A. baumannii*, and *C. albicans* microorganisms. All microorganisms were sensitive to *E. angustifolium* extract. The efficacy of *E. angustifolium* extract on the inactivation of the *E. coli* strain inoculated with the mushroom was evaluated using the immersion incubation method. 100 µL and 150 µL of *E. angustifolium* caused an almost 2 log (99%) reduction in the number of *E. coli* on the mushroom. Also, a mixture containing 10% gelatin (Dr Gusto), 3.5% glycerol (Sigma 98%) and (10-15%) extract was used to prepare the *E. angustifolium* extract-loaded hydrogel solution. The mushroom surface was coated with the obtained *E. angustifolium* hydrogel and time-dependent deterioration was followed. As a result, it has been shown that the mushroom surface can be protected for a long time with *E. angustifolium* phenolic extract.
Keywords: *Epilobium angustifolium*, Food preservative, Antimicrobial activity

Özet
Gıda kaynaklı enfeksiyonlar insan sağlığı için ölümcül olabilecek hastalıklara neden olur. Bu nedenle gıda endüstrisinde patojenlerin bulaşma riskini azaltmak için gıda katkı maddeleri veya koruyucular kullanılmaktadır. Birçok sentetik gıda katkı maddesi yerine günümüzde doğal antimikrobiyal ajanlar daha çok tercih edilmektedir. Bu nedenle bitkiler doğal antimikrobiyal ajan olarak kullanılmaktadır. Bu bitkiler arasında *E. angustifolium* Onagraceae familyası ve dünyanın hemen hemen tüm kıtalarında bulunabilir. Ülkemizde dere ve göl kenarlarında, kayalık yerlerde, çayır, ormanlarda, ve bataklıklarda yetişir. Bu çalışmada, *E. angustifolium* bitkisinden elde edilen ekstraktın *Escherichia coli, Staphylococcus aureus, Acinetobacter baumannii,* ve *Candida albicans* mikroorganizmalarına karşı antimikrobiyal etkisi agar kuyucuk difüzyon yöntemi ile araştırıldı. Patojenler *E. angustifolium*'e ekstraktına maruz kaldıklarında hassasiyetlerinin kalıcı veya geçici (toleranslı) olup olmadığı TDtest (Tolerance Disc Test) yöntemi ile test edildi. Buna göre, *E. angustifolium* ekstraktı varlığında ve inkübasyon süreleri 48 saatin sonunda dahi mikroorganizmaların hassasiyetleri devam etti. *S. aureus*, *E. coli*, *A. baumannii*, ve *C. albicans* mikroorganizmalarında *E. angustifolium* ekstraktına karşı bir direnç tespit edilmedi. Tüm mikroorganizmalar *E. angustifolium* ekstraktına karşı hassastı. *E. angustifolium* ekstraktının, mantar yüzeyine aşılanmış *E. coli* suşunun inaktivasyonu üzerindeki etkinliği daldırma inkübasyon metodu kullanılarak değerlendirildi. *E. angustifolium*'ın 100 µL ve 150 µL'si, mantar üzerindeki *E. coli* sayısında neredeyse 2 log (%95) azalmaya neden oldu. Aynı zamanda, *E. angustifolium* ekstrakt yüklü hidrojel çözeltisinin hazırlanması için %10 jelatin (Dr Gusto), %3,5 gliserol (Sigma 98%) ve (%10-15) ekstrakt içeren bir karışım kullanıldı. Elde edilen *E. angustifolium* hidrojeli ile mantar yüzeyi kaplandı ve zamana bağlı bozulma takip edildi. Sonuç olarak mantar yüzeyinin *E. angustifolium* fenolik ekstraktı ile uzun süre korunabileceği gösterilmiştir.
Anahtar Kelimeler: *Epilobium angustifolium,* Gıda koruyucu, Antimikrobiyal aktivite

INVESTIGATION OF THE PROTECTION OF *Epilobium angustifolium* PHENOLIC EXTRACT ON MUSHROOM SURFACE

INTRODUCTION

Among the medicinal plants, *Epilobium* angustifolium belongs to the Onagraceae family. The Onagraceae (Earring Flower) family is very rich and widespread in the world, includes genera and species. In general, the genus Epilobium is called "Yakı" grass among the people. Epilobium species can be found on almost all continents of the world. It lives in the temperate and high mountain belt. In our country, it grows on the banks of streams and lakes, in open forests, on rocky places, in meadows and marshes (Aslan, 2010). Cultivated forms of this plant are found in some botanical gardens of the world (Plant Finder, 2022). Epilobium species were known that they have a great wound healing activity. Hyperoside was found as the primary active compound in parts of *E. angustifolium* such as leaves and petiole (Karakaya et al., 2020). There has been limited research on *Epilobium angustifolium* studies in the food field. It has been reported to be used in soups and salads (Kalle et al., 2016). Young sprouts can be made into a salad or cooked. The root can also be eaten raw or cooked, or dried to powder. The body part is gelatinous, can be added to soups as a seasoning, has a laxative effect, so it should not be eaten on an empty stomach. Tea is made from the dried leaves. It can be consumed as jam (Sayık, 2007). It has been suggested to be used as a food additive due to its antimicrobial properties. It is stated that *E. angustifolium* can be considered as an important natural antioxidant source, which prevents or minimizes lipid peroxidation in foods, extends the shelf life of drugs and foodstuffs, and partially as an antibacterial agent for foods (Kavaz et al., 2021).

Hydrogels are semi-fluid, soft or slightly rigid structures of cross-linked hydrophilic polymeric network (Ahmed, 2015). Physical hydrogels include hydrogen bonds of hydrophilic polymer networks, hydrophobic interactions, van der Waals interactions and ionic strengths, etc. includes hydrogels with non-covalent bonds. These include biomedical and food applications of hydrogels based on starch (Chen et al., 2019), pectin (Mehrali et al., 2019) or soy protein polysaccharide (Birch et al., 2015).
The aim of this study is to evaluate the antimicrobial activity of the acetone extract of the *E. angustifolium* plant, which is widely used for medicinal purposes. At the same time, the plant was used as a food coating hydrogel and the bacterial inhibition was calculated logarithmically. The food to be chosen is mushroom and it is aimed to cover the mushroom surface with this plant's hydrogel.

Materials and Methods

Preparation of the extract

The plant was collected from the stream side of Çamlıyayla Gülbahçesi station in 2021. After drying, it was ground and 100 g/mL extracts (24 h) were prepared in acetone. It was then filtered and used for experiments.

Antimicrobial screening

The inoculums of *Staphylococcus aureus, Escherichia coli*, *Acinetobacter baumannii*, and *Candida albicans* were prepared in 3 mL Tryptic soy broth for bacteria and 3 mL Sabouraud Dextrose Broth for yeasts and they incubated at 37ºC during overnight. After one day incubation, the microorganism suspensions were adjusted to 0.5 McFarland Turbidity and stored at +4 °C until tests.

Well Diffusion test and Modified TD test

According to well diffusion test, 6 mm wells are opened in the solid medium first and microorganisms are cultivated on the entire medium surface. 50 µL samples were placed in 6 mm wells. All samples were incubated for 24 hours at 37ºC. The results were evaluated by measuring the zone diameters in the Images program.
TD (Tolerance Disc Test) test was created by expanding the disc diffusion with a few simple techniques. This method consists of two steps: For this, 6mm diameter wells were drilled into the middle of Mueller–Hinton_agar plate, and the cultures at the stationary phase were spread the plates. The 50 µL (0.1g/mL) of *E. angustifolium* extract was placed in the wells and incubated at 37°C for 24 hours. Then, as second: 50 µL glucose solution of 10% was placed in the well, which discharged because of the diffusion of the extract into the agar. The petri dishes were re-incubated during 37°C for 24 hours and IZs was measured again and compared with the clear zone in the primary step. TD test, which *E. angustifolium* extract was replaced with the glucose, allows re-growth and detection of the surviving microorganisms on the agar surface. According to this method, it is interpreted as susceptible strain if inhibition zone were found around the well after glucose addition (Gefen et al., 2017).

Coating the mushroom surface with the extract

Inactivation of the *E. coli* strain inoculated on the mushroom surface 1 cm^2 pieces of the mushroom were prepared and sterilized using alcohol and hydrogen peroxide. For this, the mushroom was dipped in ethanol and hydrogen peroxide, respectively, and dried in a sterile environment. 10 µl of *E. coli* (Mcfarland) was inoculated on it and then dried. Then, the extract was dripped onto it and samples were taken after 15 minutes, transferred to a stomacher bag containing 1 ml of phosphate buffer and homogenized for 2 minutes.

Bacteria count was calculated logarithmically using serial dilution on the agar surface. The same procedure was performed using water instead of the extract (negative control) (Palmer et al., 2001).

A mixture containing 10% gelatin (Dr Gusto), 3.5% glycerol (Sigma 98%) and (13%) extract was used to prepare the *E. angustifolium* extract-loaded hydrogel solution. The hydrogel-converted *E. angustifolium* extract was transferred to a beaker while hot and the mushroom was immersed in the beaker. Fungi were immersed in the prepared gel solution and 10-day controls were made. The experiment is repeated 3 times, the most obvious results are photographed.

Statistical analysis

Statistical analyses and significance were measured by Tukey test in one way analysis of variance for IZs using SPSS 25. Differences were considered significant at $p \leq 0.05$.

RESULT and DISCUSSION

The results showed that *E. angustifolium* extract was effective against *A. baumannii, E. coli, S. aureus and C. albicans* by agar well diffusion method (Table 1). There was no statistically significant difference between the IZs of *E. angustifolium* against the pathogens. The highest inhibition zone was found with 23.0 mm on *C. albicans* while the lowest IZ with 11.5 mm on *S. aureus* and also it was 13.2 mm on *A. baumannii* and 22.4 mm on *E. coli* at the end of the 24-h incubation by agar well diffusion test ($p<0.05$). At the end of the 48-h incubation, the inhibition zones were not changed and all pathoges were susceptible (Table 1).

Table 1: IZ (mm) of *E. angustifolium* extract against *A. baumannii, E. coli, S. aureus and C. albicans.* Res: Response of microorganisms in step 2 according to TDTest, S: Susceptible strain

Microorganisms	IZ (24 h)	IZ (48 h)-Res
A. baumannii	$13.2^{a} \pm 3.6$	$13.2^{a} \pm 3.6$-S
E. coli	$22.4^{a} \pm 3.6$	$22.4^{a} \pm 3.6$-S
S. aureus	$11.5^{a} \pm 2.5$	$11.5^{a} \pm 2.5$-S
C. albicans	$23.0^{a} \pm 2.3$	$23.0^{a} \pm 2.3$-S

The average IZs were expressed with the standard deviation (±) and significance level (ANOVA, 25; 0.05, Tukey test). Values on the same column with the same superscript letters don't differ statistically at the 0.05 level.

Similarly, in a study, the anti-microbial effects of *E. angustifolium*, ethanol extract on gram-positive and gram-negative bacteria, yeasts and fungi were determined in vitro. The dry extract showed anti-microbial effect. *E. angustifolium* inhibited a broad spectrum of bacteria, yeasts and fungi (Battinelli et al., 2001). Yüksel et al., (2021) demonstrated *E. angustifolium's* effectiveness against *S. aureus* as 7.0 mm and *E. coli* as 8 mm. Previous studies have shown that its activity is due to the synergistic effect of multiple compounds, not from a single component in the aromatic plant (Lee and Bae, 2017). It has been stated that the phenolic compounds responsible for the activity (Kavaz Yüksel et al., 2021). The highest logarithmic reduction and percent inhibition on the pathogens were shown as 2 $CFU.cm^{-2}$ Log and 99% with 150 µL of the plant, respectively, in *E. coli*, while the lowest logarithmic reduction and percent inhibition were 0.4 $CFU.cm^{-2}$ and 66%, respectively, with 50 µL of the plant.

In the 100 µL of the extract, logarithmic reduction and percent inhibition were 1.8 CFU.cm^{-2} and 98.6%, respectively (Table 2).

Table 2: Log reduction in *E. coli* exposed to *E. angustifolium* on the mushroom surface.

	E. coli		
	50µL	100µL	150 µL
Log reduction (CFU/cm^2)	0.4	1.8	2
Percent reduction (%)	66	98.6	99
Control*		~1.5 x 10^8	

*Starting population

Covering the mushrooms with *E. angustifolium* hydrogel and some of the 10-day observations were photographed (Figure 1). According to the figure, the hydrogel coating of the mushrooms gave a shine to the surface and the direct contact with the air was cut off compared to the control. After a few days, deterioration was observed in both mushroom, but blackening was greater in the uncoated mushroom.

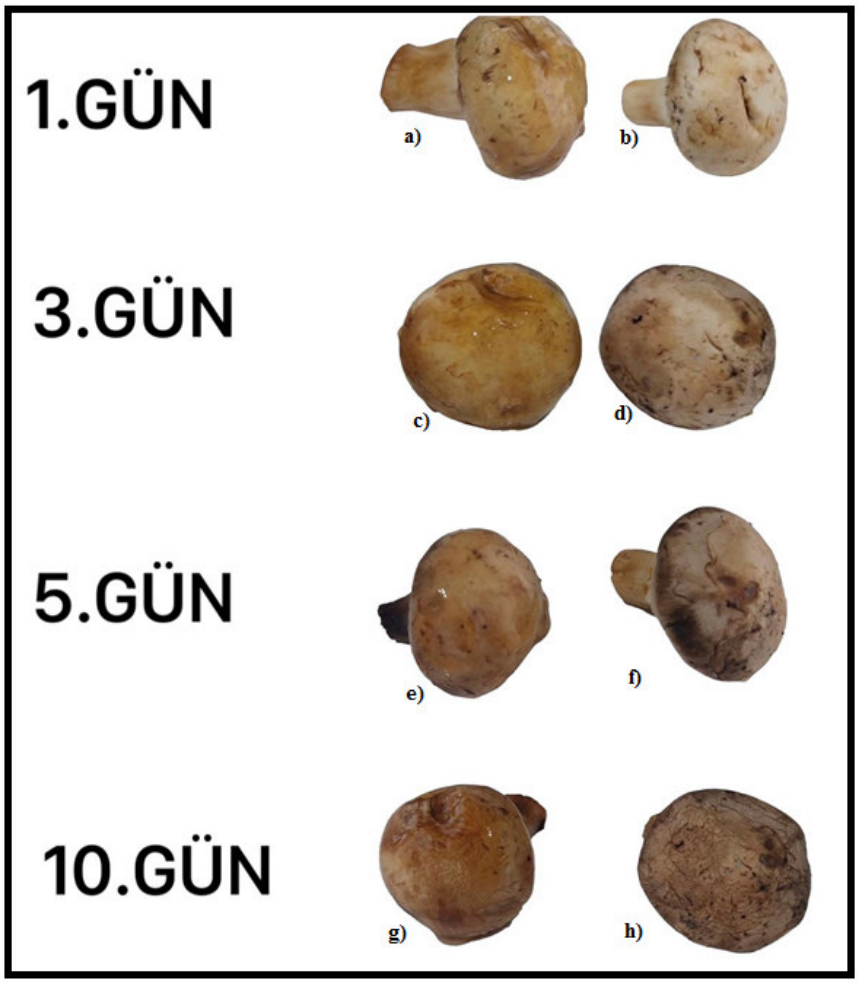

Figure 1. 10-day change in mushroom surface with hydrogel (a,c,e,g) and non hydrogel (b,d,f,h).

As a result, *E. angustifolium* with antimicrobial potential can be used in food coatings to extend the shelf life of foods and to inhibit pathogens. Furthermore, more studies is needed.

Acknowledgements
This project was supported by Mersin University Scientific Research Unit. Project no: 2022-1-TP2-4657

REFERENCES

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. Journal of advanced research, 6(2), 105-121.

Aslan, S., (2010). Yakı otu türleri ve önemi. Uludağ Arıcılık Dergisi. 116-118.

Battinelli, L., Tita, B., Evandri, M. G., & Mazzanti, G. (2001). Antimicrobial activity of Epilobium spp. extracts. Il Farmaco, 56(5-7), 345-348.

Birch, N. P., Barney, L. E., Pandres, E., Peyton, S. R., & Schiffman, J. D. (2015). Thermal-responsive behavior of a cell compatible chitosan/pectin hydrogel. Biomacromolecules, 16(6), 1837-1843.

Chen, J., Chen, L., Xie, F., & Li, X. (2019). Drug delivery applications of starch biopolymer derivatives (pp. 133-137). Springer.

Lee, G., Bae, H. (2017) Therapeutic effects of phytochemicals and medicinal herbs on depression. BioMed Res. Int. 17, 1–11.

Gefen, O., Chekol, B., Strahilevitz, J., Balaban, N.Q., (2017) TDtest:Easy detection of bacterial tolerance and persistence in clinical isolates by a modified disk-diffusion assay. *Sci Rep*, 7 41284.

PlantFinder https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?taxonid=297622. (30.11.202)

Kalle, R., Sõukand, R. (2016). Current and remembered past uses of wild food plants in Saaremaa, Estonia: Changes in the context of unlearning debt. Economic Botany, 70(3), 235-253.

Karakaya, S., Süntar, I., Yakinci, O. F., Sytar, O., Ceribasi, S., Dursunoglu, B., ve diğerleri. (2020). In vivo bioactivity assessment on Epilobium species: A particular focus on *Epilobium angustifolium* and its components on enzymes connected with the healing process. Journal of Ethnopharmacology, 262, 113207.

Kavaz Yüksel, A., Dikici, E., Yüksel, M., Işık, M., Tozoğlu, F., & Köksal, E. (2021). Phytochemical, phenolic profile, antioxidant, anticholinergic and antibacterial properties of Epilobium angustifolium (Onagraceae). Journal of Food Measurement and Characterization, 15(6), 4858-4867.

Mehrali, M., Thakur, A., Kadumudi, F. B., Pierchala, M. K., Cordova, J. A. V., Shahbazi, M. A., ... & Dolatshahi-Pirouz, A. (2019). Pectin methacrylate (PEMA) and gelatin-based hydrogels for cell delivery: converting waste materials into biomaterials. ACS applied materials & interfaces, 11(13), 12283-12297.

Sayık, A. (2007). Yakı otu (*Epilobium angustifolium*) bitkisinin kimyasal yapısının incelenmesi. Unpublished MSc Thesis. Istanbul University Turkey

Smith-Palmer, A., Stewart, J., Fyfe, L. (2001). The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiology, 18, 463-470.

Vitalone, A., & Allkanjari, O. (2018). Epilobium spp: pharmacology and phytochemistry. Phytotherapy Research, 32(7), 1229-1240.