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# Biological Activities and Chemical Composition of Turkish Sweetgum Balsam (Styrax Liquidus) Essential Oil

## Türk Sığala Balzamı (Styrax Liquidus) Uçucu Yağının Biyolojik Aktiviteleri ve Kimyasal Bileşimi

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### ABSTRACT

**Objective:** The purpose of this present study was to make a chemical analysis of the composition of essential oil obtained from sweetgum balsam and to examine its antimicrobial, anticholinesterase, α-glucosidase inhibition, and antioxidant activities.

**Methods:** The essential oil obtained by the hydrodistillation method was analyzed by gas chromatography-mass spectrometry systems. The antimicrobial activities of the essential oil were evaluated by disc diffusion and resazurin microplate methods, the anticholinesterase effect was determined by using *in vitro* AChE and BChE enzymes inhibition assays, and the antioxidant effect was evaluated by ABTS and CUPRAC methods.

**Results:** The main components of the essential oil were determined as styrene (92.6%) and α-pinene (2.2%). The essential oil showed weak antimicrobial activity against *K. pneumoniae*, *S. epidermidis* but strong antimicrobial activity against *A. baumannii, C. glabrata*. It showed moderate inhibitory activity to AChE and BChE enzymes, and $IC_{50}$ values were calculated as 36.5 µg/mL and 69.5 µg/mL, respectively. It also showed low inhibition of α-glucosidase ($IC_{50}$ value was 637.2 µg/mL) and a similar antioxidant effect in the CUPRAC and ABTS method ($A_{0.5}$ value was 637.2 µg/mL and $IC_{50}$ value was 632.2 µg/mL, respectively).

**Conclusion:** Styrax Liquidus essential oil can be considered a natural antimicrobial agent due to its strong antimicrobial activity capacity against *A. baumannii* and *C. glabrata* strains.

### ÖZ

**Amaç:** Bu çalışmada, sığala balzamından elde edilen uçucu yağın bileşiminin kimyasal analizinin yapılması, antimikrobiyal, antikolinesteraz, α-glukozidaz inhibitörü ve antioksidan aktivitelerinin incelenmesi amaçlanmıştır.

**Yöntemler:** Hidrodistilasyon yöntemiyle elde edilen uçucu yağ, gaz kromatografisi kütle spektrometresi sistemleri ile analiz edilmiştir. Uçucu yağın antimikrobiyal aktiviteleri disk difüzyon ve resazurin mikroplak yöntemleri ile; antikolinesteraz etkisi *in vitro* AChE ve BChE enzimleri inhibisyon testi ile; antioksidan etkisi ise ABTS ve CUPRAC yöntemleri ile değerlendirilmiştir.

**Bulgular:** Uçucu yağın ana bileşenleri stiren (%92,6) ve α-pinen (%2,2) olarak tespit edilmiştir. Uçucu yağ, *K. pneumoniae, S. epidermidis* kökenlerine karşı zayıf, *A. Baumannii, C. glabrata* kökenlerine karşı güçlü antimikrobiyal aktivite göstermiştir. AChE ve BChE enzimlerine orta derecede inhibitör aktivite gösterip $IC_{50}$ değerleri sırasıyla 36,5 µg/mL ve 69,5 µg/mL olarak hesaplanmıştır. Ayrıca düşük oranda α-glukozidaz inhibisyonu ($IC_{50}$ değeri 637,2 µg/mL) ve CUPRAC ve ABTS yönteminde benzer bir antioksidan etki (sırasıyla $A_{0,5}$ değeri 637,2 µg/mL ve $IC_{50}$ değeri 632,2 µg/mL) göstermiştir.

**Sonuç:** Styrax liquidus uçucu yağı, *A. baumannii* ve *C. glabrata* suşlarına karşı güçlü antimikrobiyal aktivite kapasitesi nedeniyle doğal bir antimikrobiyal ajan olarak değerlendirilebilir.

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**Keywords:** Styrax liquidus, essential oil, antimicrobial, anticholinesterase, α-glucosidase, antioxidant

**Anahtar Sözcükler:** Styrax liquidus, uçucu yağ, antimikrobiyal, antikolinesteraz, α-glukozidaz, antioksidan

## Introduction

Styrax liquidus is a resinous exudate (sweetgum balsam) obtained from the wounded trunk of *Liquidambar orientalis* Mill. from the Altingiaceae family. The species is found only in southwestern Turkey and on the island of Rhodes. *L. orientalis* is an endangered relict species (1,2). In history, essential oil of Styrax liquidus was known as Egyptian Queen Cleopatra's "love elixir" and used as perfume oil. It has been used as a medicine since the Hippocrates. Ancient Egyptians used this oil during mummification. Amphoras filled with balsam extracted from sunken Phoenician ships indicated that the Styrax liquidus had an important place in the Mediterranean trade in the past (3).

The Styrax liquidus has been used for the treatment of various ailments in Turkish folk medicine. It is used to treat wounds, asthma, bronchitis, upper respiratory tract diseases, skin diseases like scabies and it is used as expectorant and antifungal. It used as fumigation, in the form of powder and pastille, and in the form of pomade and plaster (4-7). The essential oil of Styrax liquidus has been used in the pharmaceutical and cosmetic industry particularly in perfumery. The essential oil constitutes 0.5-1% of the balsam (8).

In the literature review, it was determined that balsam had antibacterial, antioxidant and antiulcerogenic effects (9-11). Styrax liquidus essential oil has been reported to be inhibitory on the central nervous system as well as having an antimicrobial activity (12,13).

The present study was performed to evaluate the antimicrobial, antioxidant, anticholinesterase, and α-glucosidase inhibitory activities of Styrax liquidus essential oil and investigate its chemical composition.

## Methods

### Extraction of Essential Oils

Styrax liquidus was obtained from local people in Marmaris, Muğla. The essential oil of Styrax liquidus was obtained by hydrodistillation in the Clevenger apparatus for 3 hours. Essential oil, thus obtained was stored in sealed vials at +4 °C until analyzed and tested.

### Gas Chromatography (GC) and Gas Chromatography-mass Spectrometry Analysis (GC/MS)

The composition of the essential oil was determined by using gas chromatography (GC) and GC/mass spectrometry (GC/MS).

The GC analysis was performed on the Agilent 6890N GC system at an FID detector temperature of 300 °C. To achieve the same elution order as GC/MS, the simultaneous automatic injection was performed on a replicate of the same column, applying the same operating conditions. The relative percentage amounts of the separated compounds were calculated from the FID chromatograms. The analysis results are given in Table 1.

The GC/MS analysis was performed on an Agilent 5975 GC/MSD system on an Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) using helium (0.8 mL/min) as carrier gas. The GC oven temperature was held at 60 °C for 10 minutes and programmed at 220 °C at 4 °C/min, and kept constant at 220 °C for 10 minutes, then programmed to 240 °C at 1 °C/min. The division ratio was set to 40:1 and the injector temperature was set to 250 °C. Mass spectra were recorded at 70 eV. The mass range was m/z 35 to 450.

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index to a series of n-alkanes. Commercial (Wiley GC/MS Library, MassFinder Software 4.0) (14,15) and in-house (Başer Library of Essential Oil Constituents) mass spectral libraries built up by genuine compounds and components of known oils were used.

### Antimicrobial Activities

The essential oil was screened for their antimicrobial activities against *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 49461, *Acinetobacter lwoffii* ATCC 19002, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeuroginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans ATCC 66027* and *Candida glabrata ATCC 2001* microorganisms by disc diffusion and resazurin microplate methods.

### Disk Diffusion Test

Fresh passages from the microorganisms were taken first to be tested using Disk Diffusion Tests in our study. The next day, suspensions were obtained from standard strains in saline water with a turbidity level of McFarland 0.5 ($10^8$ microorganisms/mL). The Mueller Hinton Agar plate was seeded with a sample taken from these suspensions using a sterile swab.

The essential oil of sweetgum balsam was first passed through a 0.22 µm filter for sterilization. Then, the paper discs obtained from Whatman paper that we would prepare were impregnated with the essential oil of sweetgum balsam placed on the plates. Moreover, various antibiotic discs with known sensitivity were also placed in Petri dishes for comparison. During this process, care was taken to keep a 22 mm space between the discs and a 14 mm distance from the edge of the Petri dish to ensure that the zones formed did not overlap. The inhibition zones were then measured by incubating the media for 18-24 hours at 37 °C.

### Resazurin Microplate Assay (REMA)

Resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide) microplate method was used to determine the antibacterial activities and minimum inhibitory concentrations (MIC) of the essential oil of sweetgum balsam *in vitro* against the standard origins of *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 49461, *Acinetobacter lwoffii* ATCC 19002, *Acinetobacter baumannii* ATCC 19606 *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeuroginosa* ATCC 27853 *Escherichia coli* ATCC 25922, *Candida albicans ATCC 66027* and *C. glabrata ATCC 2001*. The activity determination was planned as two replicates. Streptomycin and fluconazole were used as the standard drugs. Stock solutions of the studied substances were prepared with DMSO at a concentration of 1,024 µg/mL and sterilized by passing through a 0.22 µg membrane filter. To begin, each well was filled with 100 µL of Muller Hinton Broth medium. Serial dilutions of the prepared solutions were made by adding 1,000 µg/mL to the first well of 96-well microplates (MIC range of chemicals 0.3-1,000 µg/mL). The final concentration of the standard drug streptomycin was adjusted to 83 µg/mL and other standard drug fluconazole was adjusted to 30 µg/mL, and serial dilutions were made by adding 50 µL to the first well. Only DMSO was placed in one column of the plate as a negative control, and only standard bacteria as a positive control in the other column, both of which were 50 µL, and serial dilutions were made. A suspension of McFarland 0.5 turbidity was prepared from 1-2 day old colonies of microorganisms and then diluted 1:100. 10 µL of these final suspensions were added to the plate wells. Plates were covered with parafilm and incubated in a normal atmosphere for 24 hours at 37 °C. After the incubation, 10 µL each of 33.75 mg resazurin and 20% Tween 80, which were dissolved in 5 mL distilled water, were added to all wells, and plates were left to incubate for 2-4 hours, and the results were evaluated visually. The MIC value was determined to be the lowest concentration value preventing the color change from purple to pink.

**Inhibitory Activities Against AChE and BChE**

The anticholinesterase activity of essential oil was determined by using *in vitro* AChE and BChE enzymes inhibition assays. AChE and BChE inhibition activities were determined using the method found by Ellman et al. (16). Galantamine is used as reference compound. The $IC_{50}$ was determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of seven different concentrations. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates in the reaction, and 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used as a reagent. Stock solutions of essential oils and galantamine were prepared with methanol at a 4,000 µg/mL concentration. Acetylthiocholine iodide at 7.085 mM concentration and butyrylthiocholine iodide at 0.785 mM concentration were prepared. Hundred and fifty µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution, and 20 µL of AChE (2,476x10-4 U/µL) (or 3,1813x10-4 U/µL of BChE) solution were mixed. It was incubated at 25 °C for 15 minutes. Ten µL of DTNB solution at a concentration of 5 mM was added. The reaction was initiated by the addition of 10 µL acetylthiocholine iodide (or butyrylthiocholine iodide). In this method, the activity was measured by following the yellow color produced as a result of the presence of thio anion which was produced by the enzymatic hydrolysis of the substrate with DTNB. Also, methanol was used as a control solvent. The hydrolysis of the substrates was monitored using a BioTek Power Wave XS at 412 nm (17). All experiments were done in triplicate and inhibition activity was calculated as;

Inhibition (%) = $(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample})}/\text{Absorbance}_{\text{Control}}$ x100

$IC_{50}$ values were calculated using Graphpad Software.

**α-Glucosidase Inhibition Assay**

Commercially available α-glucosidase from *Saccharomyces cerevisiae* (Sigma, G5003) was selected as the target protein using p-nitrophenyl-α-D-glucopyranoside (pNGP, Sigma, N1377) as substrate. The essential oil and genistein were dissolved in DMSO, and the enzyme and substrate were dissolved in potassium phosphate buffer (0.05 M, pH 6.8). The enzymatic reaction mixture consisting of α-glucosidase (0.02 U, 20 µL), substrate (1.25 mM, 30 µL), essential oil solution (10 µL), and potassium phosphate buffer (140 µL) was incubated at 37 °C for 30 minutes. Next, the absorbance of the yellow color produced due to p-nitrophenol formation was measured at 405 nm using a Synergy H1 (BioTek, USA) 96-well microplate reader (18). All experiments were done in triplicate. α-glucosidase inhibition activity was calculated as;

Inhibition (%) = $(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample})}/\text{Absorbance}_{\text{Control}}$ x100

$IC_{50}$ values were calculated using Graphpad Software.

## Antioxidant Activity Assay

**ABTS Cation Radical Scavenging Assay**

ABTS cation radical scavenging activities of the essential oil were determined using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (19). A stock solution of 1,000 µg/mL of essential oil was prepared. Two, 5, 10, and 20 µL of this stock solution were taken and their volume was made up to 40 µL with ethanol, and 160 µL of 7 mM ABTS cation radical solution was added to them. After the reaction was kept in the dark for 6 minutes, absorbance was measured at 734 nm. ABTS cation radical scavenging activity was calculated by evaluating the absorbance values of the essential oil sample against the control. The percent inhibition was calculated from the following equation;

Inhibition (%) = $(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample})}/\text{Absorbance}_{\text{Control}}$ x100

$IC_{50}$ values were calculated using Graphpad Software.

**CUPRAC Assay**

In the presence of antioxidant compounds in the essential oil, Cu(II)-Neocuproin (Nc) complex was reduced to colored Cu(I)-Nc chelate, and the absorbance of this chelate was measured at 450 nm. Butylated hydroxytoluene (BHT) was used as standard. $CuCl_2$, neocuproin, and $NH_4OAc$ buffer were added to the essential oil and BHT with final concentrations of 10, 25, 50, 100 µg/mL, and absorbance was measured at 450 nm after 1 hour (20). The absorbance values of the essential oil sample were evaluated against the standard. The study was performed in three replications.

## Results

### Composition of the Essential Oils

The analysis of essential oil obtained by the hydrodistillation method was carried out with GC/FD and GC/MS systems. Comparative analysis results are given in Table 1.

### Antimicrobial Activities

The essential oil was screened for its antimicrobial activities against *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 49461, *Acinetobacter lwoffii* ATCC 19002, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeuroginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans ATCC 66027* and *Candida glabrata ATCC 2001* microorganisms by disc diffusion method and resazurin microplate methods. According to our results; the essential oil showed weak antimicrobial activity against *K. pneumoniae,* and *S. epidermidis* but strong antimicrobial activity against *A. baumannii,* and *C. glabrata* (Table 2).

**Table 1.** The essential oil composition of Styrax liquidus

| No | RRI | Compound | (%) |
|---|---|---|---|
| 1 | 1032 | *α*-Pinene | 2.19 |
| 2 | 1035 | *α*-Thujene | 0.06 |
| 3 | 1076 | Camphene | 0.01 |
| 4 | 1118 | *β*-Pinen | 0.5 |
| 5 | 1203 | Limonene | 0.07 |
| 6 | 1218 | *β*-Phellandrene | 0.05 |
| 7 | 1272 | Styrene | 92.59 |
| 8 | 1466 | *α*-cubebene | 0.18 |
| 9 | 1497 | *α*-Copaene | 0.15 |
| 10 | 1541 | Benzaldehyde | 0.08 |
| 11 | 1549 | *β*-cubebene | 0.18 |
| 12 | 1611 | Terpinen-4-ol | 0.02 |
| 13 | 1612 | *β*-caryophyllene | 0.03 |
| 14 | 1671 | Acetophenone | 0.43 |
| 15 | 1704 | *γ*-Murolen | tr |
| 16 | 1740 | *α*-Murolen | tr |
| 17 | 1773 | *δ*-Cadinene | 0.19 |
| 18 | 1804 | Myrtenol | tr |
| 19 | 2049 | 4-Ethylguaiacol | 0.06 |
| 20 | 2065 | Benzenepropanol | 0.87 |
| 21 | 2068 | *(E)*-Cinnamaldehyde | 0.09 |
| 22 | 2113 | Cumin alcohol | 0.48 |
| 23 | 2157 | *(E)*-Ethyl cinnamate | 0.05 |
| 24 | 2195 | 4-Ethyl phenol | 0.67 |
| 25 | 2219 | *α*-Muurolol | 0.24 |
| 26 | 2308 | Cinnamyl alcohol | tr |
| **Total** | | **99.19** | |
| **Oil yield** | | **0.57** | |

RRI: Relative retention indices calculated against *n*-alkanes, % calculated from FID data, and tr: Trace (<0.01%).

### AChE and BChE Inhibition

The essential oil moderately inhibited cholinesterase enzymes. Galantamine was used as the standard substance. The calculated $IC_{50}$ values are given in Table 3.

### α-Glucosidase Inhibition

Styrax liquidus essential oil showed a low rate of α-glucosidase inhibition compared to genistein, which had strong inhibitory properties against α-glucosidase. Calculated $IC_{50}$ values are given in Table 4.

### Antioxidant Activity

Antioxidant effect was examined by CUPRAC and ABTS methods. Activity results were evaluated by comparison with standard BHT. It was observed that essential oil had a very low antioxidant effect when compared to standard (Table 5).

**Table 2.** Investigated microorganisms for antimicrobial effects

| Microorganisms | Minimal inhibitory concentration of the essential oil of Styrax liquidus (µg/mL) |
|---|---|
| *Enterococcus faecalis* ATCC 29212 | 125 |
| *Streptococcus pneumoniae ATCC 49619* | 250 |
| *Stapyhlococcus aureus* ATCC 29213 | 125 |
| *Stapyhlococcus epidermidis* ATCC 49461 | 62.5 |
| *Escherichia coli* ATCC 25922 | 125 |
| *Pseudomonas aeruginosa* ATCC 27853 | 125 |
| *Klebsiella pneumoniae* ATCC 70063 | 62.5 |
| *Acinetobacter baumannii* ATCC 19606 | 31.25 |
| *Acinetobacter lwoffii* ATCC 19002 | 125 |
| *Candida albicans* ATCC 66027 | 125 |
| *Candida glabrata* ATCC 2001 | <3.9 |

## Discussion

Styrax Liquidus is known to have various biological effects due to its use in folk medicine and scientific studies. A limited number of studies has reported the biological effects of essential oil Styrax liquidus. On GC-GC/MS analysis of the essential oil, 26 compounds representing 99.19% of the total oil were identified where styrene (92.59%) and α-pinene (2.2%) were identified as the major components (Table 1).

There are several previous publications with different results in the literature regarding the chemical content of Styrax liquidus. Analysis by GC-MS resulted in cinnamyl cinnamate (21.5%), phenyl propyl cinnamate (7.5%), cinnamic acid (4.0%), cinnamyl alcohol (2.0%), styrene (0.5%) and phenyl propyl alcohol (0.5%). However, in this study, approximately 60% of the content was analyzed and different results were thought to be due to this (21). Another study reported that the main components of essential oil were styrene (89.5%), α-pinene (7.2%), and β-pinene (1.1%) (22). These results are very close to our findings.

In a different study, among 58 components representing more than 99.4% of the essential oil, styrene (70.4%), α-pinene (19.4%), and β-pinene (4.3%) were determined as the main components (23).

The benefits of Styrax liquidus have been known for years, especially by local people. It has been used to treat skin problems, peptic ulcers, parasitic infections (4,7,24). The antibacterial, antiulcerogenic, antioxidant, and cytotoxic effects of styrax were investigated by researchers (10,25,26).

Our aim in this study was to reveal the potential for antioxidant, antidiabetic, anticholinesterase, and antimicrobial effects of the essential oil of sweetgum balsam.

**Table 3.** *In vitro* inhibition $IC_{50}$ for AChE and BChE activities

| Test sample | AChE $IC_{50}$ (µg/mL) | BChE $IC_{50}$ (µg/mL) |
|---|---|---|
| Styrax liquidus essential oil | 36.5 | 69.5 |
| Galantamine | 0.55 | 3.56 |

**Table 4.** α-Glucosidase inhibition $IC_{50}$ values

| Test sample | $IC_{50}$ (µg/mL) |
|---|---|
| Styrax liquidus essential oil | 637.2 |
| Genistein | 12.279 |

**Table 5.** CUPRAC and ABTS values

| Test sample | CUPRAC $A_{0.5}$ (µg/mL) | ABTS $IC_{50}$ (µg/mL) |
|---|---|---|
| Styrax liquidus essential oil | 637.2 | 632.2 |
| BHT | 3.275 | 1.242 |

BHT: Butylated hydroxytoluene

Anti-α-glucosidase compounds have received great attention due to their potential use in treating diabetes (27). Our study is the first report on anticholinesterase and α-glucosidase activities of essential oil of Styrax liquidus. The $IC_{50}$ values against AChE and BChE were 36.5 µg/mL and 69.5 µg/mL, respectively. The $IC_{50}$ values of enzyme inhibition results of galantamine were AChE =0.55 µg/mL and BChE =3.56 µg/mL, respectively. The obtained essential oil appeared to inhibit cholinesterase enzymes at a moderate level. It showed a low rate of α-glucosidase inhibition ($IC_{50}$ value was 637.2 µg/mL, Genistein = $IC_{50}$ value of was 12,279 µg/mL) and similar antioxidant effect on CUPRAC and ABTS method ($A_{0.5}$ value was 637.2 µg/mL, BHT was 3,275 µg/mL, and $IC_{50}$ value was 632.2 µg/mL, BHT was 1.242 µg/mL, respectively).

A previous study investigated the antibacterial potential of Styrax liquidus against several bacteria strains by using the agar diffusion method. Different concentrations of balsam are potent on some bacterial strains. In one of the most comprehensive studies, its antibacterial activitywas studied. The concentration of 10% balm was effective against *Bacillus brevis, B. cereus, B. subtilis, Corynebacterium xerosis, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumoniae, Micrococcus luteus, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescent,* and *Staphylococcus aureus.* The concentration of 0.4% balm was effective against *E. aerogenes, P. vulgaris*. The concentration of 0.2% balm was effective against *E. aerogenes,* and *P. vulgaris*. Besides, the concentration of 0.1% did not show any antibacterial effect reported (11). In our study, the essential oil of Styrax liquidus showed weak antimicrobial activity against *Klebsiella pneumoniae, Staphylococcus epidermidis* but strong antimicrobial activity against *Acinetobacter baumannii, Candida glabrata* (Table 2).

Due to their strong antimicrobial activities, plant-derived secondary metabolites are known to be important in the treatment of various diseases (28). In a study investigating the antiadhesive efficacy of coated with Styrax liquidus surgical silk sutures against common oral pathogenic microorganisms, the highest antiadhesive efficacy was observed against *S. aureus* (29).

Styrax liquidus is obtained from *L. orientalis.* This species is considered endangered due to threats such as agricultural activities, fires, pollution, polluted water, heavy tourism investments, and overgrazing. It was therefore recorded as "Critically Endangered" on the 2017 European Red List (30).

In order to ensure continuity in the supply of natural products in pharmaceutical research, it is necessary to protect and cultivate medicinal plants.

### Study Limitations

Strong antimicrobial effects of Styrax liquidus essential oil were determined. However, evaluation of its use as an antimicrobial agent was limited, since studies on cytotoxic effects could not be performed.

## Conclusion

As in all balsams, the content of Styrax liquidus includes resin, essential oil, and free cinnamic acid. The resin containing "cytorezinol" constitutes 30-40% of the balm and 20-25% of the essential oil. The traditional medicinal use of the balm in various diseases and the low biological activity of the essential oil alone show that the components in the balm have a synergistic effect. In this study, the most effective biological activity of essential oil is found as the antimicrobial effect. It is important to search for alternative antimicrobial agents because of resistance to existing antimicrobial drugs over time. Styrax liquidus essential oil can be considered a natural antimicrobial agent due to its strong antimicrobial activity against *A. baumannii* and *C. glabrata* strains.

**Ethics**

**Ethics Committee Approval:** Our study does not require ethics committee approval.

**Peer-review:** Externally peer reviewed.

**Authorship Contributions**

Concept: B.B.A., G.I., B.D., Design: B.B.A., Data Collection or Processing: B.B.A., Analysis or Interpretation: B.B.A., G.I., B.Z.K., B.D., Literature Search: B.B.A., Writing: B.B.A., G.I., B.Z.K., B.D.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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