

POSSIBILITY OF USING SIĞLA TREE (*LIQUIDAMBAR ORIENTALIS* MILL. VAR *ORIENTALIS*) STORAX AS BACTERICIDE IN THE SOAKING FLOAT

by

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ABSTRACT

An increasing amount of research work has been directed to limiting the use of synthetic biocides for preventing microorganism formation on finished leather, and the develop of more suitable substitute products. Much of this work has been directed towards the protective effects of natural bioactive materials.^{1,2,3,4} The know antibacterial impact of storax against a variety of bacteria has now put this technology on the leather industry research agenda. In the present study, Siğla (*Liquidambar orientalis* Mill.var *orientalis*) storax was investigated for antibacterial activity during the soaking float. In this investigation, 1% , 2% , 5% of storax and 1% essential oil of *Origanum onites* mixed with the storax were tested for their antimicrobial activity in parallel with 50 % organosulfur compound as commercial bactericides commonly used in the leather industry. The results showed that storax has an antibacterial effect in soaking with increasing concentrating rates and when it is mixed with the oil of oregano, the bactericidal activity was increased. It was found that Siğla Storax contains 1.39% essential oils and styrene accounts for 96.55% of this oil. These findings suggested Siğla storax and oregano essential oil has synergic effect and the mixture of Siğla storax and oregano essential oil could be used as bactericidal agents in leather industry.

RESUMEN

Un creciente volumen de trabajos de investigación se ha dirigido a limitar el uso de biocidas sintéticos para prevenir la formación de microorganismos en el cuero acabado, y para el desarrollo de más productos de sustitución adecuados. Gran parte de este trabajo se ha orientado hacia los efectos protectores de materiales naturales bioactivos. El efecto antibacteriano conocido del storax contra una variedad de bacterias ha puesto a esta tecnología en la agenda de investigación de la industria de cuero. En el presente estudio, Siğla (*Liquidambar orientalis* Mill.var *orientalis*) storax fue analizado por su actividad antibacteriana en el baño de remojo. En esta investigación, el 1%, 2%, 5% de storax más el 1% de aceite esencial de *Origanum onites* mezclado con el storax, fueron analizados para determinar su actividad antimicrobiana en paralelo con el 50% de un compuesto organosulfurado como bactericidas comerciales de uso común en el cuero. Los resultados mostraron que el storax tiene un efecto antibacteriano en el remojo con el aumento de las tasas de concentración y cuando se mezcla con el aceite de orégano, la actividad bactericida es incrementada. Se encontró que Siğla Storax contiene aceites esenciales en 1,39% y el estireno conforma un 96,55% de este aceite. Estos resultados sugieren que Siğla Storax y el aceite esencial de orégano tiene un efecto sinérgico y la mezcla de Siğla storax y el aceite esencial de orégano puede utilizarse como agentes bactericidas en la industria del cuero.

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Manuscript received July 9, 2009, accepted for publication October 11, 2009

INTRODUCTION

Soaking is the first process of rehydrating and cleaning hides. Due to extended times in soaking periods or when the number of bacteria with proteolytic activity increases very much, hides and skins need to protection against bacterial attacks. Currently synthetic preserving agents are used for this aim. However, the bactericidal agents that are currently used in the industry are generally harmful to human health and nature, and their use has been either restricted or banned in certain countries. For example, Pentachlorophenol (PCP) has been banned lately due to its toxicity problems despite the fact that it was in the past known as a common chemical widely used in control of bacterial and fungal contamination in leather industry.⁵ The use of polyhalogenated phenolic compounds (TCP / TBP) has also been restricted in certain countries to that end.⁶ In addition to legal restrictions, leather manufacturing companies are also paying a great attention to eco labeling such as Blue angel, although they do not actually imply any legal restrictions. According to the most recent self regulation reported in the Blue Angel (RAL UZ 117): Low-emission preserving agents for upholstered furniture must only be used for intermediary protection during transport and storage and the applicable threshold values for finished leathers should be PCMC < 600 mg / kg, OIT < 250 mg / kg ,OPP < 1000 mg / kg , TCMTB < 500 mg/kg etc.⁷ Although every company is allowed to determine the value on its own, many downstream companies such as those in the automotive industry foresee the mentioned threshold values, which impels the entire supply chain to abide by the same parameters. For this reason product formulations and application processes are under continuing development. Moreover alternative products are also being investigated.

The objective of this study is to determine whether the 100% natural products, namely Sığla storax, could be used as alternative bactericides in the leather industry. In this research, oregano essential oil is also used because it has been identified as an effective bactericidal agent in soaking process.^{1,3} This study aims to test whether oil of oregano as a synergic impact on bacteria together with storax. Storax was also tested in the present research for its bactericidal activities against a commonly used antibacterial agent.

The species of Liquidambar, an important genus of Hamamelidaceae family, are known for their balsamic exudations. Of the species, Liquidambar orientalis Mill. is a native of Asia minor and has a local distribution in the southwestern coastal district of Türkiye, especially growing in Köyceğiz, Fethiye, Marmaris and Ula.^{8,9} The balsam produced by injuring of the trees is known in commerce as Levant Storax. Balsam production from the living trees requires a special procedure of many stages.^{9,10} Storax is a semi-solid, sticky material characterized by a balsamic and styrene-like odor; it is used in floral-type perfumes.¹¹

Storax has been used for centuries in folk medicine for the treatment of some skin diseases, diseases of respiratory system and as a neurotonic drug.^{9,10,12} Storax has antibacterial activity and the most sensitive bacterium to storax is *B. cereus* and for this reason it was reported that Sığla storax can be used for protection against bacteria as a topical antibacterial agents in some cases.¹³ The researchers reported that *Bacillus subtilis*, *Bacillus cereus*, *Bacillus pumilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus sphaericus*, *Bacillus brevis*, *Bacillus laterosporus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Micrococcus rubens*, *Kurthia variabilis*, *Pseudomonas aureginosa*, *Staphylococcus epidermidis*, and *Micrococcus candidus* were isolated from the raw hide.¹⁴ Table 1 shows the inhibitory effect of sığla storax at different concentrations against bacteria and the bacteria which can grow in leather also marked.¹³ From this results it seems that storax can be used in soaking process as bactericidal agents and this study has been designed accordingly.

TABLE I
Inhibitory effect of Sığla storax at different concentrations against bacteria (diameter of inhabitation zone in mm)¹³

Test Bacteria	10	1	0,4	0,2	0,1
<i>Bacillus brevis</i> *	12	—	—	—	—
<i>Bacillus cereus</i> *	16	13	—	—	—
<i>Bacillus subtilis</i> *	10	8	—	—	—
<i>Corynebacterium xerosis</i>	8	—	—	—	—
<i>Enterobacter aerogenes</i>	12	10	8	6	—
<i>Enterococcus faecalis</i>	13	—	—	—	—
<i>Klebsiella pneumoniae</i>	6	—	—	—	—
<i>Micrococcus luteus</i> *	14	—	—	—	—
<i>Mycobacterium smegmatis</i>	11	—	—	—	—
<i>Proteus vulgaris</i>	12	8	6	6	—
<i>Pseudomonas aeruginosa</i> *	12	—	—	—	—
<i>Pseudomonas fluorescens</i>	11	9	—	—	—
<i>Staphylococcus aureus</i> *	14	—	—	—	—

- not detectable

* The bacteria isolated from hide ¹⁴

EXPERIMENTAL

Materials and Methods

Raw skin

Native brand sheep (salted-dry) raw skins were selected for the investigation. From each skin, seven pieces (weighing

about 100 grams) were carefully cut and used in soaking tests run in parallel in the following order; 1st. row (soaking float) contained a known 0.3% bactericidal agent (50% organosulfur compound), 2nd, 3rd and 4th soaking rows contained 1%, 2%, 5% storax, 5th row contained the oregano oil, 5th row contained mixture of 1% storax and 1% oregano essential oils and the 7th row was the control respectively. The test was repeated twice.

Soaking Process

Throughout the experiments, all washing procedures were performed in sterile distilled water (DW). Pre-washing steps (for 1h) of the leather samples and the consecutive soaking steps (5 min. to 24h) took place in 1L volumes of sterile DW. The aim of the pre-washing process was to let the water diffuse through the hides, removing the substances like dirt, blood, conservation salt, dust etc. Necessary additions (levant storax, essential oil, mixture (1% O.o+1% L.o.) and the tester bactericide) were made prior to subsequent soaking steps.

Identification of the essential oil content of *siğla storax*

The traditionally acquired *siğla storax* was taken from Dalaman Municipality, and its composition was examined. Traditionally, *siğla storax* is obtained with this procedure: The trunks of trees are injured in spring (early April) in order to obtain *siğla storax*. Beginning from July, the trunks are shaved by special knives and the balsam and barks are taken, these are soaked in boiling water for 10 to 30 minutes and then they are squeezed, separated from balsam barks and offered for sale.⁹ *Siğla* balsam was distilled in Clevenger apparatus as described in the European Pharmacopeia for 3 hours, and the essential oil was obtained.¹⁵

Essential oil of storax composition analysis

The analysis to identify the compound of essential oil was conducted on HP 5973 selective mass detector and HP-INNOWax capillary column (60mx 032mm i.d., film thickness 0.5 μ m) which were connected as combined to Hewlett-Packard (HP) 6890 gas chromatography. GC-MS identification was conducted through an ionization system with 70 eV ionization energy by using helium with 1 ml/min flow speed as the carrier gas. The furnace temperature was programmed from 70 °C to 210 °C/min. Injector temperature was 150 °C. Essential oil sample was diluted with 1/100, h/h ratio ethyl acetate and injected as 1.0 μ l through auto-sampler in splitless mode.¹⁶ The essential oil composition was identified by comparing the relative retention times of components and the mass spectrums of commercial standard essential oil components. The results were confirmed through computerized comparison of the components included in the data library of Wiley 275 L mass spectra. The relative quantities of compounds were calculated by the help of the areas of peaks included in the gas chromatogram.

Oregano essential oil and bactericidal agent

The essential oil; *Origanum onites* (Turkish oregano, potmarjoram) was purchased from a commercial company. The composition of the *Origanum onites* essential oil, which is used in the study, is explained in Table 2.¹⁷ The storax and the oil were prepared in ethanol. 50% organosulfur compound was chosen as the control bactericidal agent commonly used in leather industry.

TABLE II

Composition of oregano essential oil¹⁷

Constituent	<i>Origanum onites</i> ¹⁶
Carvacrol	84,48
Gamma-terpinen	2,58
P-cymene	2,01
Thymol	1,86
Linalool	1,64
Caryophyllene oxide	1,52
Beta-bisabolene	1,52
Caryophyllene	1,07
(+) - Borneol	0,79
Terpinen-4-ol	0,77
Alpha-terpinen	0,67
Myrcene	0,64
Alpha-thujen	0,46
Alpha-pinene	0,35
Beta-phellandrene	0,35
Linalylester	0,21
(+)-Aromadendren	0.21

Growth Media

PCA (Plate Count Agar-Oxoid) medium was used for total bacterial counts (aerobic mesophyllics). 1 ml aliquots were taken from each row of the 5 series of soaking waters after 5 min. and 24h respectively. The aliquots were further diluted (10^1 to 10^7) in sterile saline and 0.1ml samples in duplicates were poured into PCA medium (kept ready in liquid form at 55 C° in a water bath). The Petri dishes were incubated for 48 hrs at 37 C° and the bacterial counts were averaged and expressed as cfu/ml.

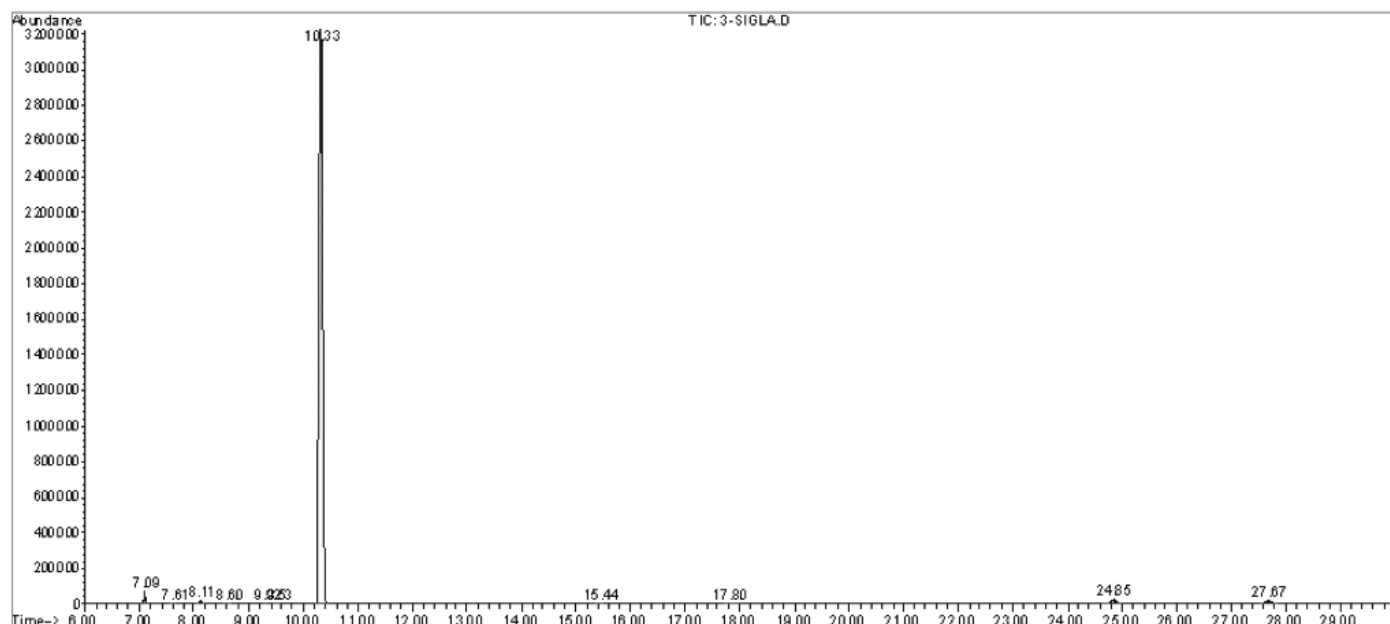


Figure 1. – GC-MS–Chromatogram of the Sığla (*Liquidambar orientalis* Mill. var *orientalis*) storax.

RESULTS AND DISCUSSION

The results of the GC-MS analysis indicated that the essential oil extracted from storax is composed of α -pinene (Rt:7.09) by 1.53%, β -pinene (Rt:8.11) by 0.36%, styrene (Rt:10.33) by 96.55%, 3-phenylpropanol (Rt:24.84) by 0.91% and 4-ethylphenol (Rt:27.68) by 0.64%. Harzemşah Hafizoğlu *et. al.* also identified as a result of their research that sığla storax is composed of styrene by 89.5%.⁸

TABLE III

Total bacterial counts averaged in the beginning and end of 24 hours soaking periods for six different skin samples

Biocides	Beginning (0 h)	24 h'
Control	1.20x10 ⁴	7.86x10 ⁶
Biocide B	2.61x10 ⁴	2.76x10 ⁵
O.o (1%)	1.18x10 ³	2.22x10 ³
L.o.(1%)	6.04x10 ³	1.01x10 ⁷
L.o.(2%)	3.6x10 ⁴	8.12x10 ⁶
L.o.(5%)	5.14x10 ³	3.51x10 ³
L.o.(1%)+ O.o (1%)	1.05 x10 ²	4.90 x10 ²

Bacterial counts obtained from seven different soaking tests of the two sets with beginning and 24 h intervals have been averaged and are presented in Table III.

As can be seen, although an antibacterial impact is not noticed when sığla storax is given to the soaking float by 1%, an antibacterial impact was identified when the dose was increased by 2 times and the impact increased significantly when the dose was increased by 5 times. It is thought that the composition of the active portion of sığla storax with antimicrobial impact has essential oil by 1.39%. In this case, when 5% sığla storax is used; it means approximately 0.069% sığla essential oil is actually used and it is more efficient during the processes than the bactericide with 50% organo sulfur which is used by 0.3%.

On the logarithmic scale, the bacteria development in the soaking water at the beginning and 24-hours periods indicate that both the size and direction of the change at different times are not same for every group. Also, when the number of bacteria that developed during the soaking process was examined at the beginning of the process, it was indicated that each one yielded different values. In the control it is observed in *Liquidambar orientalis* (1) and *Liquidambar orientalis* (2) groups that the bactericide increases from the beginning of the process to the 24th hour, while the level remains unchanged in *Origanum onites*(1), *Liquidambar orientalis* (1)+*Origanum onites* (1) and *Liquidambar orientalis* (5) groups during this period. This can be considered as a meaningful group-time interaction ($p<0.05$).

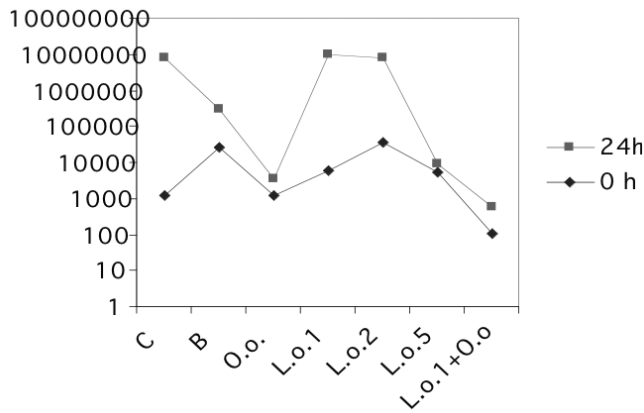


Figure 2. – Comparison of the total number of bacteria by beginning of the soaking and 24 hours soaking periods at semi-logarithmic scale.

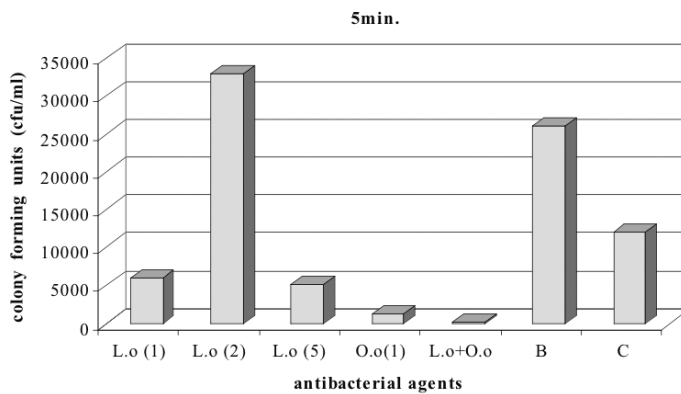


Figure 3. – Total number of aerobic bacteria at the end of 5 minutes soaking period (B: 0.3% bactericide, L.o.(1): 1% storax (*Liquidambar orientalis*), L.o.(2): 2% storax (*Liquidambar orientalis*), L.o.(5): 5% storax (*Liquidambar orientalis*), O.o.: 1% *Origanum onites* oil, C: control, L.o+O.o: 1% *Origanum onites* oil +1% storax (*Liquidambar orientalis*)).

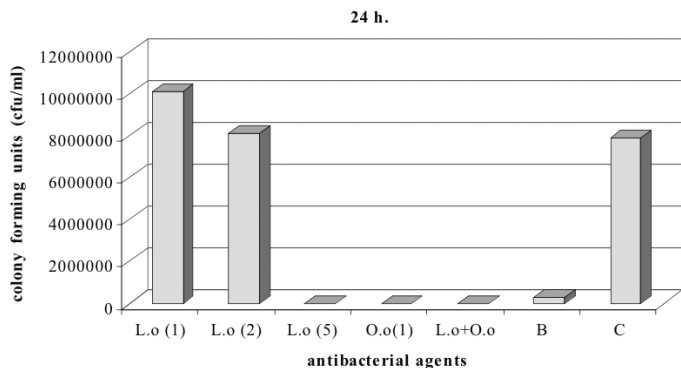


Figure 4. – The total number of aerobic bacteria at the end of 24 hours soaking period (B: 0.3% bactericide, L.o.(1): 1% storax (*Liquidambar orientalis*), L.o.(2): 2% storax (*Liquidambar orientalis*), L.o.(5): 5% storax (*Liquidambar orientalis*), O.o.: 1% *Origanum onites* oil, C: control, L.o+O.o: 1% *Origanum onites* oil +1% storax (*Liquidambar orientalis*)).

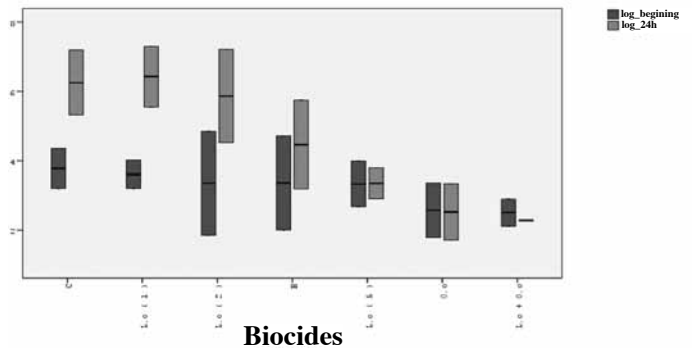


Figure 5. – Comparison of the total number of bacteria by the beginning and end of 24 hours soaking periods at logarithmic scale (Editor note: Original color coding of data pairs; left = beginning, right = 24 hours.).

In the beginning of the soaking process, the differences between groups were evaluated with Oneway Anova, while the differences between groups on the 24th hour were corrected according to the beginning values and evaluated with Ancova (Analysis of Covariance). As a result, although there was not any difference among the groups in the beginning ($p>0.925$), a meaningful difference was found among them on the 24th hour ($p<0.05$).

The differences among groups on the 24th hour are as follows: meaningful difference was found between the control and *Origanum onites*(1), *Liquidambar orientalis* (1)+*Origanum onites* (1) and *Liquidambar orientalis* (5) groups; between *Liquidambar orientalis* (1) and *Origanum onites*(1), and *Liquidambar orientalis* (1)+*Origanum onites* (1) and *Liquidambar orientalis* (5) groups; between *Liquidambar orientalis* (2), and *Origanum onites*(1), *Liquidambar orientalis* (1)+*Origanum onites* (1) and *Liquidambar orientalis* (5) groups ($p<0.05$).

As a result of the study, using the essential oil in sığla storax after distillation may sound more reasonable. However, the essential oil of sığla is not directly marketed in trade, and as it would mean extra cost and time, it is not considered as practical on the basis of the industry. Therefore, the essential oil amount and content of sığla storax was analyzed, but storax was used in combination with the essential oil of oregano. In this study, the most important advantage of storax use is considered as the facilitation of fleshing process in practice as it penetrates into the flesh layer of the skin. As for its use on fur processing, its impact on the hair should be researched.

Meanwhile, the strong antibacterial impact of *Origanum onites* was reconfirmed with this study and the mentioned impact increased further after its combination with 1% *Sığla storax*.^{1,18} Here, the interesting point is, although the soaking

float does not show an antibacterial impact when 1% storax is given, it increased the antibacterial impact of the oil of oregano when it was combined with the essential oil of oregano with this ratio. Quite interestingly, even 0.0139% of essential oil part of the 1% sığla storax added strengthens the antibacterial effect. As a hypothesis, this synergic effect can be explained as follows: Carvacrol is responsible for the biological activities of oregano such as antimicrobial activity.¹⁹ Carvacrol, which is found by 84% in *Origanum onites*, exhibits a synergic interaction with the styrene, which accounts for 96.55% of the essential oil of sığla. And this increases the antimicrobial activity. Certain studies in the field of food products also support this thesis: it was reported that Styrene can be produced naturally, usually by the decarboxylation of cinnamic acid, a common plant acid. Cinnamic acid is also used as an anti-bacterial agent in food products, and this has led to cases of food becoming contaminated with styrene, due to the activity of yeasts such as *Pichza carsonii*.²⁰ Carvacrol, thymol and cinnamaldehyde in vapor phase showed highly potent antimicrobial activity against Gram negative bacteria (*Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis*), Gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*), molds (*Penicillium islandicum* and *Aspergillus flavus*), and yeast (*Candida albicans*).^{21,22}

CONCLUSION

As a result of the study, it can be concluded that the mixture of storax and oregano essential oils can be used as new types of bactericides which are not harmful to nature or human health due to their completely natural structure. The use of world-standard products and production of leather which are not harmful for the human health in a country strengthens the position of that country in terms of commerce.²³ Oregano essential oils display both antifungal and antibacterial activities and such properties are very important for their use in leather production.¹ Although the higher cost of natural components compared to synthetic biocides seems like a disadvantage, that disadvantage may be eliminated through the co-use of natural products with synergic effect. This study clarifies that the joint use of natural compounds with antibacterial properties increase the antibacterial effect; as a result, they can be used at lower rates. So, the input costs will decrease and the demand for such products will increase. The greatest advantage of these products in addition to being 100% natural is their beneficial effects rather than harmful effects on human health. It is thought that these existence of these components with antioxidant properties on the leather will eliminate free formaldehyde formation on the leather

and our studies in this regard are currently continuing. The method is expected to find a significant place in leather industry in near future.

ACKNOWLEDGEMENTS

The authoress would like to express her gratitude to Prof. Dr. N. Gürdal Alaeddinoğlu, Prof. Dr. K. Hüsnü Can Başer, Prof. Dr. Harzemşah Hafizoğlu, Prof. Dr. Ediz Ulusoy, Martin Kleban and Hartmut Rehbein for the information they have provided and for the interest they have shown in the study. The authoress also expresses her gratitude to Assistant Prof. Bintuğ Öztürk for his help in extracting essential oil from sığla storax and in GC-MS analyses during the study. The authoress' heartfelt thanks are also due to Türer Tarım and Nurşen Aslan Çopuroğlu- Grup Dogayla Baris (Group for Peace with Nature- NGO) and Dalaman municipal corporation for providing essential oils and Sığla storax and also Taner Aksu for his help in lab. study.

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