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The Investigation of Antibacterial Activities of Some Essential Oils in Wet Blue Leather

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Abstract

In this study the applicability of *Origanum sp.* and *Schinus molle* essential oils as bactericide against bacteria that grows on wet blue process was examined. During the microbiologic tests, the growth of bacteria species like *Bacillus cereus* (CCM 99), *Pseudomonas aureginosa* (ATCC 27853), *Escherichia coli* (ATCC 27999) and *Staphylococcus aureus* (ATCC 6538) were investigated against these essential oils.

As a result of this study, it has been determined that oregano essential oil has antibacterial activity for *Bacillus cereus* (CCM 99) and *Staphylococcus aureus* (ATCC 6538).

Key words: oregano, essential oil, wet blue, leather

INTRODUCTION

Leather is an organic material that contains many nutrients for microorganisms. After the death of the animal, thousands of microorganisms can grow on the hide. Since the raw materials of the tanners are not sterile[1], at the start of the soaking process strict controls for microbial activities should be applied. During the soaking process, bacterial attacks to the hides lead to putrefactive odors and hair slips and depending on the extent of the damage, tiny abrasions known as 'pin holes' may develop. The leather at this stage peels off its grains[2], and with further damage on epidermis layer, matt and lusterless grain, loss of the skin substance, etc. can also occur. For longer soaking process it is therefor necessary to use bactericidal agents to prevent such undesirable results. However, the bactericidal agents that are currently used in the industry are generally harmful to human health and nature, and their use have 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 [3]. The use of polyhalogenated phenolic compounds (TCP / TBP) has also been restricted in certain countries to that end [4].

DMDTC (Dimethyldithiocarbomate), TCMTB (2-Thiocyanatomethylbenzthiazole), N-OITZ (N-octyl-isothiazolinone), OPP (o-phenylphenol), PCMC (p-chloro-m-cresol), Carbendazim, Mercaptobenzothiazole, Methylenbisthiocyanate, TCP (tri-chlorophenol), p-Nitrophenol are some of the well known biocides currently used in leather industry.Among these, TCMTB is known as a skin irritant and is strongly toxic. Mercaptobenzothiazol is treated as a suspicious cardigoenic agent in USA. Methylenbisthiocyanats are known to cause high acute toxicity. As can be seen from these particular examples, it is essential to discover new chemicals that can be used safely in leather industry for undesirable affects of microorganisms.The new approach is referred to as "ecologic leather production" and hence the discovery of environmentally friendly substances has gained much importance. It has been known for many years that essential oils are 100 % natural chemicals displaying varying degrees of antimicrobial activity. These oils are volatile compounds of plant secondary metabolism, and may be used as phytoproctective agents [5] Ibn-i Sina was the first man obtaining the essential oils by steam distillation techniques to be used further (980-1037) [6].

It has already been found that the *Origanum minutiflorum* essential oil has antifungal activity during pickling and wet blue processes and its effectiveness improves with increasing concentration rates[7]. The objective of this study was to determine whether the essential oils could be effective also against bacteria in wet blue process. Essential oils of *Origanum sp.* and *Schinus molle* were tested in the present research for their bactericidal activities against test bacteria. This preference was based on the earlier reports pointing out to their use in food industry and health related issues as antimicrobial agents [7-13].

MATERIALS AND METHODS

Materials

Raw skin

In this study, dry salted Turkish domestic sheep skins were used in order to obtain chrome-tanned leather.

Bacteria

Two Gram-positive and two Gram-negative bacteria were chosen. These were *Bacillus cereus* (CCM 99), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 27999) and *Pseudomonas aureginosa* (ATCC 27853).

Media

Nutrient Broth (N.B.) (Oxoid) was used for growing of bacteria and Nutrient Agar (N.A.) (Oxoid) was used for microbiological tests.

Essential Oils

O*riganum sp.* essential oil was taken from a commercial company. There are many components in oregano oil and the most effective compound of these is carvacrol. As the result of the laboratory tests, it was determined that carvacrol had a strong antimicrobial effect. These test were performed at Georgetown University and carvacrol showed much stronger effect than penicillin, streptomycin and even vancomycin (considered to be the strongest of all antibiotics) [14]. Some of the main components of oregano essential oil are presented in Figure 1 [15].

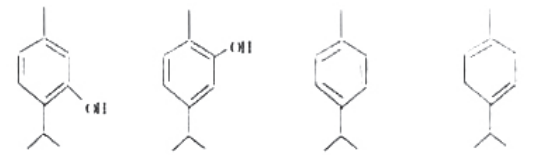

Figure 1. Some of the main components of oregano *essential oil* [15].

Schinus molle essential oil was obtained by distillation of its fruits. The fruits were picked up from the tress in Ege University and dried in a cool place. They were grinded and put in an apparatus of Clevenger type. Pure essential oil was obtained by distillation.

METHODS

Obtaining the Leather Samples Used in the Experiment

The leathers were processed in order to obtain chrome-tanned leathers, which were the main materials of the study. For all leather production, one standard garment leather processing method was applied.

The features of the wet blues were as follows:

1. Chrome tanned leather without fungicide.
2. Chrome tanned leather obtained by using ***Origanum sp.*** essential oils (1%)
3. Chrome tanned leather obtained by using ***Schinus molle.*** essential oils (1%)

The leather samples used in the experiments were cut in a sterile room under sterile conditions. The laboratory punch was used to punch 3 circular specimens, measuring 3 cm in diameter, out of the leather samples to be tested. All equipments and tools were cleaned with ethanol and were flamed before and after changing samples. The leathers were processed with essential oil for 4 hours after being cut. Every trial was duplicated and repeated twice.

Microbioiogical Test Standards:

The Preparation of Inoculation Solution

In order to count the bacteria, Nutrient Broth (Oxoid) was prepared separetly in four tubes. The amount of bacteria was preapered as 10^7 cfu/ml to get the standardization [16].

The Bacteria Inoculation in Petri Dishes with Nutrient Agar to the Wet Blue Leather

When the N.A. was cooled down to 45°C, after the autoclave, it was mixed immediately with bacteria separetly in four rox bottles as 10^7 cfu/ml. 20 ml of this preparation was put to each petri-dishes. Leather specimens were placed in the middle of each inoculated nutrient agar, and incubated 24 hours. The incubation temparature was 27°C for *B. cereus* and *P. aureginosa* and 37°C for *E. coli* and *Staphylococcus aureus.*

The Bacteria Growth in the Petri Dishes with Nutrient Agar and Observation of Inhibition Zone

The wet blue specimens without essential oil were tested by putting them over Nutrient Agar, inoculated with bacteria. They were incubated 24 hours. The media without leather were incubated for control.

The wet blue specimens, contained essential oils were put over Nutrient Agar and incubated 24 hours. As the results of the incubation, inhibition zones were observed around some wet blue leather specimens that contained *Origanum sp.* The *Bacillus cereus* inhibition zone in wet blue leather that contained *Origanum sp.* was showed Figure 2. In the figure, the specimen at the top without essential oil, the specimen at the left didn't contain leather, the specimen at the right which contain *Origanum sp.* had an inhibition zone.

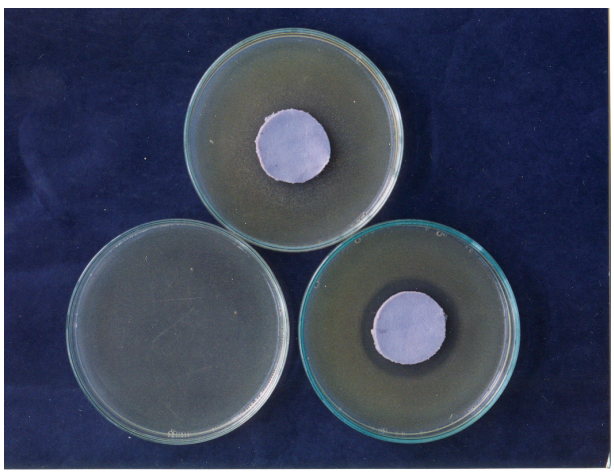

Figure 2. The growh of *Bacillus cereus* in petri dishes and inhibition zone

RESULTS AND DISCUSSION

As the results of 24 hours of incubation period, the leather containing Origanum sp. Bacillus cereus and Staphylococcus aureus had inhibition zones. Table 1 gives the average of inhibition results. On the other leather samples, no inhibition zone was determined. The inhibition zones and the control were seen in Figure 2.

The antibacterial activity of Origanum minutiflorum essential oil was searched during soaking period and its antibacterial effect was determined. This effect was better than %7-25 phenol, 4-chloro-3-methyl containing commercial bactericide[17].

Table 1. Zone of Inhibition (Average)

Essential oils in wet blue	Microorganisms	Zone of inhibition (cm)
***Origanum sp.* (%1)**	*Bacillus cereus*	4
	Staphylococcus aureus	4.5
	Escherichia coli	-
	Pseudomonas aureginosa	-
***Schinus molle* (%1)**	*Bacillus cereus*	-
	Staphylococcus aureus	-
	Escherichia coli	-
	Pseudomonas aureginosa	-
Control (without e.o.)	*Bacillus cereus*	-
	Staphylococcus aureus	-
	Escherichia coli	-
	Pseudomonas aureginosa	-

Bacillus cereus is an aerobic, Gram positive, spore forming, motile, in the shaped of rod bacteria. It is an opportunistic pathogene. The inhibition zone that *Bacillus cereus* formed was shown in Figure 3. The wet blue specimen contained *Origanum sp.* essential oil and zone of inhibition was 4 cm. *Staphylococcus aureus* showed similar properties. *Staphylococcus aureus* is a Gram positive bacteria, too. Its inhibition zone was 4.5 cm when the wet blue specimen contained *Origanum sp*. essential oil.

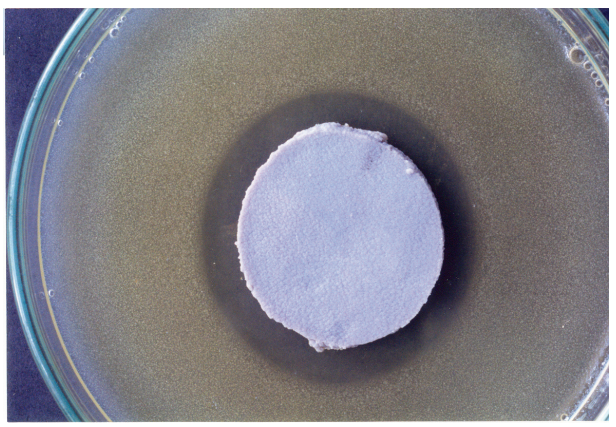

Figure 3. Zone of inhibition of *Bacillus cereus*

The wet blue leather specimens that contained *Origanum sp.* essential oil showed strong antibacterial effect to Gram positive bacteria. This result was supported by many researchers. Bacteria showed more resistance than yeast and moulds to essential oils and spice. Generally Gram-positive bacteria are more sensitive to essential oils than Gram-negative bacteria [18]. Furthermore, spores are more resistant than vegetative cells [19].

Schinus molle didn't show any antibacterial effect in this research altough it had a mild effect to storage fungi[20] and the toxic effect to animal pathogens. The reason may be sourced from the inadequate concentration. The reseachers advice the usage by combination of essential oils with each other. This combination had a preventing effect over the growth of microorganisms. Especially this synergistic effect had an influence over the growth of target microorganisms [19]. Lambert et al [21] showed oregano essential oil as an example to the mixed effect of carvacrol and thymol. The reason of this kind of an inhibition was damaging the integrity of the membrane, and effecting the inorganic ion equilibrium and pH homeostasis.

Economical consideration of the problem is of importance and some basic calculations should also be made. It was found that 2% of oregano essential oil showed best effect for fungi [7]. The amount of current fungicide which contains TCMTB will be measured as 0.048 kg, if a thousand sheepskins weigh approximately 1200 kg and fungicide is used at 0.04%. The additional cost of using fungicide is thought to be approximately 0.072$, if it is assumed that one coat is obtained from five skins and the price of commercial fungicide per kg is approximately 30$. The amount of oregano essential oil will be measured as 24 kg, if a thousand sheepskins weigh approximately 1200 kg and oregano oil is used at 2%. The additional cost of using oregano essential oil as fungicide is thought to be approximately 6$, if it is assumed that one coat is obtained from five skins and the price of oregano essential oil per kg is approximately 50$. This additional cost can be considered to be low for an ecologically produced coat [7].

CONCLUSION

As the result of this study, it was found that the wet blue leather specimens that were treated with 1% *Origanum sp.* essential oil showed antibacterial effect to Gram-positive bacteria. *Origanum minutiflorum* essential oil had an antifungal effect to wet blue. Because the bacteria more resistant to the essential oils than yeasts and moulds, the result of this study supported the others[19, 22].

Oregano essential oils display both antifungal and antibacterial activities and such properties are very important for their use in leather production. The method of killing microorganisms by essential oils and aromatherapy has gained much emphasis especially in the field of medicine. The method is also expected to find a significant place in leather industry in near future.

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