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Use of various plant extracts to provide hygiene in mattresses and antibacterial film production

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ABSTRACT

It has been known for many years that microorganisms can grow and proliferate on textile material. Other than clothes, the objects that humans mostly contact with are their mattresses. In this study, it was aimed to produce an antibacterial film in order to prevent growth and proliferation of various bacteria which are dangerous for human health in mattresses and to make mattresses hygienic since they cannot be changed in a short time. In the study, extracts were first obtained from *Saponaria officinalis* L., *Oxalis acetosella* L., *Althaea officinalis* L., *Lavandula officinalis* L., *Aesculus hippocastanum* L., *Thymus vulgaris* L., *Syzygium aromaticum* (L.) Merr at Perry plants. The extracts were used individually and combined to form experimental groups. The antibacterial effects of the extracts were examined by disc-diffusion method applied on Gram-positive rods, Gram-negative rods, Gram-positive cocci and Bacilli colonies obtained from mattresses. In addition, the colony counts were also carried out in total MAB culture. Based on the results of the study, it was determined that *S. aromaticum* and L. *officinalis* + all extracts had the highest inhibitory effect. By using *S. aromaticum* extract, xanthan gum, propylene glycol, tween-20 and distilled water, an antibacterial film, which may provide long-term hygiene in mattresses, was produced. The obtained gel was lyophilized and made available for use. As a result of this study, it was foreseen that with the development of the obtained product, 4-5 million people (e.g., dorm, hospital, hotel) could be protected from the diseases transmitted from mattresses, the money spent on the chemicals used for hygiene and the damages caused by these chemicals on human and environmental health could be reduced, mattresses used for long periods of time could be made healthy, and mattresses can be made hygienic in a cost-effective, practical and natural manner.

1. Introduction

Today's modern living and working conditions provide ideal conditions for the microorganisms to reproduce rapidly. The excessive reproduction of some microorganisms causes the deterioration of products (food, textile, healthcare products…), the formation of diseases and infections in the human body, and the increase of allergic reactions [1]. It has been known for many years that microorganisms can grow and proliferate on textile materials. Other than clothes, the objects that humans mostly contact with are their mattresses. In a study, it was reported that 317 million living organisms were found in one gram of the samples taken from two different hospitals, elderly and child deaths increased in those hospitals, and intestinal infections and diseases like hepatitis A were transmitted through mattresses. In the study conducted by Department of Genetics and Bioengineering of Yeditepe University, Faculty of Engineering and Architecture, it was reported that the examination on mattress samples obtained from 19

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different hospitals, hotels and dormitories had revealed that the mattresses became a biotope comprised of dust mites, scabies, molds and bacteria, and this was a significant threat to human health [2]. In Turkey, the student capacity of the dormitories operating as part of the Higher Education Credit and Hostels Institution (KYK) under the Ministry of Youth and Sports is 601.019 [3]. The student capacity of 2591 dormitories operated by the Ministry of National Education is 483,941 [4]. In addition, according to 2016 data, the bed capacity of 865 state hospitals in Turkey is 217.771 [5]. Hotels are the other areas posing risk for human health. In 2016, the total bed capacity of the hotels in Turkey was 312.912 [6]. However, it is now stated that this figure has reached 1 million as of today. Considering private dormitories and private hospitals, the number of beds and the number of people who benefit from them, that is the people who are under risk, has reached 4-5 million. If we also take into account our beds and mattresses in our homes, it can be said that people face this danger every day.

After the infection incidents occurring in hospitals, more importance has been placed on the hygiene of beds and mattresses, and the application of systems using ultraviolet rays has become a topic of discussion. However, the implementation of such systems is costly and difficult. Nowadays, devices that absorb these wastes with vacuum systems are used more commonly since they are more cost-effective [2]. These devices are for cleaning purposes, but the important thing is to prevent the growth and proliferation of microorganisms. Therefore, antibacterial and antifungal products should be used. Today, arsenic, iron, lead, tin, mercury, silver, vegetable and animal extracts are used as antibacterial agents. In particular, the use of heavy metals (Pb, Hg, As...) causes increase in various health problems, such as cancer, poisoning, skin rash, and itching. For instance, after the widely used tin compounds were examined by the EPA (Environmental Protection Agency), the use of these compounds was banned in Japan and in some European countries [7].

Today, human health constitutes the focus of antibacterial applications. For this reason, high efficiency and non-toxic substances have been introduced to the use of the textile industry with the help of advancing technology. For example, this feature of Bamboo, which is known as an antibacterial tree in the Far East, has been transported to clothes over time. In addition, the products obtained from algae are also known as natural antibacterial agents [8]. Antibacterial and antifungal effects have been primarily investigated in the studies conducted with plants [9-19]. Antibacterial studies have also affected bed and mattress companies and a large number of antibacterial mattresses have been put on the market. One of those companies produced antibacterial mattresses that provide better sleep quality by combining visco material and soybean oil, whereas another company combined Indian oil and visco material to reach the same result [20]. Only the upholstery of these mattresses has antibacterial properties and their prices are quite high. The aim of this study was to produce a cheap and natural antibacterial film in order to 1- prevent growth and proliferation of various bacteria, which are dangerous for human health, in mattresses, 2- and to make mattresses hygienic since they cannot be changed in a short period.

2. Materials and Methods:

2.1. Material:

In the study, *Saponaria officinalis* L., *Oxalis acetosella* L., *Althaea officinalis* L., *Lavandula officinalis* L., *Aesculus hippocastanum* L., *Thymus vulgaris* L., *Syzygium aromaticum* (L.) Merr at Perry were used as materials.

2.2. Method:

2.2.1. Obtaining extracts from Syzygium aromaticum (L.) Merr at Perry, Thymus vulgaris L. and Lavandula officinalis L. plants:

By using a Clevenger device, extracts were obtained from *S. aromaticum*, *T. vulgaris* and *L. officinalis* plants via steam distillation method [21, 22].

2.2.2. Obtaining extracts from Althaea officinalis L. and Aesculus hippocastanum L. plants:

50 g *A. officinalis* and 200 ml sterile water were put into a closed container and kept for 2 days (25 °C incubator) under dark environment. After filtration, the extract was sterilized in autoclave (121 °C) for 20 minutes [23]. The same procedure was also performed for the barks of *A. hippocastanum*.

2.2.3. Obtaining extracts from Oxalis acetosella L. plant:

The leaves of *O. acetosella* were ground and six 10-gram cartridges were prepared. After the cartridges were placed in the Soxhlet device, hexane, methylene chloride, ethyl acetate and ethyl alcohol were extracted. At the end of extraction, ethyl alcohol was removed by using a rotary evaporator and the extract was obtained [24].

2.2.4. Obtaining Saponin from **Saponaria officinalis** *L. plant:*

The pieces taken from the root of *S. officinalis* were ground and 3 cartridges (10 g x 3) were prepared. The prepared cartridges were treated with petroleum ether (200 ml) for 6 hours in the Soxhlet device. After the cartridges were dried, the process was continued for 24

hours in Soxhlet device with 80% ethanol. Ethyl alcohol was removed by using a rotary evaporator and the concentrated extract was taken to the beaker. The beaker was taken into an ice container, and acetone was added dropwise to the beaker in order to obtain Saponin precipitate with a white color. After the precipitation was complete, filtration was carried out by using filter paper and the filtrate was dried at room temperature to obtain Saponin [25].

2.2.5. Medium preparation and obtaining samples from mattresses:

At this stage of the study, Müller-Hinton agar (Merck 1.05437) growth medium was used to incubate microorganisms found on beds and mattresses. Petri dishes containing growth medium were placed upside down on the mattresses covered with clean linens for 1 hour, and then incubated at 35 °C for 24 hours. This procedure was repeated every two days in 3 separate beds for two weeks. After incubation, the bacterial colonies were identified [26]. In general, four different colonies were identified in the Petri dishes. These colonies, classified as Gram positive rods (e.g., *Lactobacillus, Listeria, Eubacterium*), Gram negative rods (e.g., *Enterobacteria, Pseudomonas, Acinetobacter*), Gram positive cocci (e.g., *Streptococcus, Staphylococcus*) and Bacilli (e.g., *Bacillus*), can be produced by various species [27, 28]**.** Since bacteria could not be identified individually based on their species, the study was carried out based on total bacteria count.

2.2.6. Cultivation of mesophilic aerobic bacteria culture:

In the literature review performed to obtain a clearer result in this study, it was seen that Chinese (CTITC) and Japanese (JTIA) Textile Inspection centers conducted their studies on antimicrobial mattresses in mesophilic aerobic bacteria culture (MAB) or *Staphylococcus* [28]. MAB culture contains bacteria such as *Lactobacillus* and *Staphylococcus* [27]. At this stage of the study, Brain-Heart Infusion Medium (Merck 1.10493) was used for MAB culture inoculation. Inoculation was performed 24 hours after the medium was prepared and left for incubation at 35 °C for 24 hours. After the incubation, 1 loop (~ 10^9 bacteria) was taken from MAB culture and transferred to 100 ml Bufferd Peptone Water medium.

2.2.7. Investigation of Antibacterial Effects of Plant Extracts:

The antibacterial effects of the extracts were investigated by two different methods.

In the first stage, Disc-Diffusion Method was used to investigate the antibacterial activity of the extracts on the samples taken from the mattresses [29]. The discs to be used in the method were previously prepared and sterilized by being kept in a dry sterilizer for 1 hour at 180 ºC. 28 experimental groups were formed from plant extracts (**Table 1**). In addition, deionized water was used as a negative control group and Amoxicillin Clavulanate antibiotic was used as a positive control group [30]. By using physiological saline solution, 10^{-2} dilutions were prepared from the colonies developed in Petri dishes, and 50 microliters were taken from the dilutions and inoculated onto Petri dishes. 3 discs soaked with 25 micron of extract were placed into these Petri dishes. Petri dishes were incubated at 35 ºC for 24 hours and the changes were observed.

The resulting zones were measured by caliper and their averages were taken. The study was carried out in 3 repetitions.

In the second stage, Total Bacterial Count was performed in MAB culture. Bacteria inoculated into BPW medium were used for this process. 0.1 ml of bacterial cultured BPW was taken and inoculated into tubes containing 9 ml Maximum Recovery Diluent (physiological saline solution) and 1 ml plant extract. After inoculation, tubes were kept at room temperature for 30 minutes and total bacterial counting was performed [31]. After counting, data was obtained by using the following formula (Table 2).

$$N = C / [V(n_1 + 0{,}1 \times n_2) \times d] \quad (1)$$

(N: number of colonies (gram or ml), C: total amount of colonies counted in Petri dishes, n_1: number of Petri dishes in first dilution, n_2: number of Petri dishes in second dilution, d: dilution coefficient of the first counted Petri dish).

2.2.8. Preparation of Antibacterial Film:

In order to prepare antibacterial film, the most effective plant extract (10-15%), Xanthan Gum (1-3%), Propylene Glycol (10%), Tween-20 (5%) and Distilled water (100g) were used. Firstly, ratio of the water was determined according to the percentages and half of the water was added to xanthan gum. The gum was allowed to stand overnight so that it could expand and be homogenous. Propylene glycol was then added. The rest of the water was used to dissolve the Tween-20. The extract was added dropwise on dissolved Tween-20. This mixture was added to Xanthan gum and then taken to a petri dish and dried in a 40 °C incubator. The obtained gel was lyophilized.

3. Results and Discussion

Based on the measurements made by the disc-diffusion method on the experimental groups, it was observed that the plant extracts had different antibacterial properties (Table 1).

When the results obtained by the disk-diffusion method were examined, it was seen that *A. officinalis, L. officinalis + A. officinalis* extracts had no inhibitory effect on bacteria, and the least inhibitory effect was seen in *A. hippocastanum* extract.

It was determined that *S. aromaticum* extract had an efficient antibacterial effect on all four different colonies identified in the mattresses. Other extracts with high inhibitory effect were *L. officinalis* + all, *S. aromaticum* + all, *T. vulgaris + S. officinalis* and *L. officinalis + S. officinalis*. At the end of the MAB counting, it was determined that the results in the experimental groups were different (Table 2).

In the studies related to colony count, the counting is performed over 15-300 colonies and these values are taken as criteria [32]**.** When table 2 is examined with respect to the Positive control group, it was seen that *L. officinalis, T. vulgaris, A. officinalis, A. hippocastanum, L. officinalis+ A. officinalis, L. officinalis+ A. hippocastanum, T. vulgaris + A. officinalis* and *T. vulgaris + A. hippocastanum* extracts had no inhibitory effect on mesophilic aerobic bacteria. Similar to the disk diffusion method, it was determined that *S. aromaticum* extract had also the highest antibacterial effect on MAB culture. Other extracts with high antibacterial effect were *L. officinalis* + all and *L. officinalis* + all + salt mixtures. The data obtained from this study is also supported by previous studies [13-16, 18].

Table 1: Results of Disc-Diffusion Procedure Performed on Plant Extracts (mm)

Extract	**Gram(+) Rods**	**Gram (-) Rods**	**Gram (+) cocci**	**Bacilli**
L. officinalis	1.4	1.6	1.8	1.6
S. aromaticum	9.5	9	9.2	9.4
T. vulgaris	6	5.5	5	6.2
S. officinalis	4	5	3	3
O. acetosella	3	2	2	2
A. officinalis	0	0	0	0
A. hippocastanum	0.5	0	0.5	0
L. officinalis +S. officinalis	6.30	6	6.8	5
L. officinalis +O. acetosella	4	5	4	4
L. officinalis+ A. officinalis	0	0	0	0
L. officinalis+ A. hippocastanum	1	0.5	0.5	1
L. officinalis +all	8.5	8	7.5	8
L. officinalis + all+ salt	4.5	4	3	3.5
*L. offi*cinalis+all+ carbonate	4.5	5	4	5
S. aromaticum+S. officinalis	6	6.5	5.5	6
S. aromaticum +O. acetosella	6.5	7	6	6.5
S. aromaticum+ A. officinalis	3	2	2	2.5
S. aromaticum+ A. hippocastanum	4	4.5	4.5	3.5
S. aromaticum +all	7	6	6	6.5
S. aromaticum+all+ salt	4	4	5.5	5
S. aromaticum+all+ carbonate	3.5	4	4	3.5
T. vulgaris +S. officinalis	6	6	7	6
T. vulgaris +O. acetosella	4.5	3.5	4	4
T. vulgaris + A. officinalis	2	1.5	2	2
T. vulgaris + A. hippocastanum	2	2	2.5	2.5
T. vulgaris +all	5	6	4.5	5.5
T. vulgaris +all+ salt	5	6	6	6
T. vulgaris +all+ carbonate	3	3.5	2.5	3.5
Negative group	0	0	0	0
Positive group	10.5	11.5	10	12

Table 2: Antibacterial effects of plant extracts in MAB culture (cfu/g).

Extract	MAB
L. officinalis	>300
S. aromaticum	8
T. vulgaris	>300
S. officinalis	14
O. acetosella	20
A. officinalis	>300
A. hippocastanum	>300
L. officinalis +S. officinalis	20
L. officinalis +O. acetosella	15
L. officinalis+ A. officinalis	>300
L. officinalis+ A. hippocastanum	>300
L. officinalis +all	10
L. officinalis+all+ salt	11
L. officinalis+all+ carbonate	16
S. aromaticum+S. officinalis	13
S. aromaticum +O. acetosella	16
S. aromaticum+ A. officinalis	27
S. aromaticum+ A. hippocastanum	18
S. aromaticum +all	16
S. aromaticum+all+ salt	19
S. aromaticum+all+ carbonate	41
T. vulgaris +S. officinalis	19
T. vulgaris +O. acetosella	47
T. vulgaris + A. officinalis	>300
T. vulgaris + A. hippocastanum	>300
T. vulgaris +all	19
T. vulgaris +all+ salt	21
T. vulgaris +all+ carbonate	26
Negative group	>300
Positive group	0

In terms of cost, it was decided to produce antibacterial film by using clove extract and the sample film was produced as described in the Materials-Methods section. It was determined that by using this film, Gram positive cocci which cause various infections in hospitals, gram-negative rods which cause various diseases such as pneumonia and antibiotic resistance, and gram-positive rods which cause skin and soft tissue infections [33] can be prevented from settling and proliferating in beds and mattresses.

Based on the results of this study; it was foreseen that by using the produced antibacterial film on mattresses and various surfaces, natural resources of Turkey can be better utilized and 4-5 million people can be protected from the diseases transmitted from mattresses, the money spent on the chemicals used for hygiene and the damages caused by these chemicals on human and environmental health can be reduced, and mattresses can be made hygienic in a cost-effective, practical and natural manner. It is recommended that the sample antibacterial film produced in this study should be further developed by experts in the field and put into the practice as soon as possible (Figure 1).

Figure 1. Use of antimicrobial film on mattresses

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