



Determination of Microbial Load Accumulating in Paper Mouth and Face Masks and Obtaining Antimicrobial Properties with Chamomile Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to determine the microfungi and bacteria accumulated in different usage periods of paper mouth and face masks which are used for protection against microfungi and bacteria in many areas and to give antimicrobial properties to these masks by impregnating Chamomile extract with high antimicrobial properties. Disc diffusion test was applied in this study. The mouth and face masks used for different periods (3-6-12 hours) were cultured on the media to calculate the microbial load. Nutrient Agar and Malt Extract Agar were used to determine bacterial loads. Then, the paper mouth and face mask were impregnated with different concentrations of chamomile extract and microbial loads were compared. The results of the study show that the use of chamomile extract in paper mouth and face masks, where fungus flora is low and bacteria flora is rich, has the effect of reducing microbial load. These paper masks impregnated with chamomile extract concentration can be used in first aid units, clinics, surgery, dental units and so on. places can be used to prevent the spread of infection. At the same time, this study is expected to be the source of similar studies.

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1. INTRODUCTION

Fungal spores and hyphae, which are intensely indoors and outdoors, cause a wide range of symptoms and cause inflammatory reactions. For this reason, the identification of the fungi is important in determining the molecular mechanisms involved in the action of the immune cells of the fungi. [1]. *Aspergillus* sp., *Alternaria* sp., *Cladosporium* sp. and *Penicillium* sp. microfungus species, which are commonly carried by air, have been reported to cause significant symptoms [1-4]. Antimicrobial products are produced in order to minimize the effects of bacteria and fungi in our lives. In this context, many studies have been carried out to determine the essential oils found in plants and the antimicrobial substances contained in these oils [5-10]. In a study of commercially available extracts, antimicrobial activities of five different extracts against gram positive and gram negative bacteria were investigated. The extracts used in this study were reported to have more antimicrobial properties than antibiotics [10]. Known as the largest family in the world, the Asteraceae (chamomile) family is an annual plant, with more than 1765 genera and 2773 species. There are many species of chamomile that grow naturally in our country (Turkey) [11-12]. Chamomile extracts are used for many medical purposes among the public in different regions [13]. In this study, microbial load at different usage times of paper product commonly used as mouth and face mask will be determined. Then, these masks will be impregnated with chamomile extract to give antimicrobial properties against bacteria and fungi.

2. MATERIALS AND METHODS

Paper mouth and face masks are sold commercially. This masks have been supplied from the market. This product has been

preferred because it is low cost and easily accessible.

2.1 Determination of Bacterial and Fungal Loads of Paper Mouth and Face Mask Used at Different Times

Samples were taken at different times (3, 6 and 12 hours) to determine the microbial load of the paper mask sample. For microorganism isolation, the mask sample was homogenized in 10 ml of isotonic solution. 1000 µl were taken from this liquid medium and seeded to Nutrient Agar (NA) medium for bacteria. 1000 µl were taken from this solution and bacteria were planted on Nutrient Agar medium. For the fungus and yeast samples, Malt Extract Agar (MEA) and Rose Bengal Agar (RBA) were cultured with the same method. The media for bacteria were incubated at 37° C and 24 hours. For fungi and yeast strains, the media were incubated at 27° C and 7-10 days. Then, colonies formed as a result of incubation were counted [14].

Total counts (colony forming unit/m³ or cfu/m³) = [Total colonies× 1000]/250.

2.2 Making Identification Microfungi

The total number of microfungi was determined according to the macroscopic appearance obtained at the end of incubation. Some sources have been used to identify microfungi [15-20]. Identification of genera and species, macroscopic and microscopic structures were examined under light microscope.

2.3 Determination of Antimicrobial Activity

Essential oils or sample extracts obtained by appropriate methods are adsorbed onto empty sterile 6 mm diameter discs by micro pipette (Schleicher & Shüll No: 2668, Germany).

Table 1. Features of mouth and face mask

General features	Technical specifications	
3 Layer	Fabric	Nonwoven-Spunbond PP
Disposable	Filter	Meltblown
Box contents 100 pcs	Tire	Gipe Tire
High filter capacity	Needlework	Ultrasonics Needlework
Full face placement	Product Type	3 Layer (PE film+Airlaidpaper+nonwoven spunbond)
Latex-free	Color	White

Essential oils are impregnated with 0.1–100 µl and the extracts are impregnated with 20, 25, 30 µl or 50 µl. Sometimes this amount can reach up to 150-200 µl. Standard antibiotic discs are used as a control sample for comparison [21-24]. In this study, commercially sold chamomile oil extract was impregnated to empty discs. In this study, 3 different bacteria and 5 different species of fungi were used. Bacteria were incubated at 37° C for 24 hours, fungi and yeast strains were incubated at 27° C for 48 hours in the incubate under suitable conditions. Then the formed zones were evaluated.

2.4 Used Media and Species of Microorganisms

Bacterial samples used in the study were obtained from Kilis 7 Aralık State Hospital Microbiology Laboratory. Fungus / yeast strains were obtained from Kilis 7 Aralık University, Faculty of Science and Letters, Biology Laboratory in Turkey. In study, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* were used as bacterial strains. *Candida albicans*, *Acremonium implicatum*, *Cladosporium* sp., *Cladobotryum dendroides*, *Penicillium brevicompactum* were used as fungus strains.

2.5 Disc Diffusion Method

Disc diffusion method was used to determine antimicrobial activity [24-29]. The prepared extracts were impregnated with micropipettes into 10 µl of empty sterile 6 mm diameter Whatman papers. For the determination of antimicrobial activity, were cut to 1 cm² from the mask sample and 25 and 50 µl chamomile extract was impregnated. Sterile Whatman no: 1 discs, used as standard in antimicrobial studies, were also used for comparison. At the same time, two antibiotic discs, blank paper(Whatman no: 1) and unused mask samples were used as controls. The antimicrobial activity study was conducted in triplicate.

2.6 Making Statistical Analysis

Descriptive statistics of total, average and standard deviations of the findings obtained before and after the study were obtained.

3. RESULTS AND DISCUSSION

Chamomile extract used in the study has been found to have antimicrobial properties

against bacterial strains in paper mouth and face masks.

Chamomile extract has the highest effect against *S. aureus* strain at different doses in both paper mask and whatman paper. It has been determined that the Paper Mask sample can be treated with chamomile extract to obtain effective results against microorganisms that can pass through the air and cause bacterial infections in the user. These results are more effective than the antibiotics used in the study. Fig. 1. shows the antimicrobial activity findings against bacterial strains.

Chamomile extract was found to be effective on fungus strains, but it was not effective against *C. albicans* yeast species. It was determined that the chamomile extract used in the study gave parallel results to the antibiotic discs. Conclusion of Study shows that chamomile extract will be beneficial against fungal infections. In this study, it was observed that chamomile extract has a microbial load reducing feature on paper mouth and face masks. It has been reported that chamomile extracts are used as in vitro in antimicrobial activity studies and positive results are obtained [30-33]. Fig. 2. shows antimicrobial activity findings against fungus / yeast strains.

In a study with *Matricaria chamomilla* L., it was stated that methanol extracts showed more effective antimicrobial activity [30]. In another study, the relationship of essential oil fractions of the same sample with different antibiotics was investigated. In this study, it was reported that *Matricaria chamomilla* L. extract has a synergistic effect against *S. aureus* and *C. albicans* isolates [33]. In this study, the microbial load of the paper mask sample was determined at different usage times (3-6-12 hours) and antimicrobial property was given to this paper mask by using chamomile extract. In this study, the average bacterial load was 31.67 and the fungal load was 9.67. At the same time, it was determined that bacterial and fungal burden increased with increasing paper mask usage time. Fig. 3. shows the microbial load of the mask sample at different usage times.

Samples were taken at different times (3, 6 and 12 hours) to determine the microbial load of the paper mask sample. In the study, it was seen that the burden of bacteria and fungi increased with increasing use of paper mask. In addition, bacterial load was found to be higher than fungal load. Allergen species were determined among the fungi. It is important to

treat the paper mask sample with chamomile extract to give antimicrobial properties and to protect the users from microbial diseases to which they are exposed.

Gabriel et al. (2016) reported that *A. alternata* is a common allergen species [3]. The same species was identified in our study. Levetin et al.

(2016) in their study on fungal allergens, they gave information about the common genus and species [4]. *Aspergillus*, *Cladosporium* and *Alternaria* species that we identified in our study were found to have common allergen properties (including allergen proteins). In addition, these species were evaluated in airborne fungi species [14].

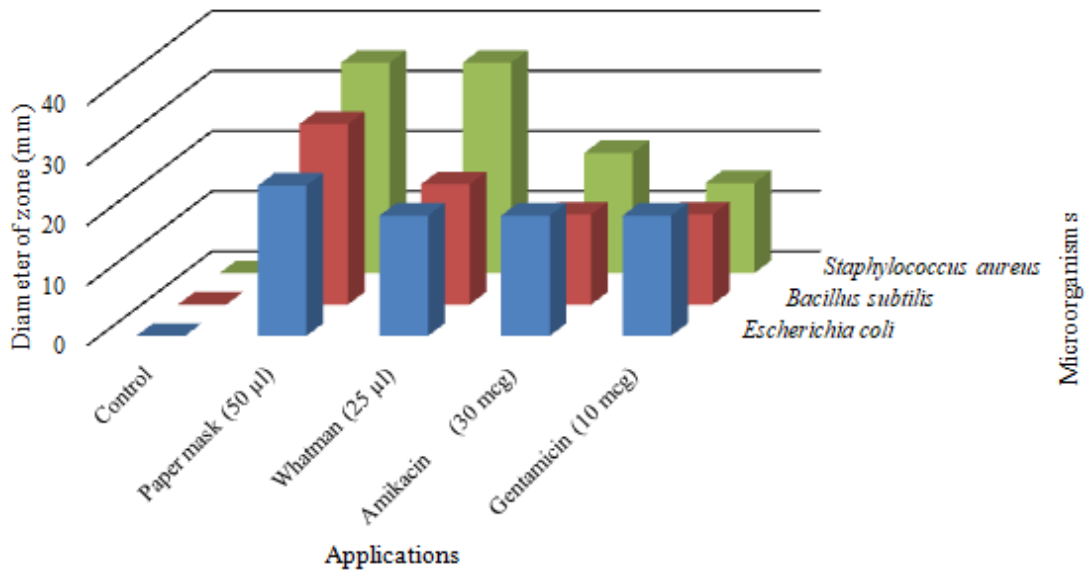


Fig. 1. Antimicrobial activity findings against bacterial strains

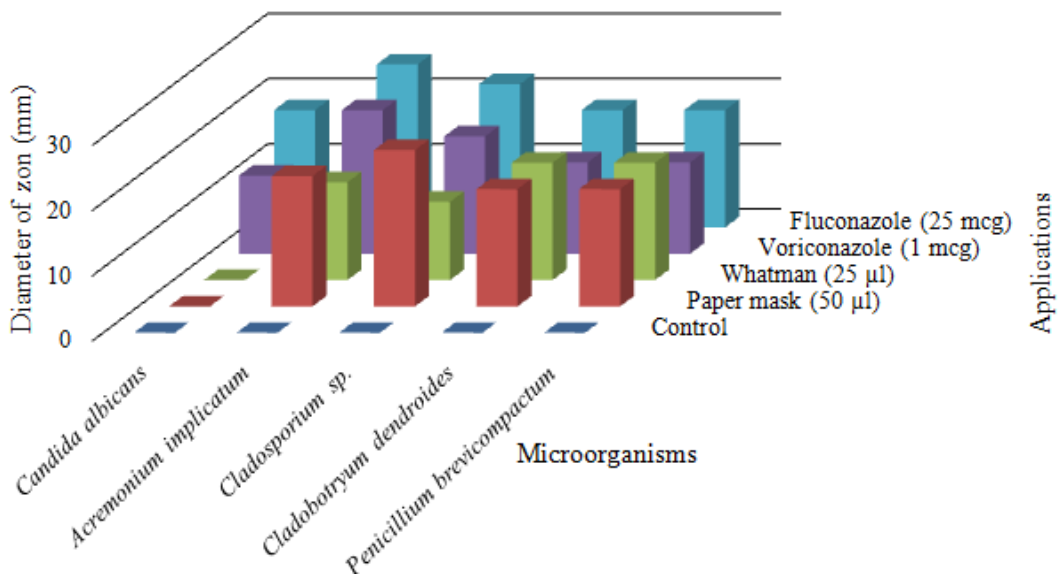


Fig. 2. Antimicrobial activity findings against fungi/yeast strains

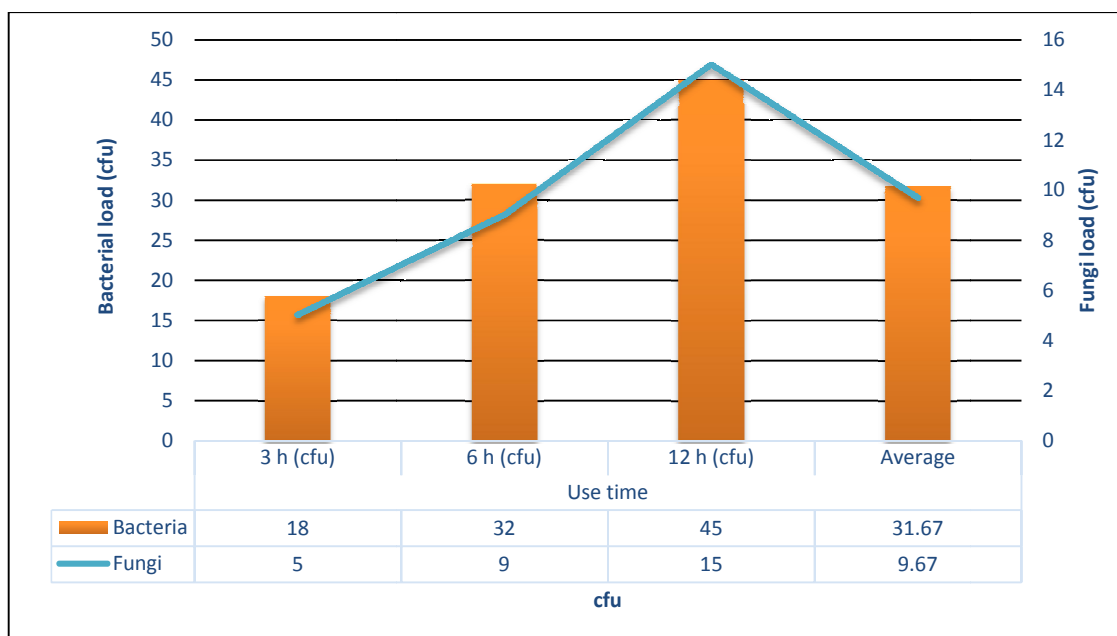


Fig. 3. Microbial load of paper mask sample at different usage times

Table 2. Microfungus species identified in the study

Feature	Microfungus Genus and Species	Resources
<i>Aspergillus</i> Mich. Ex Fr.		
Allergen	<i>Aspergillus niger</i> van Tieghem.	Hasenekoğlu (1991), [15]
Allergen	<i>Aspergillus flavus</i> Link ex.Gray. 1821	Klich (2002), [18]
	<i>Aspergillus japonicus</i> Saito 1906	Samson and Pitt (2000), [17]
Allergen	<i>Aspergillus fumigatus</i> Fresenius 1863	Walsh et al. (2018) [29]
<i>Alternaria</i> Nees ex Fr.		
Allergen	<i>Alternaria alternata</i> (Fr.) Keissl.	Hasenekoğlu (1991), [15]
<i>Penicillium</i> Link ex Gray.		
	<i>Penicillium</i> sp.	Hasenekoğlu (1991), [15]
	<i>Penicillium funiculosum</i> Thom	Samson and Pitt (2000), [17]
	<i>Penicillium soppii</i> W. Zalesky 1927	Klich (2002), [18]
	<i>Penicillium commune</i> Thom.	Walsh et al. (2018), [29]
	<i>Penicillium thomi</i> Maire 1917	Campbell and Johnson, (2013), [19]
<i>Cladosporium</i> Link: Fries		
Allergen	<i>Cladosporium cladosporioides</i> (Fres) De Vries 1952	Hasenekoğlu (1991), [15]
Allergen	<i>Cladosporium herbarum</i> (Pers) Link ex. Gray 1821	Campbell and Johnson, (2013), [19]

4. CONCLUSIONS

As a result of the study, it was determined that the microbial load increased as the usage time of the paper mask sample increased. The applicability of chamomile extract was tested to reduce microbial load. Chamomile extract used in this study has been shown to have antimicrobial properties in the use of paper mouth and face masks. It

was found that chamomile extract used as effective as antibiotics. It was determined that the use of chamomile extract in paper mask production stage may have protective effect against microorganisms. It is thought that the development of paper mask products within the scope of R & D and the treatment with chamomile extract will significantly affect the health level of the users.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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