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Antimicrobial socks for orthosis-prosthesis users

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This study aims to solve the problem of orthosis-prosthesis users and these socks will be preferred under plaster-splint after body fractures. It might be also a solution for diabetics' wound
Stump socks will keep the users drier during hot days and promote skin integrity and hygiene. As a result, the patients will be offered a better quality of life as it will be reduced the daily care period and prevented fungal infections

Abstract: Orthosis are a device used to correct, accommodate or enhance the use of a body part. Prosthesis is an artificially made limb or part of the body that is used to replace a part of the body that is missing either due to amputation or lack of development. Orthotics and prostheses are usually worn over the cotton stump-socks which should be washed every day. Also the stump-area must be cleaned and maintained regularly. Fungal infections are common on the stump of orthosis-prosthesis users who cannot perform regular care. Considering that 70% of patients using orthosis-prosthesis have such problems, it is understood that patients cannot provide adequate hygiene or care in the problematic areas. Antifungal stockings are aimed to find solutions to this problem and to facilitate patient life. Due to the high price of the existing antibacterial copper and silver ion socks and the presence of heavy metal ions, as an alternative solution, cotton stump-socks might be treated with natural plant extracts such as tragacanth of *Astragalus nitens*, an endemic Turkish-plant. *A.nitens'* extract-treated samples showed antifungal activity against tested three fungus strains, before and after five cycles of washing.
This study aims to solve the problem of orthosis-prosthesis users and these socks will be preferred under plaster-splint after body fractures. It might be also a solution for diabetics' wound. Stump socks will keep the users drier during hot days and promote skin integrity and hygiene. As a result, the patients will be offered a better quality of life as it will be reduced the daily care period and prevented fungal infections.

Keywords: Orthotics, prosthesis, *Astragalus nitens*, tragacanth, antibacterial, stump socks

1 Introduction

Although there are advances in technology and health, orthopedic diseases caused by chronic diseases, aging populations, war, trauma or congenital causes are still an important health problem. Prosthesis-orthotic applications and rehabilitation are important for improving the function and independence for disabilities. For existing problems, socks treated with silver or copper ions are used. However, it is known that such heavy metal ions have a toxic side effect even for healthy people.
Although there are antibacterial effects of these existing socks, there are concerns about their effectiveness against mold and fungi. In addition, because of their high prices, their uses are very limited. Therefore, there is no long-term, hygienic and cheap solution.
In order to find a solution to this problem and facilitate patient life, antifungal socks treated with natural/plant extracts might be a succesfull alternative strategy. There are various naturally antibacterial additives such as clove-, oregano-, cinnamon- and thyme essential oils and components (Saeed 2019). Treating the socks with tragacanth of *Astragalus* would be the prominent one of the plants.
Astragalus, which is named as "geven" is a plant with a wide range of (about 2500) species. Some of the species have white, yellow, purple or pink flowers and fruits in different shapes and sizes.
Astragalus nitens which is a species of the legumes family (Fabaceae) grow in the arid and semi-arid regions of Asia (Fig 1 and Fig 2). They also grow in eastern and the interior of Anatolia in 1300-3500 meters altitude. It is frequently seen in the mountainous regions of Sivas.
The trunk is hollow and grows vertically in the season; as it grows it becomes oblique or flat. It grows 70-120 cm in one season. It consists of a dense in florescence from pale yellow to white. The seeds are in the shape of a sac. The roots and trunk remain intact throughout the winter under snow.
It is resistant to living in wild life due to its structure. It is found in humid regions and stream sizes in Europe (Finland, Sweden, Spain and Russia) (Karadag et al. 2005).

Fig. 1 Astragalus nitens (Geven - in Sivas region)

Fig. 2 The root of *A. nitens* (Tragacanth, in the middle of the root)

The active ingredients of *Astragalus* root are characterized as follows: polysaccharide cycloartane glycoside fractions (astragalosides I-IV and trigonosides I-III), isoflavonoids (formononetin, ononin, calycosin and its glycoside), saponins, several isoflavonoids, biogenic amines and triterpenoids (SigmaAldrich).

Geven plant is effective in strengthening the immune system and increasing the ability of the body to fight diseases. It does not only increase the resistance to flu, but also shortens the duration of the disease. Many species are used among the people for their protective, antioxidant, immunostimulant and antiviral properties. These pharmacological activities were found to be caused by three groups of chemicals: These are Polyholosites, slings and phenolics (Rios and Waterman 1997).

Usage areas: Protecting the soil in sloping areas; maintaining biodiversity; in beekeeping (benefit from the aroma); as animal feed; in medicine sector (pharmacy) and as raw material in the paint and paper industry. In addition, it would also be prefered for socks used for stabilization after diabetes patients and body fractures (Ayubi-Rad 2020, Wang et al. 2019, Wang et al. 2016, Pistelli et al. 2002, Teyeb et al. 2012, Solgar 2020)

The aim of this study was to give antimicrobial properties to the cotton samples by applying the solution of *Astragalus*' tragacanth, and determining the antibacterial and especially antifungal activities of them.

2 Materials and Method

In this study, knitting interlock cotton (100%) fabrics 230 g/m^2 were used and were treated with *A. nitens* solutions according to conventional method (Cotton samples of 5g were used throughout this work).

In June, the plant (*A. nitens*) was collected around Sivas region in Turkey. The tragacanth (gum) which was in the centre of *Astragalus*' root was separated. Then, a solution (10g/L) was prepared by using this tragacanth.

2.1 Exhausting method

Pre-treatment: The 100% cotton fabrics were first scoured with 0.5 % non-ionic detergent in the bath with Fabric to Liquor ratio (F:L) 1:20.

Treatment: The procedures were all conducted in glass beakers (250 mL) in atmospheric conditions.

The fabric to the extract ratio was 1:20 (w:v) and the percentage of the *A. nitens*' tragacanth in the solution was 10%. The temperature was raised from the initial degree of 20°C up to 60°C within a period of 10 minutes (4°C/min) and maintained that condition for 30 minutes. The bath was then cooled; the treated samples were taken out, rinsed with cold water thoroughly and allowed to dry in the open air.

Then, the extract and treated cotton samples were evaluated for their potential antimicrobial activities.

2.2 Antimicrobial Tests

2.2.1. Antimicrobial activities (for extract)

Microorganisms: The American Type Culture Collection (ATCC) fungal standard strains of the yeast, *Candida albicans* ATCC 10231, and the molds *Aspergillus flavus* ATCC 204305 and *Trichophyton rubrum* ATCC 28188 were used in the experiments. Inoculums of fungi were prepared with 2-5 days cultures, for producing a concentration of 1 x 10^7 colony-forming units (cfu/ml).

Media: RPMI-1640 medium (Sigma) buffered to pH 7.0 with morpholine propane sulfonic acid (MOPS, Sigma) were used to determine the minimum inhibitory concentration (MIC) of yeast and molds, and Saboroud dextrose agar (SDA, Difco Laboratories) was used for colony counts.

Determination of minimum inhibitory concentrations (MIC): In vitro antifungal activities of extract against yeast *C. albicans* ATCC 10231, and molds *A. flavus* ATCC 204305 and *T. rubrum* ATCC 28188 were investigated. MICs of extract was determined by microbroth dilution technique as described by the Clinical and Laboratory Standards Institute (CLSI, 2000). Serial two-fold dilutions of extract was prepared in 96 well polistiren microplate, with RPMI-1640 medium. Each well was inoculated with 50 µL of a 2-5 days fresh culture that gave a final concentration of 5×10^3 cfu/mL in the test tray. The trays were covered and placed in plastic bags to prevent evaporation, and incubated at 25°C for 2 or 7 days for yeast and molds, respectively. The MIC was defined as the lowest concentrations of extract producing complete inhibition of visible growth. Ketaconazole was used as reference antifungal for standardization of the study.

In vitro antibacterial activities (MIC values) of extract against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352 were determined by microbroth dilution technique as described by the CLSI (2012). Serial two-fold dilutions of extract were prepared in Mueller-Hinton broth

(MHB, Difco), and each well was inoculated with 50 µL of a 4-6 h broth culture that gave a final concentration of 5 × 10^5 cfu/mL. The trays were covered and placed in plastic bags to prevent evaporation, and incubated at 37°C for 18-24 h. The MIC was defined as the lowest concentrations of extract producing complete inhibition of visible growth. Ciprofloxacin was used as reference antibiotic for standardization of the study.

2.2.2. Antimicrobial Tests (for treated cotton fabric)
The ISO 20645:2004 method (Agar diffusion method) was used for determining the antimicrobial properties of the cotton samples (ISO 20645, 2004).
The antimicrobial activities of the samples were tested before and after washing (5 times) which were performed by using the Standard Test Method ISO 105-C06 A1S, (2010). The A1S washing test was carried out at 40°C for 30 min in a 150 ml soap solution (4g/L) containing 10 steel balls. After washing, the samples were rinsed in deionized cold water, dried in the open air, and then tested for antibacterial properties as mentioned above (A TERMAL B21606E model washing machine was used for washing of the samples).

3 Results

In this study, it was investigated the antibacterial and antifungal effects of *A. nitens*' extract and extract-treated cotton samples. Untreated cotton samples were prepared for comparison.
Altought there is no significant effect has been observed for the antibacterial activity of *Astragalus* extract, the MIC values of the extract was determined as 1/4 dilution against *C. albicans* ATCC 10231 and *T. rubrum* ATCC 28188, and 1/8 dilution against *A. flavus* ATCC 204305 (Table 1). The MICs of Ketaconazole were within the accuracy range in CLSI quality control breakpoints (CLSI, 2016) throughout the study.

Table 1 Antifungal activities of Astragalus extract (%)

Extract	Fungus		
	C.albicans	***A.flavus***	***T.rubrum***
A. nitens (%100)	1/4 dilution (25%)	1/8 dilution (12.5%)	1/4 dilution (25%)
After five cycles of washing	(10%)	(5%)	(10%)

C.albicans: Candida albicans, ATCC 10231, *A.flavus*: *Aspergillus flavus* ATCC 204305, *T.rubrum*: *Trichophyton rubrum* ATCC 28188

The antifungal properties of the treated samples against *C. albicans*, *A. flavus* and *T. rubrum* were measured before and after 5 cycles of washing.
When the results were examined, the untreated cotton sample was not effective against any tested bacterial or fungal strains, the extract-treated samples showed antifungal activity against tested three fungus strains.
The treated sample, which exhibited a 16 mm zone against *A. flavus* and an 11 mm zone against *C. albicans* and *T. rubrum* before washing. It was observed that the extract and extract treated sample were more effective against *A. flavus* (Fig 3).
Five cycles of washing decreased the antibacterial properties of all the samples about 40-50%, but all the samples were still antifungal after washing.

Fig. 3 Antifungal effect against *A. flavus*

4 Discussion

According to the 2008 data of the World Health Organization (WHO 2015), 0.5% of any population globally requires prostheses and orthoses and rehabilitation treatment (ICRC 2013). This gives an idea of the challenge countries are facing. It has been estimated that 0.5% of the world population would correspond to 35–40 million people globally who require prosthetics and orthotics services. Over the next few decades, the number of people requiring prosthetics and orthotics services is bound to rise because both the world's population and life expectancy are growing. As a larger proportion of the ageing population will be affected by disability (WHO 2015), he need for services will rise proportionally.
The world is witnessing significant increases in musculoskeletal conditions and noncommunicable diseases such as diabetes and stroke, which will greatly add to the need for prosthetics and orthotics.
Thus, by the middle of this century, the proportion of the world's population that requires services is likely to be closer to 1%.
An estimated 415 million people were reported to have diabetes in 2015, and this figure is expected to rise to 642 million in 2040 (WHO 2016; Diabetes atlas 2016). As 60–70% of people with diabetes lose sensation in their feet, they are at risk for injury. Furthermore, 12–15% of people with diabetes will develop a foot ulcer (Singh et al. 2005; Cavanagh et al. 2005), which increases their risks for infection, amputation or even premature death. According to researches, diabetic finger or leg amputations are more than amputations in accidents.
According to the Social Security Institute's data (Ministry of Labor and Social Security), approximately 30.000 prosthetic and orthotic were made last five years in Turkey.

Between 2008 and 2012, the number of diabetes patients increased by an average of 17 percent each year, increasing from 2 million 500 thousand to 5 million 200 thousand people. It is thought that the number of orthosis-prosthesis users who have diabetes patients will also increase in these rates. Accordingly, a market size is expected to reach 10 million people in 5 years and 15 million people in 10 years.
The aim of this study was to provide a better quality of life for these patients by shortening the daily care period and preventing fungal growth. For this purpose, we tried to design antifungal socks treated with *A. nitens*.
According to our results, the antibacterial effect of tragacanth-treated cotton fabric was limited against the selected bacteria, whereas the those cotton samples showed antifungal effect against all three fungus. After 5 cycles of washing, the antifungal activities were decreased about 40-50%, but the treated samples were still effective against all the tested fungus (Table 1). Similarly, there are some other researchers were found the *Astragalus* extracts have an in vitro or in vivo antibacterial or antifungal activities against standard bacterial or fungal strains (Kanaan et al., 2017; Mikaeli et al., 2012; Pistelli et al., 2002)

5 Conclusion

In view of these results, it was thought that the *Astragalus* -tragacanth solution can be good candidate for application in the medical textiles sector, especially for the stump-socks of the orthosis-prosthesis users.
The antifungal effect of *Astragalus*' tragacanth will prevent the growth and reproduction of fungi that develop in hot and moisture, while the daily care period of the stump-area will also be reduced. Accordingly, it is aimed to increase patient comfort.

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