Antibacterial Potency for Mimosa, Quebracho and Essential Oils of *Origanum* Species against *Acinetobacter pittii, Klebsiella pneumoniae* and *Bacillus cereus* from Diabetic Foot Patient

by

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Abstract

The diabetic patients may sometime suffer from foot lesions, foot ulcers and amputation that adversely affect their quality of life. In this respect, good footwear has a critical significance for diabetic foot patients. The aim of this study to evaluate the potential efficacy of mimosa, quebracho, and essential oils of *Origanum onites, Origanum onites oleum*, and *Origanum minutiflorum*, against *Acinetobacter pittii*, *Bacillus cereus*, and *Klebsiella pneumoniae*, which was isolated from diabetic foot patient. According to our results, the mimosa extracts were slightly more efficient when compared to quebracho extracts to control the bacterial growth of *A. pittii* (8.77±0.58-35.12±8.41% inhibition for three doses), and *K. pneumoniae* (21.40±0.48-47.04±0.51% inhibition) except *B. cereus* (71.1±0.31-23.51±1.66% inhibition). But these inhibition percentages remained at lower levels. On the other hand, essential oil samples of *O. onites, O. onites oleum,* and *O. minutiflorum* at tested doses have considerably high antibacterial effects against *A. pittii*, *B. cereus*, and *K. pneumoniae*. The tested essential oils almost completely inhibited *B. cereus* with percentages of inhibition ranging from 96.18±2.98-100±0.00. Also, the bacterial growth of *K. pneumoniae* and *A. pittii* was inhibited by 88.01±2.36 to 100±0.00% and 71.42±12.57 to 100±0.00%, respectively. Moreover, the essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum,* had bactericidal activity against *A. pittii,* and *K. pneumoniae* but bacteriostatic activity against *B. cereus*. This potency for essential oil of *Origanum* species may be evaluated for diabetic footwear. More detailed technical studies are required for the application of *Origanum* species to leather footwear.

Introduction

Diabetes is a disease associated with the endocrine system, with an increasing prevalence and affecting many people around the world. Therefore, it is evaluated as a global health problem nowadays. Considering the socioeconomic burden due to long-term drug use or prolonged hospital stay due to complications in diabetic patients, diabetes and diabetes-related complications have recently become an issue that needs to be emphasized.[1,2] The World Health Organization (WHO) reported 422 million patients suffering from diabetes mellitus are from low and middle-income countries.[3] Furthermore, the total cost of treating diabetic foot disease in the United States has been reported around 9-13 billion.[4] Taken into consideration the number of patients with diabetes, which is predicted to increase to 54 percent between 2015 and 2030, these costs will also be undoubtedly increased.[5] Moreover, increasing morbidity and mortality rates of these patient groups are associated with diabetic foot complications.[6] Diabetes is a major cause of foot infections, foot ulcerations, or impaired tissue integrity.[4] Unfortunately, it has been reported that at least 15% of diabetic patients may experience diabetic foot lesions at some point in their lives.[7] Foot ulceration leads to chronic foot infections and severe forms of gangrene that are responsible for 85% of amputation cases.[4] It has been suggested that one-third of these diabetic patients will probably experience amputation of the extremity. Furthermore, statistical data show that globally an amputation occurs every 30 seconds, usually due to secondary foot ulcers or lesions.[4,8,9] All these complications adversely affect the life quality of people.[10]

Some of the issues that should be especially considered in the diabetic foot are listed in the literature as choosing appropriate shoes, not cutting nails too short, not smoking, not using too many chemicals, etc.[2,11] The selection of good footwear is assessed as one of the most important parameters, especially in diabetic foot and it is stated that the prevention of foot infections or foot ulcers is possible with good foot care and screening of the risk of complications.[10] Following the guidelines of The Australian Diabetes Foot Network (2013) for footwear of patients with diabetes, advanced original experiments and guidelines have come into prominence.[12] The need for alternative footwear studies has been emphasized in primary and secondary prevention of foot ulcers.[11]

Various materials (leather, synthetic, fabric) with different properties are used in footwear production. However, it is highly

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important to select the appropriate material for diabetic shoes. In this view, leather appears to meet these criteria to be of a breathable, soft, and comfortable nature. In addition, leather has excellent moisture permeability by absorbing sweat and keeping the skin dry with its ability to take the shape of the feet of diabetics.[13] It is necessary to pay attention to these features in the production of shoes that will especially appeal to this patient group. At this point, as there is a close relationship between the skin surface of diabetics and the skin of the feet, an important point to be considered is bacterial colonization. Life-threatening foot infections result from bacterial colonization and sometimes biofilm formation on the leather/skin surface. The leather can provide suitable conditions for bacterial growth, such as temperature, humidity, and nutrients, and these bacterial populations can form a biofilm structure that is very difficult to eradicate from the environment. This scenario may potentially result in foot ulceration and amputation.[14]

Foot infections are sometimes caused by a mixed bacterial culture (aerobic bacteria and fungus) and sometimes by an individual bacterium. In the foot ulcer patients, possibly causative bacterial population may be Gram-positive (*Staphylococcus aureus, Enterococcus* species, etc.), or Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella* species, *Proteus* species, etc.), or anaerobes, with high potential for multi-drug resistance.[15, 16] Kramer et al. (2016) reported the percentages of bacteria isolated from patients with diabetic food infection as *Pseudomonas* spp. (29%), *Bacillus* spp. (3%), *Enterobacter* spp. (7%), *Staphylococcus* spp. (13%), *Acinetobacter* spp. (10%), *Enterococcus* spp. (9%) and *Klebsiella* spp. (8%). In other studies, *S. aureus, S. saprophyticus, S. epidermidis, Streptococcus pyogenes, S. mutans, P. aeruginosa, B. subtilis, Proteus sp., Escherichia coli* and *K. pneumoniae, Peptostreptococcus*, *Bacteroides fragilis,* and *Clostridium* species were reported from cases with foot infection.[17] In a study including 115 patients in 2021, the samples were taken from 94 patients with diabetic foot ulcers, and the most common bacteria were reported as *S. aureus* (30.1%), *K. pneumoniae* (21.9%), and *Acinetobacter* spp. (19.1%).[18] *Acinetobacter* spp. was also detected at a rate of 4.5% in samples taken from patients with diabetic foot ulcers. Similarly, in a study conducted in Egypt with 120 patients in 2021, 12.2% of microorganisms isolated from diabetic foot ulcer patients were found to be *Acinetobacter* spp.[19, 20] Cardosa et al. (2017), reported that the members of *Acinetobacter* spp. are major microorganisms that cause amputation in the ulcers of patients with diabetic foot.

The genus *Acinetobacter* is a Gram-negative bacterium with 68 species belonging to the *Moraxellaceae* family.[21] *Acinetobacter pittii,* belongs to the *A. calcoaceticus-A. baumannii* complex, which is responsible for hospital-acquired infections. Although *Acinetobacter pitti* is encountered less frequently than *A. baumannii*, clinical specimens of *A. pittii* have been increasingly reported in recent years.[22] Furthermore, the emergence of carbapenem-resistant strains of *A. pittii* with carbapenem-hydrolysis β-lactamases such as NDM-1 has become a major medical concern. Information on virulence factors belonging to the *A. pittii* species or their role in pathogenicity is limited.[23,24]

Klebsiella pneumoniae is a Gram-negative bacterium and a member of the *Enterobacteriaceae* family. Bacterial infections caused by the *K. pneumoniae* cannot be effectively treated due to the development of resistance of this bacterium to commonly prescribed antibiotics. This bacterium can cause serious healthcare-associated infections such as pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Furthermore, *K. pneumoniae* may cause serious infections in immunocompromised patients including diabetes.[24,25] Mukkunnath et al., (2015) reported approximately 21.7% of diabetic foot cases caused by *K. pneumoniae*.[27]

Bacillus cereus is an aerobic, spore-forming, Gram-positive bacterium that may potentially lead to infections in immunocompromised patients.[28] The cutaneous infections due to *B. cereus* may be observed in diabetic patients. *B. cereus* can be treated by several antibiotics but recently, its resistance to erythromycin and tetracycline antibiotics in Europe and in the United States was reported.[29] This bacterium can be seen in diabetic patients, albeit to a lesser extent. Michelotti and Jonathan Bodansky (2015) reported a case report for haemorrhagic superficial necrosis up to the knee due to *B. cereus* in 72-year-old man with Type 2 diabetes mellitus.[30]

In recent years, the potential bioactivities of plant-derived chemicals such as antioxidant, antibacterial, and antifungal activities have been reported.[31] There are numerous studies examining the antimicrobial efficacy of various plant extracts/the compounds/mixture of their compounds against a variety of bacteria. In the light of all this information, it is of great importance to have plant extracts or chemicals with antibacterial properties that can be used in the shoes of patients with diabetic foot. More recently, microencapsulated substances materials or components such as plant-based materials for their antimicrobial properties are tested in the footwear industry. These natural resources can help patients overcome unpleasant odors from foot complications thanks to their antibacterial properties and increase the durability of the leather. From this point, in this study, we aimed to investigate the potential efficacy of mimosa and quebracho, which are utilized as vegetable tanning agents, and also essential oils of *O. onites* (wild oregano), *O. onites oleum,* and *O. minutiflorum* (oregano) (endemic in Turkey), which have economic importance in the worldwide trade and have also various biological activities (antifungal, antimicrobial, etc.), against *A. pittii, B. cereus*, and *K. pneumoniae* which was isolated from diabetic foot patient.

Materials and Methods

Bacterial strain and test materials

A. pittii, K. pneumoniae and *B. cereus* were isolated from diabetic foot patients in Marmara University, Istanbul Pendik Training and Research Hospital. The test isolates were stored at -80°C until the experiments. Before experiments, the pure culture of the isolates were obtained on Tryptic Soy Agar at 37°C for 24 h. Mimosa and quebracho were purchased from Mimosa GS Powder Elephant Brand, UCL Company (PTY) LTD, and Unitan Atg Company, respectively. The essential from *O. onites, O. onites oleum*, and *O. minutiflorum* was purchased from Türer Bitkisel A.S., Botaniksan, Health and Sleep Company, respectively.

Antibacterial tests

Antibacterial tests were performed in sterile glass tubes containing Tryptic Soy Broth medium. Mimosa, quebracho, and also essential oils of *O. onites, O. onites oleum* and *O. minutiflorum* were added to each tube at a volume of 3.33%, 1.67, and 0.83% (v/v). Samples of mimosa and quebracho were resolved at maximum concentration. The final concentrations of mimosa extracts of stock solution were 69.2 mg/mL and quebracho extracts were 39.8 mg/mL. The applied concentrations for mimosa extract were 2.31, 1.15 and 0.58 mg/mL, and for quebracho extracts were 1.33, 0.66 and 0.33 mg/mL. The bacterial culture was added to the tubes by measuring the optical density (OD) at 600 nm and adjusting it to 0.5 Mc Farland. The tubes were incubated in a shaking incubator for 24 hours at 37°C. Optical density (OD) measurements were taken at 600 nm after the incubation period. The experiments included untreated, treated, and antibiotic-treated groups. The five antibiotics (gentamicin, apramycin, vancomycin, penicillin, and kanamycin) were tested in preliminary screening experiments. The gentamisin and apramycin were found to be effective against *A. pittii* and *K. pneumoniae,* respectively. For *B. cereus*, all antibiotics were successful except penicillin. From this respect, gentamisin and apramycin antibiotics were tested for the antibacterial efficacies against tested bacteria. The tests were performed in three replicates. The antibacterial efficacy was evaluated by comparing the test tubes to untreated groups.

Statistical analyses

The statistical analyses for the antibacterial activities of the extracts of mimosa, quebracho, and essential oil samples of *O. onites, O. onites oleum*, and *O. minutiflorum* against *A. pitti, K. pneumoniae,* and *B.cereus* were performed by One-Way Anova (Tukey) test via SPSS 16.0 program. The significance between the control (untreated) group, antibiotic treatment group, and three different doses of test materials was examined for each bacterial species. A p-value of 0.05 and below was considered significant. The statistically significant differences in analyses were given in the figures as (a,b,c,d) for *A. pittii*, (1,2,3,4) for *K. pneumoniae*, (□, ∆, Ω, ○, ⌓) for *B. cereus*.

Results and Discussion

In this study, the antibacterial potentials of the extracts of mimosa and quebracho, and also essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum* were investigated against *A. pittii*, *K. pneumoniae* and *B. cereus*. In the literature, there are numerous studies focusing on the potential bioactivities of plant extracts, essential oils and active compounds against many bacterial species. At the beginning of this study, it was prioritized that it would be more meaningful to select the bacteria to be studied directly from the foot of the diabetic foot patient. Because it is well known that many bacteria can develop antibiotic resistance against existing prescription antibiotics in the clinical course. In this respect, as emphasized in most studies, alternative agents to overcome this problem need to be discovered. Diabetic foot is of great importance because of the potential aggressive infections caused by various microorganisms, which can usually be caused by individuals themselves. Diabetic shoes have a pivotal role in the treatment of patients to ensure their quality of life. To our best of knowledge, there is no study in the literature examining the potential antibacterial efficacy of extracts or essential oils from tested plant materials against *A. pittii*, *K. pneumoniae* and *B. cereus* isolated from diabetic foot patient.

The assessment of the potency of antibiotics was performed. Then, gentamicin, and apramycin which are selected based on preliminary screening tests, were performed as positive controls. The gentamicin inhibited bacterial growth of *A. pittii* by the inhibition percentage of 94.77±0.07 whereas apramycin suppressed *K. pneumoniae* and *B. cereus* with inhibition rates of 96.10±*0*.3, and 84.44±0.21, respectively. These data were included as OD values in the figures below.

Antibacterial efficacy of mimosa extracts against tested bacteria

The extracts of mimosa had no remarkable inhibitory efficacy against *A. pittii.* The inhibition percentages of the extracts of mimosa were observed as 35.12±*8*.41, 11.76±*1*.0, and 8.77±*0*.58 for tested concentrations, respectively. Similar insufficient efficacy was observed against *K. pneumoniae*. The mimosa extracts inhibited the bacterial growth of *K. pneumoniae* by the inhibition ratios of 21.40±*0*.48%, 47.04±*0*.51%, and 21.62±1.08%, respectively. However, the antibacterial efficacy of mimosa extracts were obtained against *B. cereus* for the concentrations of 2.31 mg/mL and 1.15 mg/mL by the inhibition percentages of 70.36±0.29 and 71.10±*0*.31. In the 0.58 mg/mL treatment group, there was no notable suppressive effect against *B. cereus* (Figure 1).

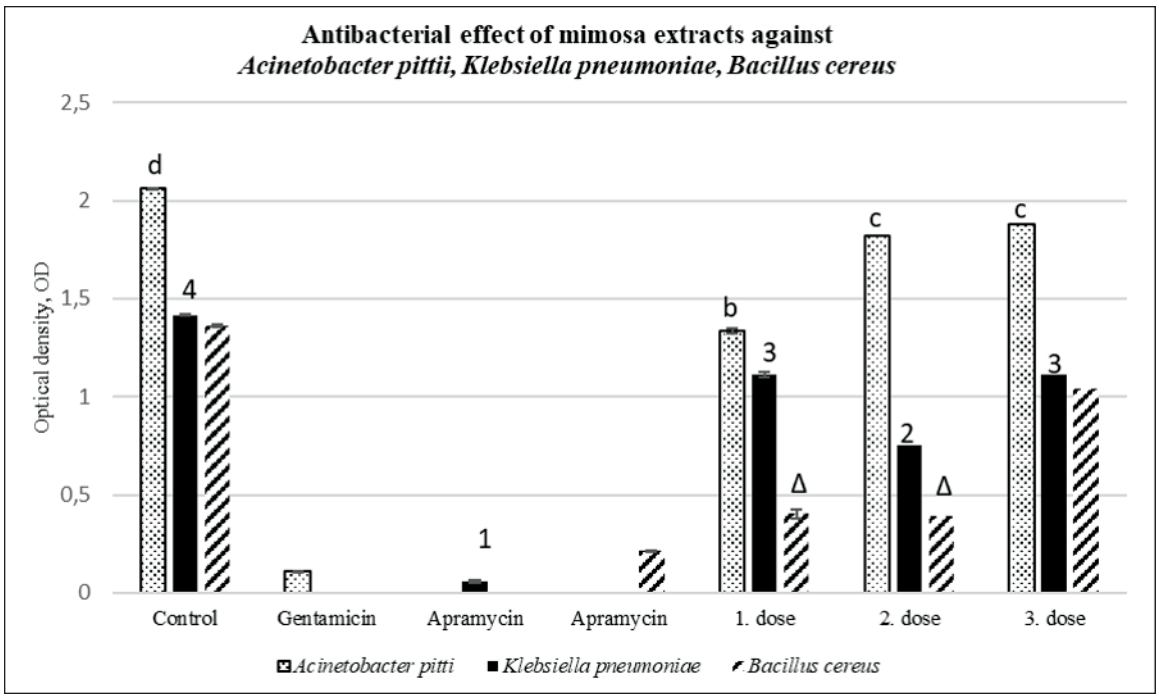

Figure 1. The antibacterial effects of mimosa extracts aganist *A. pittii, K. pneumoniae, B. cereus.* (1.dose 2.31 mg/mL, 2.dose 1.15 mg/mL and 3.dose 0.58 mg/mL). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○).

Antibacterial efficacy of quebracho extracts against tested bacteria

Similarly, as in mimosa extracts, quebracho extracts had no significant antibacterial efficiency against the bacterial growth of *A. pittii.* In comparison with untreated groups, the inhibition ratios were recorded as 16.73±0.37, 14.79±0.44, and 11.22±0.80% for tested concentrations, respectively. The inhibition percentages were detected as 49.05±0.53, 26.79±0.63 and 13.30±0.22 for the tested concentrations of the extracts of quebracho against *K. pneumoniae*, respectively. Just like in mimosa extracts, quebracho extract was found to be more effective on *B. cereus*, especially for the first dose with the inhibition rate of 62.40±1.28. The other lower test concentrations (0.66 mg/mL and 0.33 mg/mL) showed very low suppressive efficiency (29.68±0,35 and 16.03±0,92%, respectively) against this bacterium. (Figure 2).

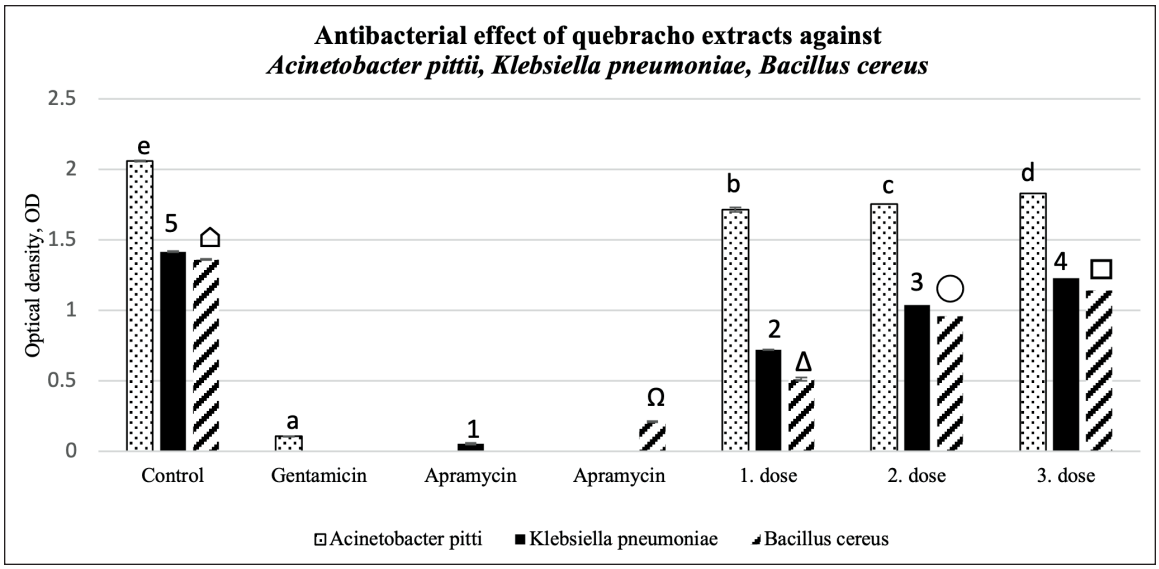

Figure 2. The antibacterial effects of quebracho extracts against *A. pittii, K. pneumoniae, B. cereus.* (1.dose 1.33 mg/mL, 2.dose 0.66 mg/mL and 3.dose 0.33 mg/mL). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○, ⌓).

Antibacterial efficacy of essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum*

We observed a considerable antibacterial effect for the essential oil of *O. onites* against *A. pittii* at the volumes of 3.33%, 1.67% and 0.83% (v/v). The inhibition percentages of the test material were recorded as 100±0.09, 86.69±1.62, and 71.42±12.57, respectively. The same pronounced efficacy was observed also against *K. pneumoniae* for tested essential oil samples (98.62± 2.07%, 100±0.00% and 100±0.00%, respectively), and *B. cereus* (100±0.00%, for each tested volumes) (Figure 3).

Similar outstanding results were also obtained for the essential oils of *O. onites oleum* and *O. minutiflorum*. The inhibitory ratios for *O. onites oleum* against *A. pittii* at the test volumes of 3.33%, 1.67% and 0.83% (v/v) were 100±0.00, 87.74±4.97, and 82.74±18.68, respectively. The potential antibacterial efficacy was also gathered by the essential oil of *O. minutiflorum* with the inhibition rates of 100±0.00, 80.04±4.97, and 78.52±2.47%, respectively (Figure 4 and 5). The notable suppressive effects of the essential oils of *O. onites oleum* and *O. minutiflorum* were observed also against *K. pneumoniae* and *B. cereus.* The inhibitory effects of essential oils of *O. onites oleum*

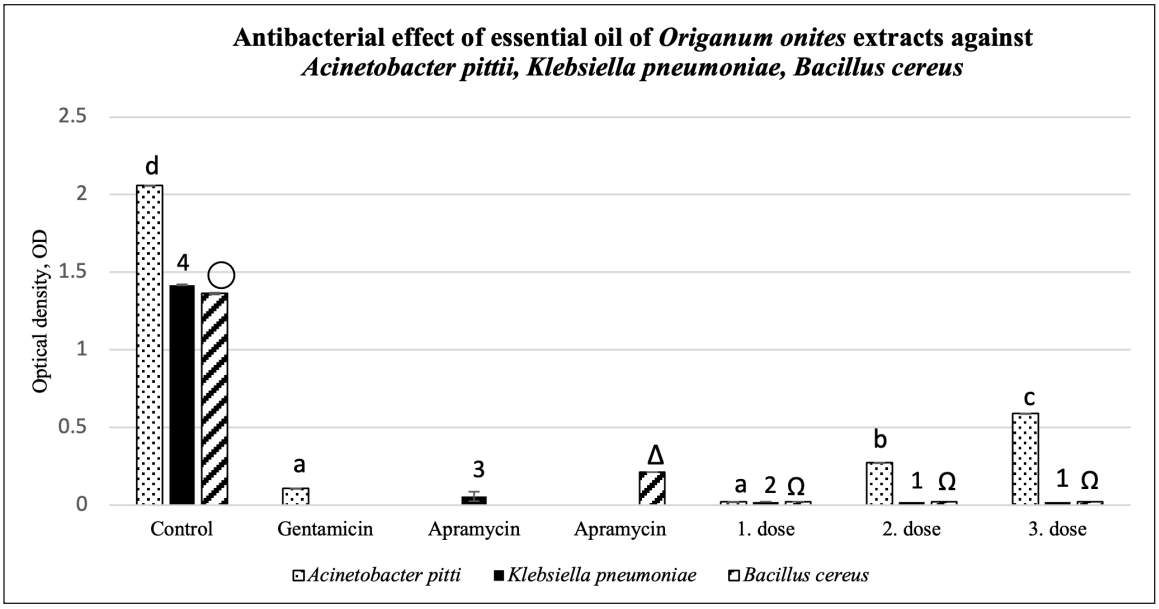

Figure 3. The antibacterial effects of the essential oil of *O. onites* against *A. pittii. K. pneumoniae, B. cereus.* (1.dose 3.33% (v/v), 2.dose 1.67% (v/v) and 3.dose 0.83% (v/v)). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○).

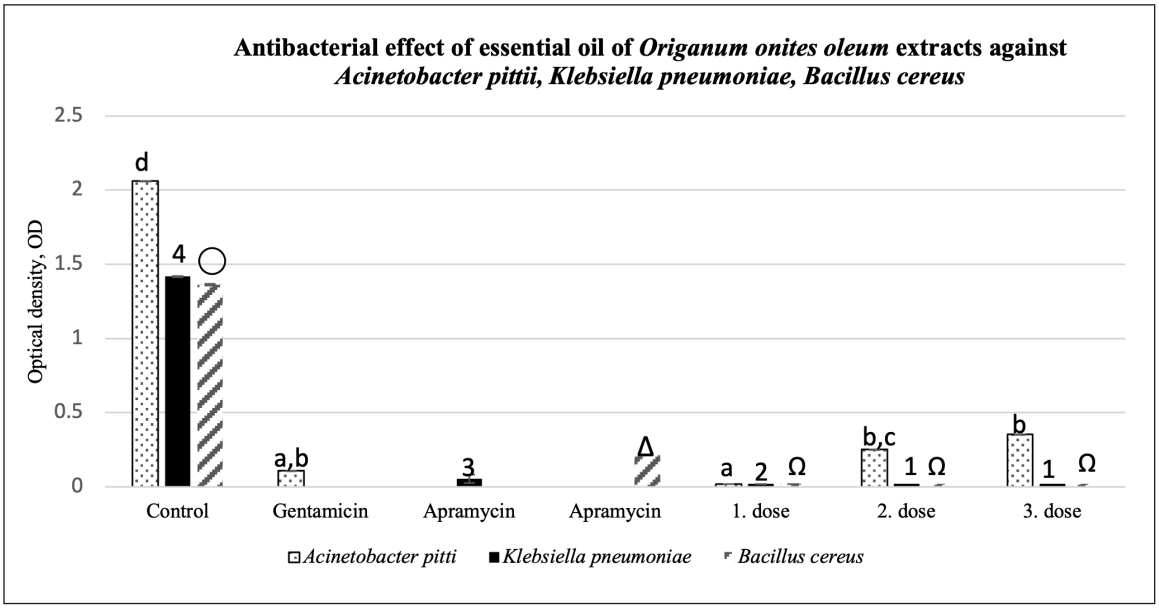

Figure 4. The antibacterial effects of essential oils of *O. onites oleum* against *A. pittii, K. pneumoniae, B. cereus.* (1.dose 3.33% (v/v), 2.dose 1.67% (v/v) and 3.dose 0.83% (v/v)). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○).

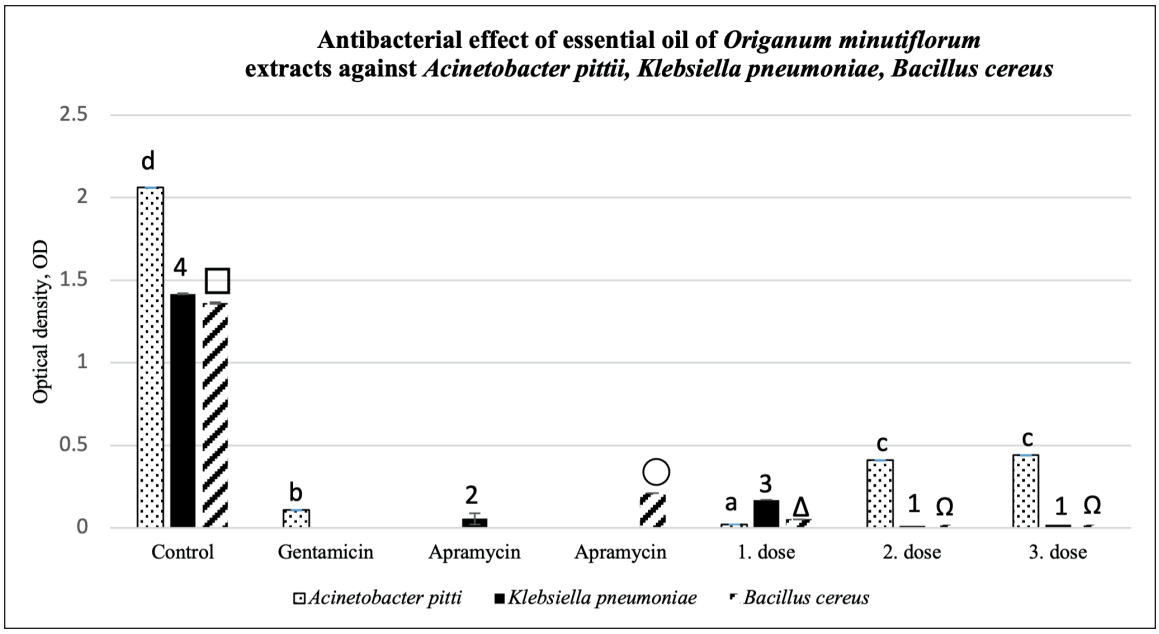

Figure 5. The antibacterial effects of the essential oil of *O. minutiflorum* against *A. pittii, K. pneumoniae, B. cereus.* (1.dose 3.33% (v/v), 2.dose 1.67% (v/v) and 3.dose 0.83% (v/v)). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, Δ, Ω, ○).

were detected as, 98.62±2.07, 100±0.00 and 100±0.00% against *K. pneumoniae* and 100±0.00% (for all tested volumes) against *B. cereus*. Also, the essential oils of *O. minutiflorum* at the test volmes of 3.33%, 1.67%, 0.83% (v/v) inhibited bacterial growth of *K. pneumoniae* with the inhibition percentages of 88.01±2.36, 99.08±1.38, and 100±0.00, respectively. Similarly, essential oil samples of *O. minutiflorum* had antibacterial efficiency against *B. cereus* and inbition ratios were recorded as 96.18±2.98%, 100±0.00% and 100±0.00%, respectively.

Statistical analyses

For *A. pittii*, *K. pneumoniae* and *B. cereus,* it was observed that all three tested concentrations of extracts of mimosa and quebracho, and essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum* had significance compared to the control (untreated) groups (p=0.000). Also, the gentamicin treatment group for *A. pittii* and the apramycin treatment groups for *K. pneumoniae* and *B. cereus* was found to be statistically more significant compared to mimosa and quebracho extract treatment groups at all tested concentrations (p=0.000). It was observed that there was no statistically significant difference between the first and second doses of mimosa for *B. cereus*. There was a statistically significant difference (p=0.000) in quebracho extract treatment groups between the first dose with the 2nd and 3rd doses. According to the gentamicin group, the first dose of *O. onites* gave similar results, and no statistical significance was observed. On the other hand, all tested doses showed statistically significant difference when compared to apramycin treatment groups for *K. pneumoniae* and *B. cereus* (p=0.000). There was no significant difference in all three doses of *O. onites oleum* in comparison to gentamicin treatment group. The first dose was statistically significant according to the second dose (p=0.035) and the third dose of *O. onites oleum* (p=0.001). No statistically significant difference was found for *B. cereus* for all administered doses of *O. onites oleum*. According to the gentamicin group, a statistically significant difference was observed in *A. pittii* for the first dose (p=0.001) and the 2nd and 3rd doses (p=0.000) of *O. minutiflorum*. On the other had, there was no statistically significant difference between the 2nd and 3rd doses of *O. minutiflorum* for *K. pneumoniae* and *B. cereus.*

Overall, our results demonstrated that the essential oil samples from *O. onites, O. onites oleum,* and *O. minutiflorum* were more successful in suppressing the bacterial growth, where a 3% (v/v) volume of essential oil samples from three tested *Origanum* species totally killed *A. pittii*. In addition, 1.67% (v/v) and 0.83% (v/v) volumes of essential oil samples from three tested *Origanum* species were also highly effective in inhibiting the bacterial growth of *A. pittii*. On the other hand, the desired effect by the extracts of mimosa and quebracho was not detected at the test concentrations. As known, mimosa and quebracho has largely been used in the leather industry in the vegetable tanning process to give reddish color.[32] There are some studies investigating the antibacterial potential of these tanning agents. Digrak et al. (1999) examined the extracts of mimosa bark against some Gram-positive and Gram-negative bacteria including *Brevibacillus brevis, B. subtilis, B. cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes, Mycobacterium luteus, K. pneumoniae, M. smegmatis, Proteus vulgaris* and they reported antibacterial efficacy against tested bacteria.[32] However, Rosiati et al. (2020)highlighted the possibility of vegetable tanned leathers as a potential growth medium for bacteria.[33] Furthermore, they tested the antibacterial efficiency of vegetable tanned (mimosa tanning agent) goat skins against *S. aureus* and reported slight inhibition zones as 11.40 mm.

Sirvaityte et al. (2011) demonstrated that the high resistance of leather tanned with mimosa to *E. coli* in comparison to quebracho group.[34] Moreover, quebracho and mimosa (50:50) mix showed a great efficacy for the inhibition of *P. aeruginosa* and *S. aureus*. However, Colak (2006)tested some vegetable tannins in soaking process for their antibacterial potencies against total aerobic bacteria at the 8th and 24th hours.[35] The researcher recorded no notable results for mimosa and and quebracho when compared to control group. These studies show that the potential antibacterial efficiency for the extracts of mimosa and quebracho may vary depending on the type of bacteria or process.

In the literature, there are studies in the leather sector by using extracts/chemical components/essential oils of various plants for the different leather-making processes. For example, the extracts of *Coridothymus capitatus, Olea europaea, Corylus avellana,* and *Juglans regia*, were tested in retanning stage to reduce chromium (VI) and satisfying results were found.[36] Haibin et al. (2011) indicated eco-friendly fungicide potency of essential oils from cinnamon, garlic, clove, and star anise in the leather sector.[37] The antibacterial efficacy for essential oils of *Lavandula officinalis* and myrtle oil (1%) in soaking process was reported in previous studies.[36,38] Our promising results are consistent with several studies evaluating different *Origanum* species for their antibacterial properties.[39-42] Aligiannis et al. (2001) tested the antibacterial and antifungal properties of essential oils obtained from two *Origanum* species against *S. aureus*, *S. epidermidis*, *E. coli*, *Enterobacter cloacae*, *P. aeruginosa, Klebsiella pneumoniae*, *Candida albicans, C. tropicalis* and *C. glabrata* and they demonstrated considerably high efficacies on tested materials.[39] Bayramoglu (2007) evaluated the antibacterial efficiency of four test materials including 1% of essential oils from *O. onites*, two different *Origanum* species and *Foeniculum vulgare* on the growth of bacterial population in the soaking process at the 8th and 24th hours. The researcher tested commercial bactericide namely 7-25 % phenol and 4-chloro-3-methyl as a control group. The study results demonstrated that 1% of essential oils from three *Origanum* species had antibacterial activity at both time points when compared to the untreated group.[43] The essential oil of *O. onites* has been also found to be successful in the fatliquoring process to eliminate free formaldehyde.[44] Bayramoglu et al. (2006) also tested the potential antifungal efficiency for essential oils of *O. minutiflorum, Laurus nobilis, Foeniculum vulgare,* and *Schinus molle* against *Aspergillus niger, Alternaria alternata, Penicillium rubrum* and *Trichoderma viride,* which are easily grown during pickling stage of leather making process. They reported the strongest antifungal effect for *O. minutiflorum*.[45]

The bioactive properties for the species belonging to *Origanum* species are reported to be relevant to the content of major phenolic compounds, especially thymol and carvacrol.[46] In our study, considerably high efficacies may have been obtained due to this chemical compound content. Further studies are needed to be performed for testing the applicability of these natural resources on diabetic footwear. It should be noted that the chemical composition of essential oils may vary depending on the place and time of harvest and the way of processing and storage. In addition, their antibacterial potency may change based on the content or concentration of the plant or its essential oil, and also the type and density of the bacteria.[47] The essential oils of *Origanum* species, obtained at the right time, from the right place, and in the right way, can be applied to the leather, which is a soft and breathable material for diabetic foot patients. This application may be done after the fatliquoring process via microencapsulation or during finishing process by spraying method of nano/microencapsules. The microencapsulation method provides protection from reactions caused by moisture, light, oxygen, ensures the controlled release of natural extracts, essential oils, active compounds, and also longer-lasting antibacterial efficacy. There are studies evaluating microencapsulated essential oils as biocides in footwear.[48-50] Thus, complaints of many diabetic foot patients such as foot infections, lesions, or ulcers can be prevented, and also the risk of amputation can be eliminated.

Conclusion

The importance of a good footwear selection in the clinical course of diabetic foot patients is stated in the literature. Recently, due to the problem of antibiotic resistance, there has been a trend towards the search for new natural resources with antibacterial effect. Controlling the bacterial population in the diabetic foot is of paramount importance to avoid the patient's history of worsening foot infections, which may lead to amputation. Studies focusing on the microbial flora of the diabetic foot have demonstrated the presence of *A. pittii*. The results of this study showed that the essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum* had remarkable inhibitory effects on the bacterial growth of *A. pittii*, *K. pneumoniae* and *B. cereus*. On the other hand, the extracts of mimosa demonstrated a very slight effect against *A. pittii* (35.12-8.77% inhibition range for three doses) and *K. pneumoniae* (47.04-21.40% inhibition range for three doses). However, the most efficacy of mimosa extracts was observed against *B. cereus*. Compared to quebracho extracts, the extracts of mimosa were found to be slightly more successful against tested bacteria. The greater than 50% inhibition was recorded at the concentration of 2.31 mg/mL of quebracho extracts against *B. cereus* whereas the same efficacy was not detected against other tested bacteria. The essential oils tested inhibited almost *B. cereus* with percentages of inhibition ranging from 96.18-100. The growth of *K. pneumoniae* and *A. pittii* was inhibited by 88.01 to 100% and 71.42 to 100%, respectively. From this point of view, these essential oils may be used in diabetic footwear by various application routes, such as microencapsulation or spraying on the leather. There is a need for studies investigating the effectiveness of these test materials against most common bacteria in diabetic foot patients when applied to the leather. The importance to study with mixed cultures should be kept in mind. Because in most cases, antibacterial agents can act on a single bacterium but sometimes this expected efficacy may not be seen on mixed cultures.

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