THE INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF DIFFERENT NATURAL AND ORGANIC AGENTS

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Abstract: The increasing need of natural antimicrobial agents has started a new field of investigation due to the global diseases. Synthetic antimicrobial agents are found to be causing skin problems and other toxic effects in long term. Therefore, antimicrobial activity of three natural extracts tea tree oil, tree moss extract, oakmoss extract and organic o-phenylphenol were evaluated in the study. These agents were added to a bar soap formulation and tested for antimicrobial activity EN 1276 standard method. The agents were added to five bar soap formulation as i) bar soap base; ii) bar soap base and tea tree oil; iii) bar soap base, tea tree oil, tree moss extract and oakmoss extract; iv) bar soap base, and o-phenylphenol; v) bar soap base, tea tree oil, tree moss extract, oakmoss extract and o-phenylphenol were prepared. As a result, in formulation (i) bar soap could not only pass the antibacterial test. The maximum antimicrobial activity was seen in formulation (v).

Keywords: bar soap, antimicrobial, antibacterial, tea tree oil, oakmoss, treemoss, o-phenylphenol

Introduction

The diseases evoking all over the world has become a global issue for humankind. The chemicals used as antimicrobial agents have entered our lives substantially. However, their effects on our skin and body have crucial risks in long term. The irritation on skin and toxicity are some of these health effects. Therefore, biocidal formulations which have low side effects has gained importance and natural ingredients are focus of interest for these formulations.

Tea Tree Oil (TTO, Tea tree oil) is an essential oil obtained by steam distillation from the leaves of *Melaleuca alternifolia (Myrtaceae)* that grows naturally in Australia. Studies have shown that tea tree oil has a broad-spectrum antimicrobial activity with its high terpinen-4-ol content (Carson et. al, 2005). In Turkey, as in Europe and North America it has also begun to be used in various cosmetic preparations and care products. In recent years, tea tree oil has been described as a safe antiseptic, more preferred due to its natural origin. TTO is regarded as an ideal disinfectant for topical use due to its antimicrobial effect against a wide range of microorganisms even at very low concentrations, easy penetration into the skin and no irritation (Çakır et. al, 2005).

TTO is currently not subject to any restrictions for use in cosmetic products. The oil is used in cosmetic ingredients, such as skin and body care products, toothpaste, mouth wash and bath oils, as well as in aromatherapy products. “Scientific Committee on Consumer Products, 2008” evaluated the use of TTO as an active ingredient used in cosmetic products. Accordingly, 0.2% in toothpaste and mouth wash products, 1.25% in skin care-moisturizers, 1.25% in body lotions, 2% in hair care - shampoo and conditioner products, 20% in nail care products, 0.7% in face washing products, 0.7% in hand washing, 2% in soap, 2% in foot spray, 1% in foot powder, 2% in shaving products, and 1.25% in post-waxing procedures may be used (Scientific Committee on Consumer Products SCCP, 2008).

Lichens are fungi living symbiotically with algae. They produce some secondary metabolites providing antibiotic, antiviral and anti-inflammatory effects (Aoussar et. al, 2017). Certain lichens are significant sources of natural extracts which are used in fragrances. The most well-known lichens are *Evernia prunastri* (oakmoss) (CAS 90028-68-5 or 68917-10-2, EINECS 289-861-3) and *Evernia furfuracea* extract (CAS 68648-41-9, EINECS 289-860-8) (Uter et. al, 2012).

o-Phenylphenol are antibacterial agents that are commonly used as bacteriostats, fungicides and disinfectants. It is an aromatic compound and organic chemical that is a white, crystalline (sand-like) solid (Centers for Disease Control and Prevention, 2020). o-Phenylphenol has biocidal properties, making it useful for a variety of preservation applications (Roberts, 2009).

In this study, ingredients TTO, o-phenylphenol and fragrance containing treemoss and oakmoss which have antibacterial activity were added to bar soap formulation with high pH and the antibacterial efficacy performance were evaluated by EN 1276 standard test method.

Materials and Methods

The soap base content is given in Table 1. TTO 1%, fragrance 0.75%, o-phenylphenol 0.15% were added to the bar soap base formulation as suitably for efficacy and compliance with regulations. Table 2 shows four different soap formulations.

According to European Cosmetic Regulation Annex V/7, o-phenylphenol is limited and its use must be under 0.2%.

Table 1. Bar soap base content values

SOAP BASE (SB)	
Total Fatty Acid Matter, % Min	78,0 min.
Free Fatty Acid (as Oleic), %	1 max.
Free Alkali (as NaOH), %	absent
Chlorides (as NaCl),%	0,5 - 1
Iodine Value, Gm of I_2/100 g	36 min.
Volatile Matter (105°C), %	14 max.

Table 2. Bar soap formulations

F1	Bar soap base
F2	Bar soap base + TTO (1%)
F3	Bar soap base + TTO (1%) + fragrance (0.75%) (including oakmoss and treemoss)
F4	Bar soap base + o-phenylphenol (0.15%)
F5	Bar soap base + fragrance (0.75%) + o-phenylphenol (0.15%)

Results

The bar soap removes the dirts and some microorganisms from the skin due to the micelles which are formed by the contact of bar soap and water. However, the performance of bar soap is not enough to remove all bacteria and it cannot kill them. Therefore, some antibacterial agents are added to bar soap formulations as other biocidal products to increase the efficacy. Synthetic antimicrobial agents cause skin irritations and other health problems in long term. Therefore, in this study; natural and organic alternatives were added to bar soap that is a natural cleaning product in order to obtain an antibacterial soap.

The results of antibacterial activity were given in Table 3. In EN 1276 test, the logarithmic reductions of four bacteria strains are evaluated: *Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 15442, Enterococcus hirae ATCC 10541, Escherichia coli ATCC 10536.* Minimum 5 log reduction is needed for a positive test result for all bacteria strains. The contact duration of the antibacterial formulation and the skin contact is standardized as 1 minute.

Table 3. Antibacterial activity results of bar soap formulations (EN 1276)

			Logarithmic reduction (cfu/ml)				
Bacteria	***Contact duration (min)***	***Activity range***	**F1**	**F2**	**F3**	**F4**	**F5**
Staphylococcus aureus ATCC 6538	1	>5	3.8	5.4	7.8	6.2	8.5
Pseudomonas aeruginosa ATCC 15442	1	>5	3.7	5.3	7.8	6.3	8.5
Enterococcus hirae ATCC 10541	1	>5	2.8	5.3	7.6	6.1	8.6
Escherichia coli ATCC 10536	1	>5	3.6	5.3	7.7	6.2	8.6
Efficacy result			**(-)**	**(+)**	**(+)**	**(+)**	**(+)**

The formulation 1 composes bar soap base and the logarithmic reduction values are under limit 5 log after 1 minute contact duration. Therefore, the bar soap does not have the necessary efficacy to kill the bacteria which place on the standard test method EN 1276. In formulation 2, TTO was added to bar soap base and the logarithmic reduction values exceeded the 5 log limit a little bit and positive result was obtained. In formulation 3, the fragrance was selected due to its content of oakmoss and treemoss. When fragrance was added to bar soap base and TTO, an increase of more than 2 log was observed. In formulation 4, the organic ingredient o-phenylphenol was added to bar soap. Normally, it has both anti-fungal and bacteriostatic activity. The maximum log reduction values were seen as 6.1 – 6.3 with formulation 4. When fragrance added to bar soap base and o-phenylphenol, an increase of more than 2 log reduction was also observed in case of formulation 5. The fragrance addition has a crucial effect on formulations 3 and 5 as 2 log that provides 100 times better antibacterial activity.

Conclusion

The different natural and organic antibacterial agents were added to bar soap formulation for antibacterial efficacy. Both TTO and o-phenylphenol provided antibacterial properties to bar soap for selected concentrations. The results may have changed with various concentrations of fragrance, TTO and o-phenylphenol.

Additionally, the fragrance which included treemoss and oakmoss increased the efficacy in significant ratios when added to TTO and o-phenylphenol. The fragrance may also have synergistic effect with TTO and o-phenylphenol on the bacteria. The main components of essential oils are mono- and sesquiterpenes, along with carbohydrates, phenols, alcohols, ethers, aldehydes and ketones, which are responsible for the biological activity and fragrances of aromatic and medicinal plants (Rotar, 2017). Both tea tree oil and oakmoss, treemoss extracts may show these activities due to rich contents. Interactions between such compounds may lead to antagonistic or synergistic effects. Therefore, a safe result may be obtained for EN 1276 test, especially if products containing natural antibacterial active are supported with components such as fragranced used in the study.

References

Aoussar, N., Manzali, R., Nattah, I., Rhallabi, N. Vasiljevic, P., Bouksaim, M. Douira, A., Manojlović, N., Mellouki, F. (2017). Chemical composition and antioxidant activity of two lichens species (*Pseudevernia furfuracea L* and *Evernia prunastri L*) collected from Morocco. *Journal of Materials and Environmental Sciences.* 8(6):1968-1976.

Carson, C. F., Messager, S., Hammer, K. A., Riley, T.V. (2005). Tea tree oil: a potential alternative for the management of methicillin-resistant Staphylococcus aureus (MRSA), *Australian Infection Control*. 10(1):32-34.

Centers for Disease Control and Prevention (2020). *ortho*-Phenylphenol. Retrieved from https://www.cdc.gov/biomonitoring/Orthophenylphenol_BiomonitoringSummary.html

Çakır, N., Kaleağası, S., Kökdil, G. (2005). Tea Tree Oil: As A Promising Antimicrobial Agent. *Ankara Ecz. Fak. Dergisi*. 34(4):315- 327.

Roberts, A. L., Yu, J. T., Coelhan, M. (2009). Presence of the biocide ortho-phenylphenol in canned soft drinks in the United States and Germany. *Food Chemistry*. 112(2):515-519.

Rotar, M.A., Cristina, A. S., Pop, C. R., Semeniuc, C. A. (2017). Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *Journal of Food and Drug Analysis* 25:403-408.

Scientific Committee on Consumer Products SCCP (2008). Opinion on Tea Tree Oil. SCCP/1155/08.

Uter, W., Schmidt, E., Lessmann, H., Schnuch, A. (2012). Contact sensitization to tree moss (*Evernia furfuracea* extract, INCI) is heterogeneous. *Contact Dermatitis.* 67: 36-41.

