International Journal of Pharmacy and Chemistry
2023; 9(3): 32-37
http://www.sciencepublishinggroup.com/j/ijpc
doi: 10.11648/j.ijpc.20230903.12
ISSN: 2575-5730 (Print); ISSN: 2575-5749 (Online)

Hurdle Technology – Approaches to Improve Cosmetic Preservation

Saima Athar[1], Anjali Gholap[1, 2], Rachna Rastogi[1, 2, *]

[1]Bregma Science LLP, Bangalore, India
[2]DPKA Universal Consumer Ventures Private Limited, Mumbai, India

Email address:
rachna@bregma.org (Rachna Rastogi)
[*]Corresponding author

To cite this article:
Saima Athar, Anjali Gholap, Rachna Rastogi. Hurdle Technology – Approaches to Improve Cosmetic Preservation. *International Journal of Pharmacy and Chemistry*. Vol. 9, No. 3, 2023, pp. 32-37. doi: 10.11648/j.ijpc.20230903.12

Received: June 25, 2023; **Accepted**: July 13, 2023; **Published**: July 27, 2023

Abstract: Increasing toxicity reports and new regulations for existing preservatives has driven a need for cosmetic formulations that are self-preserved. Use of preservatives is important to ascertain shelf life stability and minimize contamination after opening. While new classes of materials are being researched, their efficacy has been found to be substantially lower than their predecessors. Due to the variety of formulation types, it is exceedingly challenging to preserve different product formats using a handful of preservatives. Hurdle technology is being adapted by cosmetic scientists for designing formulations by modifying physico-chemical properties and use of multi-functional ingredients with antimicrobial properties to improve shelf life and minimize in-use contamination of products. This technology will also assist formulation scientists to make "preservative-free" claims for products while consumers get the advantage of using "clean cosmetics". Further, multifunctional materials help in reducing the formulation cost while enhancing product stability due to lesser number of ingredients. In this focussed review, we describe various techniques for improving preservation with their strengths and weaknesses to assist formulation scientists in making informed choices. Implementation of these methods with new preservatives will provide solutions to scientists to manage the diverse range of formulations for various benefits.

Keywords: Natural Preservatives, Personal Care Products, Multi-Functional Ingredients, Cosmetic Formulation

1. Introduction

Due to high water content and conducive pH (pH 4-9) in most formulations, cosmetic products are prone to microbial growth. Preservatives are commonly used in cosmetic products to prevent microbial contamination and ensure product safety during storage as well as after opening. [1, 2] Over the years, preservatives have been a subject of bad press due to reports of contact dermatitis, skin sensitivity and hormonal imbalance. [3, 4] Consumers and clean beauty advocates look for cosmetics formulated with safe alternatives. While multiple new preservatives and their combinations are under development [5], formulators need to look for alternate approaches to ensure product safety while following beauty trends.

Hurdle Technology is an effective preservation technique in which combination of various preservative factors or hurdles are applied in a way that minimizes micro-organism growth. Hurdle technology was first coined by Leistner in 1978 [6, 7]. An ideal preservation system (internal or external) must ensure product safety and protection of product from microbial contamination both in original closed packaging until expiry, and in an open container throughout its use [8]. The combination of various preservative ingredients and methods helps to achieve and maintain product quality while maintaining safety and stability. Different preservatives that act at different cellular targets will have greater impact on reducing organism growth. Figure 1 explains the mechanisms by which preservatives can achieve bacteriostatic/bactericidal effect.

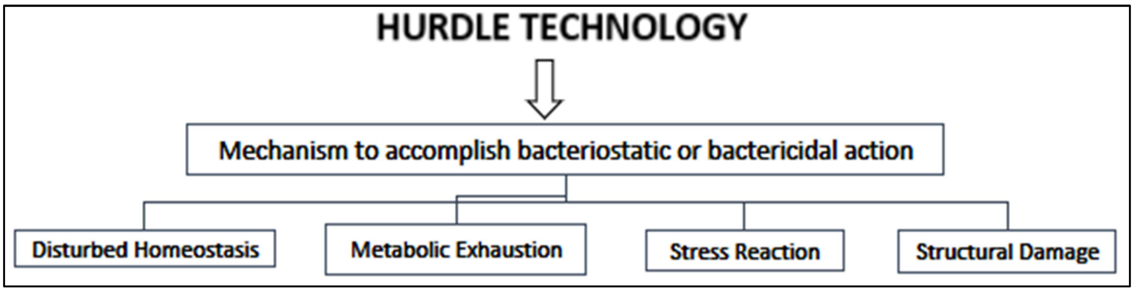

Figure 1. *Mechanism for achieving bacteriostatic or bactericidal action which will help in increasing preservation. Homeostasis is the tendency of any micro-organisms to maintain its internal balance. Disturbed homeostasis will promote Metabolic exhaustion leading to auto-sterilization. Stress reaction due to exposure to multiple stresses simultaneously activates the synthesis of various stress shock proteins, in turn making the micro-organisms metabolically weak, and Structural damage will eventually lead to leakage of cellular contents and cell death.*

2. Approach

Various methods, physical and/or chemical can be used simultaneously to achieve superior preservation. Figure 2 enlists the techniques to limit microbial contamination and growth. The methods, their pros and cons are discussed in detail further.

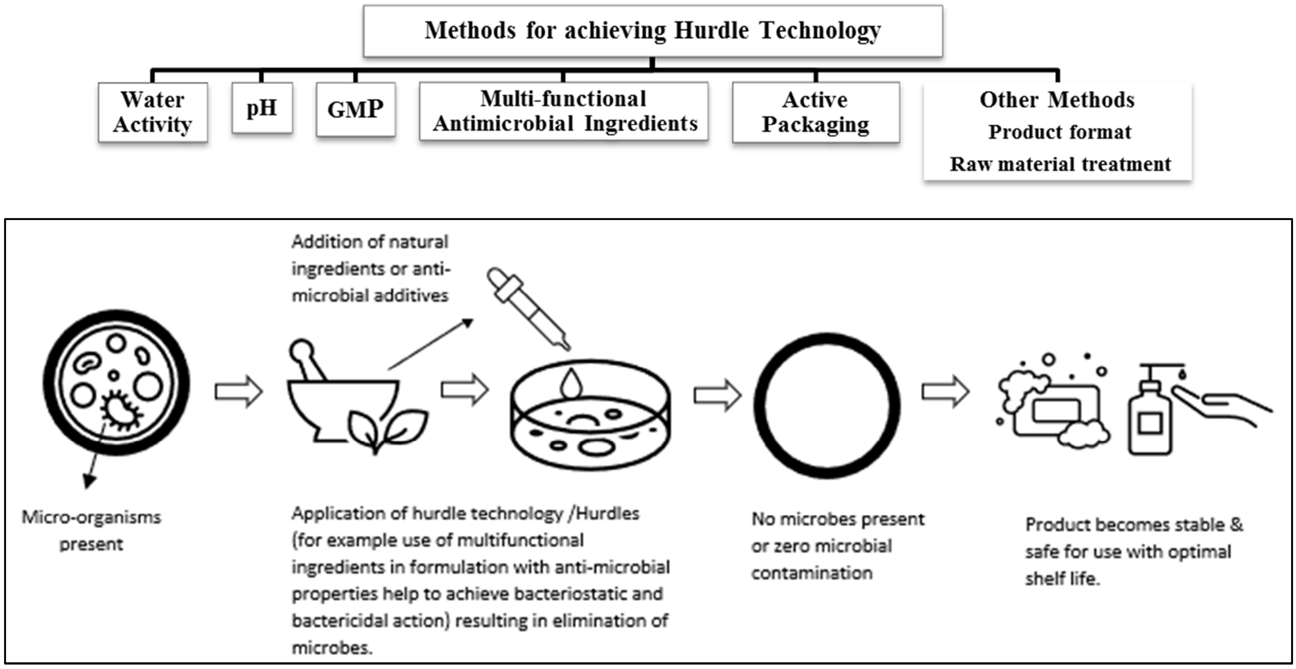

Figure 2. *Methods to achieve Hurdle Technology. When combination of different preservative factors or hurdles are applied, they deteriorate the growth of micro-organisms present making the product free from microbes, and safe for human use with optimal shelf life.*

Low Water Activity (a_w): Water activity or a_w is a measure of free amount of water available in a product [9]. Most formulations contain greater than 50% w/w of water, of which 80-90% is bound to various chemicals such as salts, thickeners and emulsifiers. The remaining 10-20% of unbound water may provide media for microbial growth. Water activity of formulation can be reduced by increase in use of polyols (e.g., glycerol, sorbitol, propylene glycol), hydrocolloids (xanthan gum, guar gum etc.), salt (sodium chloride, sodium lactate) and hydrogels (polyacrylamide) depending on the ingredient properties and formulation design [10, 11]. The minimum a_w value permitting the growth of micro-organism found or present in cosmetics is from 0.8 to 0.97. Addition of water-soluble solutes that lower the a_w to less than 0.95 will prevent growth of gram-negative bacteria (e.g., P. aeruginosa, E. coli) and solutes that decrease the a_w to less than 0.86 will help prevent the growth of most gram-positive and gram-negative bacteria [6].

Pros – Helps prevent bacterial growth or micro-organism spoilage which helps to increase the shelf-life of cosmetic products.

Cons – Impact in formulation cost as the water used will be replaced with ingredients such as polyols, hydrocolloids etc. which will increase cost. Formulations with high salt concentrations are difficult to stabilize and may require specific emulsifiers and viscosity enhancers.

pH: Formulation pH can provide favourable conditions for microbial growth [12]. Each micro-organism has a specific pH tolerance range. The optimum pH for growth of micro-organisms in cosmetic products is between 5 to 8. Thus, pH outside this range induces unfavourable conditions for the growth of micro-organisms [10]. For example, the acidic pH of cationic hair conditioners (pH = 4, approximately) can result in mild antimicrobial action of the product [10].

Pros – Cost effective technique [6].

Cons – Excess acidity or alkalinity may make products harsh or irritating (especially leave-on-products). Choice of ingredients stable under such extreme pH is limited.

Good Manufacturing Practices (GMP): GMP standards such as production under stringently aseptic conditions, periodic and controlled monitoring of water treatment/filtration, disinfection of manufacturing equipment, microbial control of raw materials and packaging components, environmental monitoring of facility, first in first out practices and properly trained personnel reduce the chance of contamination. GMP must be followed strictly during production of cosmetic products to hinder entry of micro-organisms or their spores during processing [6].

Pros: A clean, aseptic environment is created during storage of raw materials, production process, & storage of finished product resulting in low to negligible cross-contamination and fewer customer complaints.

Cons: Periodic monitoring for implementation and requirement of trained workforce has implications both on production cost and time. Certain disinfection processes such as heat sterilization may not be suited for all materials.

Multi-functional Antimicrobial Ingredients: Surfactants – Surfactants or surface-active agents can reduce surface tension, generate foam, improve wettability and solubility in water and in exceptional cases also show biocidal activity [13]. Surfactants can rupture cell membranes causing leakage of cellular contents, destabilization of lipid bilayer of bacteria inhibiting their growth [10].

Cationic surfactants with quaternary ammonium groups commonly known as quats are the most effective type of antimicrobial compounds. Quats (example – alkyl dimethyl benzyl ammonium chloride; ADBAC, alkyl dimethyl ethyl benzyl ammonium chloride; ADEBAC, and dodecyl ammonium chloride; DDAC) adsorb on cell membranes due to charge interactions followed by permeation, membrane damage and disturbance in homeostasis leading to cell death [14]. Cationic surfactants such as benzalkonium chloride [13] and cetyl pyridinium chloride are used often as they reduce or eliminate the need of additional preservative [6, 13].

Anionic surfactants such as fatty acid soaps have weak antibacterial effect under alkaline conditions and are usually active against gram positive rather than gram negative bacteria. The cell membrane in gram negative bacteria provides protection from anionic compounds, however use of chelating agents can improve their effectiveness [6].

Alcohols – Most commonly used alcohols in cosmetics, ethanol and isopropyl alcohol, have dose dependent bacteriostatic to bactericidal activity [10]. Alcohols are known to increase cell wall permeability; ethanol alters the peptidoglycan structure affecting the lipid-protein ratio causing cell content leakage [15].

Medium Chain Terminal Diols (MCTDs) – MCTDs are one of the most important types of multi-functional ingredients which are known to control microbial growth in cosmetics formulations. Most used MCTDs are ethyl hexyl glycerine, caprylyl glycol, pentylene glycol and hexylene glycol. The prominent mechanism of action is membrane disruption due to improved lipid dissolution [12]. This allows other ingredients to enter the cells and water to leak out of cells, leading to eventual cell collapse. Commercial examples of MCTDs include Lexgard® O (Caprylyl Glycol), Lexgard H (Hexylene Glycol), Ethyl hexyl glycerin (Lexgard® E) and Methyl heptyl glycerine (Lexgard® MHG Natural MB).

Fatty Acids, Fatty Alcohols and their derivatives – Medium chain saturated fatty acids such as heptanoic acid (C_7), caprylic acid (C8), capric Acid (C10), and lauric acid (C12) and their esters with glycerine and propylene glycol have been found to be active against micro-organisms. Glyceryl caprylate and glyceryl caprate have been effectively used at concentration of 0.5-1% w/w for the preservation of o/w formulations, body shower gels and shampoos [12]. Other derivatives which can be used includes capryl hydroxamic acid, C11/Undecylenic acid and glyceryl undecylenate. In a study, capric and lauric acid were found effective against Candida albicans in low doses of 2.5-5 mM in 30 minutes of incubation by altering the internal cellular turgor pressure [16].

Aromatic acids such as benzoic acid, p-anisic acid and their sodium/potassium salts and alcohols also have antimicrobial property. Phenethyl alcohol act as bacteriostatic agent. These acids and alcohols cause acidification of the cytoplasm resulting in enzyme inhibition and reduced metabolism leading to microbial cell death [12].

Plant-derived essential oils and extracts – Plant extracts and essentials oils also show antimicrobial and anti-inflammatory properties [10, 17, 18]. Numerous plant-derived extracts (such as Lonicera caprifolium extract and Lonicera japonica extract [12], Aloe vera extract [19], olive leaf extract [20], Glycyrrhiza glabra root extract [21], rosemary leaf extract [22], Citrus aurantium dulcis fruit extract, Citrus limon fruit extract, essential oils and extract of peppermint, basil, tea tree [23] show antimicrobial efficacy, when used alone or in combination with chemical preservatives for preserving cosmetic products.

Essential oils such as clove oil, cinnamon oil, eucalyptus oil, lemon oil, lavender oil, rose oil, thyme oil, tea tree oil, Calamintha officinalis, totarol, carvocol have been shown to prevent the microbial growth in cosmetics [6].

Essential oils which shows antimicrobial activity against gram positive and gram negative bacteria, and can be used in cosmetic formulation as preservatives includes: tea tree, lemongrass, oregano, clove [24] and thyme [25].

Enzymes and Ferments – In cosmetic formulations enzymes are used as exfoliants, and ferments are used to break down the molecular structure of ingredients and make the nutrients easy and quick to absorb into the skin. There are various enzymes and ferments which exhibit antimicrobial or antibacterial property and thus can be used as a multi-functional ingredient. Proteolytic enzymes such as bromelain have shown activity against P. acne and S. aureus [26].

According to the study conducted by Denkova et al, different Lactobacillus strains, Bifidobacterium bifidum and Propionibacterium freudenreichii were active against common cosmetic pathogens both bacteria and molds. The mode of action involved release of lactic, citric, tartaric acids for Lactobacilli, additional acetic acid for Bifidobacterium and propionic acid for Propionibacterium. This resulted in acidification of the media while creating a competition for nutrients between the pathogenic and test species resulting in reduced growth of the former [27].

In study conducted by Sirilun et al, a natural mouthwash solution with Lactobacillus fermented medicinal plant juice showed activity against E. coli, S. aureus and P. aeruginosa [28].

Chelating Agents – Ethylene diamine tetra acetic acid; EDTA, citric acid, lactic acid and phytic acid increase the permeability of cell membranes and make them more sensitive to antimicrobial agents. Chelating agents block the iron needed for microbial metabolism and growth enhancing the efficacy of antimicrobial agents [12].

According to the experiment conducted by Paterson et al [29] to examine the effects of different chelating agents used as bacteriostatic agents, the influence of eleven chelates (EDTA, DTPA, diethylene triamine penta acetic acid; DTPMP, diethylene triamine penta methylene phosphonic acid; MGDA, methyl glycine diacetic acid; GLDA, glutamic acid-N,N-diacetic acid; HBED,N,N-bis(2-hydroxybenzyl) ethylene diamine-N,N-diacetic acid; CAT, catechol; CHA, capryl hydroxamic acid; PO, piroctane olamine; TPEN, N,N,N',N'-tetrakis (2-pyridylmethyl) ethylene diamine; and BCS, bathocuporine disulphonic acid) and inhibitory effects on E. coli growth was studied [29]. The test ingredients sequestered specific divalent metals like Ca, Mg, Fe, Zn and Mn resulting in disturbance in cellular metal reserves affecting enzyme function and therefore imbalance in various functions like material exchange, growth and reproduction [29].

Antioxidants – The primary function of antioxidants is to delay auto-oxidation or self-oxidation of unsaturated lipids/oils in products, but they also show significant antimicrobial activity. Butylated hydroxy toluene (BHT), Butylated hydroxy anisole (BHA) and propyl gallate are the most effective antimicrobial antioxidants [12].

Pros – Use of natural ingredients with skin care benefit and preservation simultaneously.

Cons – Formula cost may increase due to multiple antimicrobial additives to achieve effective preservation. Ingredients, example essential oils have strong odour and may cause skin irritation when used at efficacious level [12].

Type of Packaging – Packing operations should take place under stringently clean conditions. Cleaning and disinfection of filling equipment should be done regularly as per GMP, cleaning packaging materials with filtered dry air can minimize contamination. Choice of closures also impacts product contamination during production and in-use. Tubes with narrow apertures, pre-sealed tubes, jars with inner lids and use of flip top caps and pumps instead of screw-on are examples of how formulators can minimize the duration of product exposure to the environment. Airless technologies based on a movable platform reduce the amount of air entrapped in a product during dispensing. These packaging reduce the availability of free oxygen in the product compared to standard systems thus minimizing the need for high preservative concentrations. Despite their benefits, Airless systems are expensive and therefore may not be economical for all products.

New studies show development of polymer coatings with impregnated nanoparticles or oxides such as silver, copper, zinc and titanium that can release free ions in their vicinity creating a zone of protection around the product. These technologies have been tested on catheters and prosthetics and may be suitable for cosmetic applications. Polycationic coating like polyethylene imine or quaternary chitin on glass surfaces can help reduce the extent of microbial growth. Studies need to be undertaken on the compatibility of these coatings with formulations and their contribution towards overall reduction in preservative content [30, 31].

Pros - Lower preservative concentrations can be effective while maintaining reduced contamination at the consumer end.

Cons - Certain formats, example, airless systems can be expensive.

Product Format -

Nano emulsions – Nano emulsions are described as the emulsions in which dispersed phase comprises of small particles or droplets, with a size range of 5nm-200nm [32]. Nano emulsions comprising of detergent, oil and water have shown broad-spectrum, non-specific activity against bacteria, viruses and fungi. The size of the emulsion droplets helps to improve the effectiveness of cosmetics. Thus, in several formulations, decrease in size of emulsion droplets, will increase the antimicrobial activity [33]. Nano emulsions have ability to fuse with the cell membranes of micro-organisms. The antimicrobial mechanism in nano emulsions is by the charge interaction of nanoparticles (having positive charge) and micro-organisms (negative charge) which destabilize the cellular permeability and lipid bilayers of cell membrane which leads to interruption in the activity of micro-organisms [33].

Emulsion Form – The risk of microbial contamination in water-in-oil emulsion is less as compared to oil-in-water emulsions as oil is the dispersion medium in the former leading to reduced biological oxygen availability in the product [10]. This restricts the product appeal and performance and therefore has limited application.

Treatment of raw materials – Raw materials to be used can be treated by different methods such as Pasteurization or different sterilisation methods (irradiation, autoclave, dry heat, filtration). This includes:

1) Pasteurization of raw materials (chemicals) to be used in formulation.
2) Sterilization of herbs and dry heat sterilization of

powdered raw materials.

3) Herbs / plant extracts and finished products can be treated by gamma irradiation [34].

Pros - Gamma irradiation is a clean, non-residual and environmentally friendly technology [34].

Cons - Materials can be thermolabile and therefore cannot be treated by heat sterilization techniques. These treatments add additional procedures to production and may impact time and cost.

3. Conclusion

Hurdle technology provides a method for combining several milder preservation techniques to attain an enhanced level of product safety and stability throughout shelf life. The aim is to interfere with different sustenance and growth mechanisms in micro-organisms simultaneously. With use of multifunctional ingredients, GMP and suitable packaging, it is feasible to obtain a 'preservative-free' product without compromising aesthetics and performance. This approach is useful for creating organic and natural products compliant to certifications where traditional preservatives are not permitted. A major stumbling block in implementing hurdle techniques is the requirement for adequate shelf life preservation studies since formulation ingredients can impact activity and effectiveness; overall increased cost and lowered speed-to-market [4].

References

[1] Alvarez-Rivera G, Llompart M, Lores M, Garcia–Jares C. Preservatives in cosmetics: Regulatory aspects and analytical methods in Analysis of Cosmetic Products (Ed. Salvador A and Chisvert A), (Second Edition), Elsevier, 2018, pp 175-222.

[2] Lundov MD, Moesby L, Zachariae C, Johansen JD. Contamination versus preservation of cosmetics: a review on legislation, usage, infections, and contact allergy. Contact Dermatitis. 2009 Feb; 60 (2): 70-8.

[3] Sasseville D. Hypersensitivity to preservatives. Dermatol Ther. 2004; 17 (3): 251-63.

[4] Nowak K, Jabłońska E, Ratajczak-Wrona W. Controversy around parabens: Alternative strategies for preservative use in cosmetics and personal care products. Environ Res. 2021; 198: 110488.

[5] Lundov MD, Johansen JD, Zachariae C, Moesby L. Low-level efficacy of cosmetic preservatives. Int J Cosmet Sci. 2011; 33 (2): 190-6.

[6] Kabara JJ. Hurdle Technology: are biocides always necessary for product protection? J Appl Cosmetol. 1999; 17: 102-9.

[7] Leistner L. Food preservation by combined methods. Food Res Int. 1992; 25 (2): 151-8.

[8] Brannan DK, Dille JC. Type of closure prevents microbial contamination of cosmetics during consumer use. Appl Environ Microbiol. 1990; 56 (5): 1476-9.

[9] Mandeep Kour, Neeraj Gupta, Monika Sood, Julie D Bandral, Fozia Hameed, Pawandeep Kour. Hurdle technology: A review. Int J Chem Stud 2019; 7 (5): 2579-2585.

[10] Halla N, Fernandes IP, Heleno SA, Costa P, Boucherit-Otmani Z, Boucherit K, Rodrigues AE, Ferreira IC, Barreiro MF. Cosmetics preservation: a review on present strategies. Molecules. 2018; 23 (7): 1571.

[11] Kerdudo A, Fontaine-Vive F, Dingas A, Faure C, Fernandez X. Optimization of cosmetic preservation: water activity reduction. Int J Cosm Sci. 2015; 37 (1): 31-40.

[12] Varvaresou A, Papageorgiou S, Tsirivas E, Protopapa E, Kintziou H, Kefala V, Demetzos C. Self-preserving cosmetics. International Journal of cosmetic science. 2009; 31 (3): 163-75.

[13] Wieczorek D, Gwiazdowska D, Michocka K, Kwaśniewska D, Kluczyńska K. Antibacterial activity of selected surfactants. Polish J Commodity Sci. 2014; 2 (38): 142-9.

[14] Falk NA. Surfactants as antimicrobials: a brief overview of microbial interfacial chemistry and surfactant antimicrobial activity. J Surf Deter. 2019; 22 (5): 1119-27.

[15] Haft RJ, Keating DH, Schwaegler T, Schwalbach MS, Vinokur J, Tremaine M, Peters JM, Kotlajich MV, Pohlmann EL, Ong IM, Grass JA. Correcting direct effects of ethanol on translation and transcription machinery confers ethanol tolerance in bacteria. Proc Nat Acad Sci. 2014; 111 (25): E2576-85.

[16] Bergsson G, Arnfinnsson J, Steingrímsson O, Thormar H. In vitro killing of Candida albicans by fatty acids and monoglycerides. Antimicrobial Agents Chemother. 2001; 45 (11): 3209-12.

[17] Pandey AK, Kumar P, Singh P, Tripathi NN, Bajpai VK. Essential oils: Sources of antimicrobials and food preservatives. Frontiers Microbiol. 2017; 7: 2161.

[18] Maccioni AM, Anchisi C, Sanna A, Sardu C, Dessi S. Preservative systems containing essential oils in cosmetic products. Int J Cosm Sci. 2002; 24 (1): 53-9.

[19] Tyowua AT, Vitalis TB, Terhemen MM, Mbaawuaga EM. Aloe Vera (Aloe barbadensis Miller) Extract as a Natural Antimicrobial Agent in Hand-Washing Liquid Soap. Niger Annals Pur Appl Sci. 2019; 2: 96-107.

[20] Al-Rimawi F, Odeh I, Bisher A, Yateem H, Taraweh M. Natural antioxidants, antibacterials from olive leaf extracts used in cosmetics, pharmaceutical, and food industries. In Qatar Foundation Annual Research Conference Proceedings (Vol. 2014, No. 1, p. HBPP0116). Hamad bin Khalifa University Press (HBKU Press).

[21] Nitalikar MM, Munde KC, Dhore BV, Shikalgar SN. Studies of antibacterial activities of Glycyrrhiza glabra root extract. Int J Pharm Tech Res. 2010; 2 (1): 899-901.

[22] González-Minero FJ, Bravo-Díaz L, Ayala-Gómez A. Rosmarinus officinalis L.(Rosemary): An ancient plant with uses in personal healthcare and cosmetics. Cosmetics. 2020; 7 (4): 77.

[23] Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999; 86 (6): 985-90.

[24] Dreger M, Wielgus K. Application of essential oils as natural cosmetic preservatives. Herb Pol. 2013; 59 (4).

[25] Manou IL, Bouillard L, Devleeschouwer MJ, Barel AO. Evaluation of the preservative properties of Thymus vulgaris essential oil in topically applied formulations under a challenge test. J Appl Microbiol. 1998; 84 (3): 368-76.

[26] Hidayat Y, Hermawati E, Setiasih S, Hudiyono S, Saepudin E. Antibacterial activity test of the partially purified bromelain from pineapple core extract (Ananas comosus [L.] Merr) by fractionation using ammonium sulfate acetone. InAIP Conference Proceedings (Vol. 2023, No. 1, p. 020067). AIP Publishing LLC.

[27] Denkova R, Denkova Z, Yanakieva V, Blazheva D. Antimicrobial activity of probiotic lactobacilli, bifidobacteria and propionic acid bacteria, isolated from different sources. In Mendez-Vilas A, editor. Microbial pathogens and strategies for combating them: science, technology and education. 2013. P. 857-64.

[28] Sirilun SA, Sivamaruthi BS, Kumar NA, Kesika PE, Peerajan S, Chaiyasut CH. Lactobacillus-fermented plant juice as a potential ingredient in cosmetics: Formulation and assessment of natural mouthwash. Asian J Pharm Clin Res. 2016; 9 (Suppl 3): 52-6.

[29] Paterson JR, Beecroft MS, Mulla RS, Osman D, Reeder NL, Caserta JA, Young TR, Pettigrew CA, Davies GE, Williams JG, Sharples GJ. Insights into the Antibacterial Mechanism of Action of Chelating Agents by Selective Deprivation of Iron, Manganese, and Zinc. Appl Environ Microbiol. 2022; 88 (2): e01641-21.

[30] Pinho AC, Piedade AP. Polymeric Coatings with Antimicrobial Activity: A Short Review. Polymers. 2020; 12 (11): 2469.

[31] Irshad K, Rehman K, Sharif H, Akash MS. Antimicrobial Polymer Coating. Polymer Coatings: Technology and Applications. In Inamuddin, Boddula R, Ahamed MI, Asiri AM (eds.) Polymer Coatings: Technology and Applications, Scrivener Publishing LLC 2020. P. 347-58.

[32] Ozgün S. Nanoemulsions in cosmetics. Anadolu Univ. 2013; 1 (6): 3-11.

[33] Hwang YY, Ramalingam K, Bienek DR, Lee V, You T, Alvarez R. Antimicrobial activity of nanoemulsion in combination with cetylpyridinium chloride in multidrug-resistant Acinetobacter baumannii. Antimicrobial Agents Chemother. 2013; 57 (8): 3568-75.

[34] Sivri NN, Özer AY, Özalp M, Atakan N, Polat M. Decontamination of cosmetic products and raw materials by gamma irradiation. FABAD J Pharm Sci. 2006; 4 (31): 198-209.