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Review Article

Self-preserving cosmetics

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Synopsis

Preservatives are added to products for two reasons: first, to prevent microbial spoilage and therefore to prolong the shelf life of the product; second, to protect the consumer from a potential infection. Although chemical preservatives prevent microbial growth, their safety is questioned by a growing segment of consumers. Therefore, there is a considerable interest in the development of preservative-free or self-preserving cosmetics. In these formulations traditional/chemical preservatives have been replaced by other cosmetic ingredients with antimicrobial properties that are not legislated as preservatives according to the Annex VI of the Commission Directive 76/768/EEC and the amending directives (2003/15/EC, 2007/17/EC and 2007/22/EC). 'Hurdle Technology', a technology that has been used for the control of product safety in the food industry since 1970s, has also been applied for the production of self-preserving cosmetics. 'Hurdle Technology' is a term used to describe the intelligent combination of different preservation factors or hurdles to deteriorate the growth of microorganisms. Adherence to current good manufacturing practice, appropriate packaging, careful choice of the form of the emulsion, low water activity and low or high pH values are significant variables for the control of microbial growth in cosmetic formulations. This paper describes the application of the basic principles of 'Hurdle Technology' in the production of self-preserving cosmetics. Multifunctional antimicrobial ingredients and plant-derived essential oils and extracts that are used as alternative or natural preservatives and are not listed in Annex VI of the Cosmetic Directive are also reported.

Résumé

La sécurité microbiologique des produits cosmétiques est un intérêt trés important pour les industries, parceque la contamination microbiologique peut provoquer des changements grands sur la composition des formules. Les agents conservateurs sont ajoutés dans les produits pour deux raisons: principalement, pour prévenir l' altération microbiologique et prolonger la date d' expiration du produit et également, pour proteger le consommateur contre les infections. La sécurité de conservateurs chimiques, qui previennent le développement de microbes, est sous de questions d' une grande partie des consommateurs. C' est pourquoi il y a de grand intérêt sur produits cosmétiques qui ne contiennent pas de conservateurs ou qui sont «auto-conservés». Dans cettes formules, les conservateurs traditionnels sont remplacés par autres ingrédients cosmétiques aux propriétés

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antimicrobiennes qui ne sont pas classifiés comme conservateurs selon l' Annexe VI de la Directive 76/768/EEC de la Commission et les directives 2003/15/EC, 2007/17/EC, 2007/22/EC. 'Hurdle Technology' est une technologie qui était utilisée pour le contrôle de la securité des produits de l' industrie alimentaire depuis 1970s. Cette technologie est utilisée aussi pour la production des produits cosmétiques 'auto–conservés'. 'Hurdle Technology' est un terme qui décrit la combinaison intelligente des facteurs conservateurs differents qui previennent le développement des microbes. Le respect des GMP, le packaging propre, la selection de la forme de l' emulsion, l'activité basse de l' eau, le pH haut ou bas, jouent un rôle principal sur le contrôle du développement des microbes dans les formules cosmétiques. Ce papier décrit l' application des principes basiques de 'Hurdle Technology' sur la production des cosmétiques «auto–conservés». Ingrédients antimicrobiens polyvalents et huiles essentielles ou extraits derivés de plantes qui sont utilisés comme agents conservateurs alternatifs et ils ne sont pas inclus dans la liste de l' Annexe VI de la Directive Cosmétique, sont reportés aussi.

Introduction

The microbiological safety of cosmetic products has always been of special interest for industries as microbial spoilage can lead to product degradation, or in the case of pathogens, an intimate contact with broken or damaged skin can cause a hazard for the health of the consumer and potentially spread infection. Except for petrolatum or purely oily preparations such as body oils or lipsticks, the rich composition of modern cosmetic formulations together with the aqueous environment is an ideal breeding ground for microorganisms.

Preservatives are antimicrobial chemicals added to cosmetics to protect them against microbial insults occurring from raw materials, manufacture and consumer use. Many questions have arisen regarding the safety of the traditional/chemical preservatives. Parabens, the most widely used preservatives worldwide, have weak oestrogen-like properties [1]. In December 2005 the Cosmetic Ingredient Review re-opened the safety assessment for parabens because a connection between the presence of parabens in breast tissue and breast cancer had been suggested [2, 3]. Although the European Scientific Committee determined that there was no need for the alteration of the original assessment, many consumers are concerned about parabens. There are also studies that cast suspicion on the other chemical classes of preservatives [4–6]. Formaldehyde-releasing preservatives such as imidazolidinyl- and diazolidinyl-urea are thought to cause skin reactions in sensitive individuals [4] and allergy to isothiazolinones has been the subject of many publications [5, 6].

In recent years, there has been a growing interest in the development of preservative-free cosmetic formulations. This term means without preservatives. Preservative-free aqueous formulations can be made microbiologically stable by sterile production and appropriate packaging. However, this approach may not work for the most aqueous cosmetics packaged in multiple-use containers. It has to be remarked that the common definition for preservative-free means that the product does not contain substances that are classified as preservatives according to the cosmetic legislation. Therefore, the term self-preserving is more appropriate than preservative-free. In self-preserving formulations traditional preservatives have been replaced by other cosmetic ingredients with antimicrobial properties. These ingredients have not yet been recognized as preservatives by the European Scientific Committee. This is the reason why the above substances are not listed in the Annex VI of the Commission Directive 76/768/EEC and the amending directives (2003/15/EC, 2007/17/EC and 2007/22/EC) with the official/traditional preservatives. Annex VI of the Commission Directive 76/768/EEC and the amending directives (2003/15/EC, 2007/17/EC and 2007/22/EC) contain all the substances which are permitted to be used in cosmetics as preservatives. The principles of 'Hurdle Technology' have been also applied for the production of self-preserving formulations. 'Hurdle Technology' has been used for the control of product safety in the food industry since 1970s [7]. This term is used to describe the combination of different preservation factors or hurdles in order to prevent the access of microorganisms into the final product and for the creation of a hostile environment within the formula which inhibits microbial growth or kills the microorganisms [8]. The goal is to block the growth of microorganisms by putting in their path various impediments that should each reduce the microorganism number but not kill the entire population. Each impediment should permit a diminished surviving population so that as the number

of impediments grow the number of survivors will be decreased and eventually reach zero. Some of the organisms may overcome the first hurdle; of those that survived, some may overcome the second and so forth until none survive the last hurdle [9]. This paper briefly reviews the techniques used for the production of nonclassically preserved cosmetics following the concept of 'Hurdle Technology'. Multifunctional ingredients, plant-derived essential oils and extracts with antimicrobial properties that are used as alternative or natural preservatives and are not listed in Annex VI of the Cosmetic Directive are also reported.

The principles of the self-preserving technology are presented below:

1 Good manufacturing practice (GMP)
2 Appropriate packaging
3 Emulsion form
4 Water activity
5 pH control
6 Multifunctional antimicrobial ingredients

Good manufacturing practice

Good manufacturing practice standards have to be adhered strictly during the production of cosmetic products when either traditional or alternative preservatives are used. Preparation of the cosmetic product under strictly aseptic conditions should hinder the ingress of microorganisms. Water filtration and radiation systems, positive pressure, microbial testing of raw materials, disinfection of the equipment and properly trained and dressed personnel can significantly reduce the danger of contamination [9, 10]. Thus, the use of strictly aseptic conditions during the production of cosmetics is proposed, especially when a self-preserving system is used in the formulations.

Appropriate packaging

Airless packaging is widely used in order to protect the product from all environmental insults. Containers and bottles have been especially designed to make the entry of the microorganisms into the products very difficult. Tubes which are widely used in the pharmaceutical industry are far better than wide-neck jars with shives. The nozzle offers a smaller and more discrete surface for contamination. There are tubes which have non-return valves so that once pressed, the tube cannot relax to permit the entry of the air. The most secure pack is the single-application pack, sachet, blister pack and the single-shot capsule [9, 10].

Low water activity

As microorganisms require water for growth, formulations that limit the availability of water for microorganisms help control microbial growth and become one of the hurdles placed in the organism's path. The water activity (a_w) describes the amount of biologically available water within cosmetic formulations and is determined by comparing the vapour pressure of the formula containing water with the vapour pressure of pure water. Water activity may be reduced by the use of water binding substances, such as salts, polyols, protein hydrolysates, amino acids and hydrocolloids [11]. Different classes of microorganisms have different tolerance to low water activity; bacteria generally have higher water requirements than yeasts, and yeasts higher requirements than molds [12]. Gram-negative bacteria show more susceptibility to low a_w values than gram-positive. Sorbitol and glycerol, in concentrations of around 20% w/w, are most commonly used to reduce water activity. However, high concentrations of polyols tend to give the product a sticky feeling [13]. Recently, a glycerylpolyacrylate gel consisting of water, sodium polyacrylate and polyols such as glycerin and ethoxydiglycol has been described for the successful preservation of oil in water (O/W) and aqueous formulations [14]. This new family of transparent, highly viscous hydrogels absorbs water from its surroundings and therefore deprives microorganisms of the free water necessary for their survival. Furthermore, the hydrogels are non-toxic, non-irritant and exhibit high skin moisturizing properties.

Emulsion form

It has been suggested that a W/O emulsion, where the oil is the continuous phase, is less prone to microbial spoilage, compared to an O/W formulation [15]. This might be true but it does not exclude the need for a preservative system. However, it places another obstacle in the microorganism's approach.

pH control

Hydrogen ion concentration (pH) is a crucial factor for the viability of microbes. Each organism has an

optimum pH for growth. The growth rate of microorganisms generally decreases as the pH departs from neutrality. Although many yeasts and molds are able to tolerate acidic pH conditions, many microorganisms are metabolically injured or stressed by extreme pH conditions of less than 4 or greater than 10. Products with low or high pH i.e. α-hydroxyacids creams or depilatories and hair dyes, respectively, are less susceptible to microbial contamination. On the other hand, adjustment of the pH to low or high values is a difficult problem especially for leave-on products as excess acidity or alkalinity may cause skin irritations [10, 11].

Multifunctional antimicrobial ingredients

According to European regulation, the only permitted preservatives are those that are listed in Annex VI of the 7th amendment of the Cosmetic Directive. However, many cosmetic ingredients, such as alcohols, essential oils, extracts and surfactants have antimicrobial properties. These materials which are used for their beneficial effect on the skin and may coincidentally contribute to the preservation of the formulation are not listed as preservatives in Annex VI. By a careful selection of these ingredients, it is possible to decrease or to eliminate the use of traditional/chemical preservatives and to formulate cosmetics with improved dermocosmetic properties. Below are listed some alternative preservatives.

Middle chain polar compounds

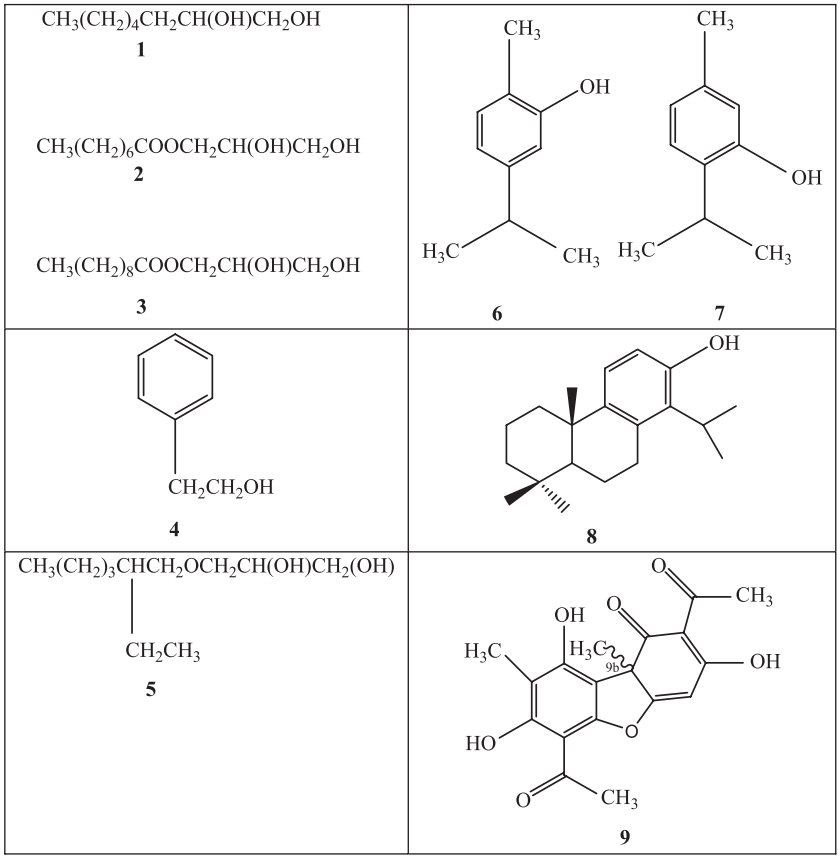
Caprylyl glycol

The antimicrobial activity of vicinal diols starts at short chain lengths and increases from butandiol to octandiol. The potency of the 1,2-diols with longer chains decreases rapidly because of their limited water solubility. 1,2-Octandiol or caprylyl glycol (Compound 1, Table I) is a C8 linear diol with moisturizing properties. In addition, because of its amphiphilic character and medium size, it exhibits very interesting viscosity modulating properties especially in O/W emulsions. These primary functions of caprylyl glycol are complemented by its antimicrobial properties which can support the activity of chemical preservatives [16]. The first example of such action was the combination of caprylyl glycol with Phenonip® (Parabens/Phenoxyethanol Clariant UK Ltd, Leeds, UK) described by Rigano [16] in the mid 1990s. According to the experiments performed by Jänichen [17] regarding an O/W formulation, the combination of caprylyl glycol 0.3% (w/w) with the traditional preservatives Phenonip® or Euxyl K702® (phenoxyethanol/benzoic acid/dehydracetic acid: Schülke & Mayr GmbH, Norderstedt, Germany) at approximately 50% of the recommended use levels led to a significant improvement in the antimicrobial activity of the preservatives. As shown in Fig. 1a, 0.5% (w/w) Phenonip® after 28 days was not sufficient to control the growth of fungi and *Escherchia coli*. On the contrary, the addition of 0.3% (w/w) caprylyl glycol substantially improved the control of the growth of these microorganisms. The supporting activity of caprylyl glycol in the case of Euxyl K702® is presented in Fig. 1b. Whereas 0.3% (w/w) Euxyl K702® alone was not sufficient against *E. coli* and *Aspergillus niger*, the addition of 0.3% (w/w) caprylyl glycol led to the complete elimination of *E. coli* and to a significant reduction of *A. niger*. Recently, it has been reported that caprylyl glycol as sole preservative at a concentration of 0.5–1% (w/w) is sufficient for the preservation of a variety of O/W and aqueous formulations [18].

Fatty acids and their monoesters

Medium chain saturated fatty acids, such as heptanoic (C_7), caprylic (C_8), capric (C_{10}) and lauric acid (C_{12}) and their esters with glycerin or propylene glycol have been found to possess activity against enveloped viruses and various bacteria and fungi *in vitro* [19–21]. The monoglycerides are active and diglycerides and triglycerides are inactive. Regarding the glyceryl monoesters, the equilibrium between the lipophilic and hydrophilic portion is decisive. There is a shift from emulsifier to antibacterial activity at chain lengths from C_8 to C_{12}. Inhibitory properties in terms of mininum inhibitory concentration (MIC) values reach a peak with C_{12} aliphatic chains and decrease rapidly at values less than 8 or greater than 12 [17]. On the contrary, the emulsifying ability of glyceryl monoesters of caprylic, capric and lauric acid is totally lost. The mechanism by which the monoglycerides kill bacteria has not been completely elucidated yet, but electron microscope studies indicate that they disrupt cell membranes, leaving the bacterial cell intact [19–21]. Glyceryl caprylate and glyceryl caprate (Compounds **2** and **3**, Table I) have been effectively used at

Table I Chemical structures of a) multifunctional antimicrobial ingredients i.e. caprylyl glycol (1), glyceryl caprylate (2), glyceryl caprate (3), phenethyl alcohol (4) and ethylhexylglycerine (5) and b) components of herbal extracts with antimicrobial properties i.e. carvacrol (6), thymol (7), totarol (8) and usnic acid (9)



concentrations of 0.5–1% (w/w) for the preservation of O/W formulations, body shower gels and shampoos [18, 22].

Phenethyl alcohol

The use of phenethyl alcohol (Compound **4**, Table I) as a bacteriostatic agent was first reported by Lilley and Brewer [23]. According to the experiments performed by Silver and Wendt [24], phenethyl alcohol causes a rapid and reversible breakdown in the permeability barriers of bacterial cells. This alteration of membranes leads to the disruption of many intracellular functions and the inhibition of DNA synthesis.

The combination of phenethyl alcohol with caprylyl glycol shows a synergistic antimicrobial effect as the wetting ability of caprylyl glycol may enhance the intracellular penetration of phenethyl alcohol. A solution consisting of 56–60% (w/w) phenethyl alcohol and 44–40% (w/w) caprylyl glycol shows MICs of 1750–3000 ppm against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *A. niger* and has been widely used at concentrations of 0.6–1.5% (w/w) for the preservation of a variety of formulations such as O/W and W/O emulsions and aqueous systems (Akema Formulary, http://www.akema.it; Sinerga formulary, http://www.sinerga.it).

Ethylhexylglycerine

Ethylhexylglycerine or 3-[(2-ethylhexyl)oxy]-1,2-propandiol (Compound **5**, Table I) is a glycerol

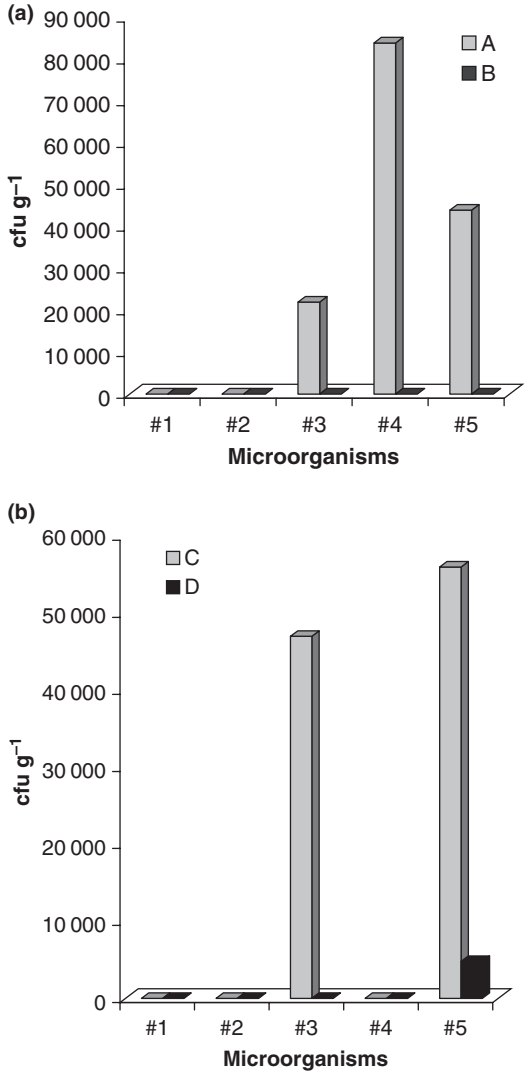


Figure 1 Comparison of challenge tests after 28 days with combinations of chemical preservatives and caprylyl glycol against #1: *Staphylococcus aureus*, #2: *Pseudomonas aeruginosa*, #3: *Escherchia coli*, #4: *Candida albicans* and #5: *Aspergillus niger*. (a) A = 0.5% (w/w) Phenonip, B = 0.5% (w/w) Phenonip + 0.3% (w/w) caprylyl glycol. (b) C = 0.3% (w/w) Euxyl K702, D = 0.3% (w/w) Euxyl K702 + 0.3% (w/w) caprylyl glycol [17].

monoalkylether, and has been used for its deodorant, emollient, humectant and perfume solubilizing properties. It is active against some odour causing gram-positive corynebacteria but activity against gram-negative bacteria, yeasts or moulds has not been found. As it has an hydrophilic-lipophilic balance of 7.4, it reduces the surface tension at the cell membrane of microorganisms and improves the contact of other antimicrobial substances with the cell membrane. Therefore, ethylhexylglycerine alone cannot preserve cosmetic products effectively but it has been used as an enhancer of the activity of alternative or chemical preservatives, such as 1,2-pentanediol or phenoxyethanol, respectively. Ethylhexylglycerine 0.5% (w/w) is incorporated often in self-preserving formulations in combination with 1,2-pentanediol 3.0% (w/w) [25].

Chelating agents

The chelators, EDTA, lactic acid, citric acid and phytic acid increase the permeability of cell membranes and make them more sensitive to antimicrobial agents. The cell wall lipopolysaccharides of gram-negative bacteria are believed to prevent the antimicrobial agents from reaching the cytoplasmic membrane [9, 26, 27]. In addition, chelating agents block the iron needed for microbial metabolism and growth. Thus chelators could be important ingredients in enhancing the efficacy of antimicrobial agents for the control of gram-negative bacteria which are known to have increased resistance to antimicrobial agents. Many publications have reported the synergistic effect of EDTA with synthetic or alternative preservatives [13]. However, because of the slow biodegradation, the use of EDTA is under consideration. Phytic acid, a fast biodegradable chelator can replace EDTA [28, 29].

Phenolic antioxidants

The primary function of phenolic antioxidants is to delay the auto-oxidation of unsaturated oils that could influence the colour and the odour of the product. Propyl gallate is rather a water-soluble molecule with antioxidant and antimicrobial activities against bacteria and fungi at a concentration of 0.5% (w/w) [13]. Caffeic, coumaric and ferulic acid have also demonstrated antimicrobial activity [29–33]. As antioxidants participate in oxidation reactions, their concentration in the formulation is gradually reduced. It has to be assured that the decrease in the antioxidant concentration does not lead to significant reduction in their antimicrobial potency in the formulation.

Plant-derived essential oils and extracts

Nature offers a broad spectrum of defence mechanisms against microbiological contamination.

Although the natural origin of a compound does not ensure a better dermatological and toxicological profile, improved biodegradability can generally be expected.

There are a number of plant-derived essential oils and extracts that possess excellent antimicrobial activities and have been used alone or in combination with chemical preservatives for the preservation of cosmetic products [34–38]. Essential oils refer to the subtle, aromatic and volatile liquids isolated from various plant parts, such as flowers, seeds, leaves, stems, bark and roots of herbs, bushes, shrubs and trees through distillation. Extracts are complex mixtures of compounds, non-aromatic, isolated from various plant parts through extraction with the appropriate solvents and techniques. Essential oils and extracts as natural preserving systems are cost-effective and in some instances may enhance the dermocosmetic properties of the final product. Thus their application as antimicrobials in cosmetic preparations is often discouraged because of the following drawbacks. First, they are much more organism-specific than synthetic preservatives and so must be carefully mixed to protect the product against the wide variety of microorganisms that can insult a cosmetic product; second, in some cases they cause dermatological allergies. In addition, essential oils have typically strong odours when used at efficacious levels which may be highly inappropriate for some kind of products i.e. make-up kits. Reduction in their antimicrobial action because of volatility and lipophilicity has in some cases been observed [39].

Thymus vulgaris essential oil

Thyme oil, the essential oil of *Thymus vulgaris* (Lamiaceae), is often incorporated into hygiene and skin care products, such as soaps, toothpastes, shower gels, shampoos, deodorants and body lotions because of its purifying and tonic properties. The oil also possesses antimicrobial properties which have been attributed mainly to the phenolic components, thymol and carvacrol (Compounds **6** and **7**, Table I) [40]. Phenols are believed to act by disrupting the proton-motive force of the cell membrane, nonspecifically denaturating the cytoplasm, the cell wall and the cell membrane.

The preservative action of thyme oil in O/W and W/O preparations has been reported. The oil at a concentration of 3% (w/w) proved to be effective against *S. aureus* and *P. aeruginosa*, satisfying the A criterion of the European Pharmacopoeia challenge test [41, 42].[1] However, it did not fulfil either the A or the B required criteria against *A. niger* in any of the formulations, or against *C. albicans*, in the O/W cream [42].

Synergistic combination of fractions of the herbs: *Origanum vulgare, Thymus vulgaris, Rosmarinus officinalis, Lavandula officinalis, Cinnamomum zeylanicum* and *Hydrastis canadensis*

The antimicrobial activity of *Origanum vulgare* (Lamiaceae) and *Thymus vulgaris* (Lamiaceae) has been attributed to the aforementioned phenolic ingredients, carvacrol and thymol. The essential oils of *Rosmarinus officinalis* (Lamiaceae) and *Cinnamomum zeylanicum* (Lauraceae) have been used for their antimicrobial properties [34, 43–45]. Preservative efficacy because of high phenol coefficients has been also reported for *Lavandula officinalis* (Labiatae) [34, 46]. The antimicrobial activity of berberine and hydrastine (*Hydrastis canadensis*, Ranunculaceae) is also known [47]. An effective antimicrobial formula containing fractions of these herbs has been developed and proved to be very effective against a variety of microorganisms including those which may be introduced into cosmetic products. The blend is called Biopein® Bio-Botanica Inc., Hauppauge, NY, USA and is active against *S. aureus*, *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *P. aeruginosa* and *C. albicans* at concentrations of 0.15–0.3% (w/w). Therefore, its composition and use as an alternative for products preservation are in the patenting process [48].

Artemisia afra oil and *Pteronia incana* oil

Essential oils of two African aromatic plants viz. *Artemisia afra* (Asteraceae) and *Pteronia incana* (Asteraceae) were evaluated for preservative efficacy in topical products. Camphor, 1,8-cineol and pinene were found to be the most frequent major compounds of these oils. However, some important compounds appeared solely in one particular oil. These were 3-thujanone, a major component of

[1]Challenge tests, products are judged adequately preserved when bacteria are reduced by more than 99% (2 log) after 2 days and more than 99.9% (3 log) after 7 days; yeasts and molds should be reduced by more than 99% (2 log for criterion A and 1 log for criterion B after 14 days.

Artemisia afra oil and *p*-mentha-8-ene-2-ol, a major component of *Pteronia incana* oil. Some of these compounds have been reported to possess antimicrobial activities [34]. According to Muyima *et al.* [49] a cosmetic cream was prepared with each individual oil as sole preservative at three different concentrations 0.5, 1.0 and 1.5% (w/w). Both oils showed microbial reduction properties, although the activity varied with the test organism species, and the type and the concentration of the oil used. These plants' essential oils can therefore be considered as alternative preservatives, the *Artemisia afra* oil proved to be the most effective.

Calamintha officinalis essential oil

The essential oil of *Calamintha officinalis* (Lamiaceae), a plant commonly found especially in dry places and used for its diaphoretic, expectorant and flavouring properties has been reported for its preservative properties in culture medium, and in cetomacrogol cream [50, 51]. Carvone, its major constituent, is widely used as a cosmetics flavouring agent and skin penetration enhancer [52].

Recently, the oil has been tested as preservative in two topical products, a cosmetic cream and a shampoo. The results of the challenge test clearly demonstrated that the *Calamintha officinallis* essential oil at 2.0% (w/w) concentration reduced the microbial inoculum, satisfying the criteria A and B of the European Pharmacopoeia regarding the O/W cream and shampoo, respectively [53].

Lonicera caprifolium extract and *Lonicera japonica* extract

Lonicera caprifolium and *Lonicera japonica* (Caprifoliaceae), with the trade name honeysuckle, have been used to treat common cold, influenza, cystitis and arthritis. The extracts of their flowers possess bacteriostatic and fungistatic activity at concentrations of 0.1–0.2% (w/w) [15, 54]. In our laboratory, we prepared a series of O/W and aqueous formulations containing *Lonicera* extracts in combination with glyceryl caprylate. All the O/W formulations (conditioning cream, peeling cream, body milk and cleansing milk) and the shampoo containing both *Lonicera* extracts 0.2%(w/w) and glyceryl caprylate 1%(w/w) fulfilled the criterion A of the European Pharmacopoeia. An example of the challenge test regarding the body milk is given in Fig. 2. As indicated the selected combination of

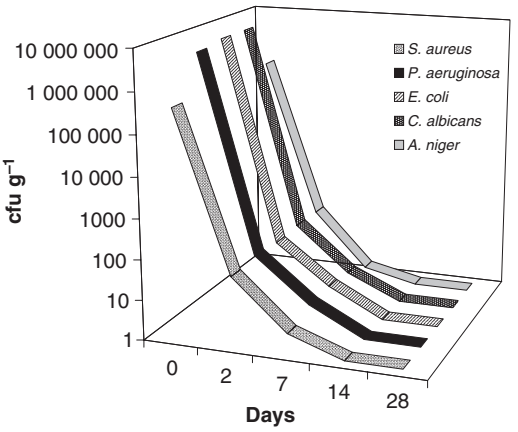


Figure 2 Effective inhibition of gram-positive, gram-negative bacteria and fungi by the combination of *Lonicera* extracts 0.2% (w/w) and glyceryl caprylate 1% (w/w) in body milk prepared in our laboratory (Unpublished data by S. Papageorgiou).

Lonicera extracts and glyceryl caprylate proved to be an effective preservation system for the emulsions and the shampoo, satisfying the criterion A of the European Pharmacopoeia. However, as shown in Fig. 3, the hydrosol which contained over 99% water and 0.25% (w/w) *Lonicera* extracts alone only met the acceptance criterion B regarding *C. albicans.*[2]

Melaleuca alternifolia essential oil

Tea tree oil is the essential oil derived from the leaves of *Melaleuca alternifolia* (Myrtaceae) and contains mainly terpinen-4-ol, α- and γ-terpinenes, 1,8-cineole and *p*-cymene. Terpinen-4-ol was identified to be the antimicrobial active ingredient in Tea tree oil. Regarding the Tea tree oil used in cosmetics, the concentration of terpinen-4-ol must be at least 30%, whereas the concentration of 1,8-cineol must not exceed 15% as it is suspected to

[2]Amounts of 30 g of the test product were inoculated with the challenge microorganisms in pure culture to reach microbial levels of not less than 106 cfu g^{-1} for bacteria and 105 cfu per fungi. The test samples were mixed and assayed at 0, 2, 7, 14, 21 and 28 days. The assays were performed on 1 g of test sample serially diluted in Letheen broth and plated in suitable agarized media. Plates were incubated at 35°C for bacteria and at 25°C for fungi. After incubation, readings of the number of colonies per gram were made.

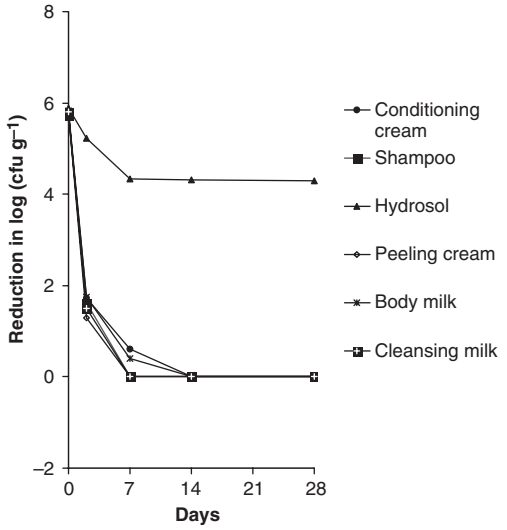


Figure 3 Effective inhibition of *Candida albicans* ATCC 10231 by *Lonicera* extracts and glyceryl caprylate in conditioning cream, shampoo, peeling cream, body milk and cleansing milk. The hydrosol was only marginally preserved with *Lonicera* extracts alone 0.25% (w/w) (Unpublished data by S. Papageorgiou).

have allergenic properties. Tea tree oil is active against a significant number of bacteria i.e. *S. aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *E. coli* and fungi i.e. *C. albicans* and *A. niger* [55, 56]. It has been incorporated into hygienic hand-washing products and formulations designed to treat moderate acne, boils and minor wound infections. It has been also used successfully at a concentration of 0.5% (w/w) as an alternative preservative [57].

Chitosan–*Inula helenium* (CI mixture)

Chitosan with the chemical name poly [β-(1-4)-2-amino-2-deoxy-D-glycose] is made by deacetylation of chitin which is extracted from the shells of crabs and shrimps. Chitin and chitosan are non-toxic materials with antimicrobial, antioxidant, anticancer and immune-activating properties. It has been suggested that the antibacterial activity of chitosan is related to the free amino group (positive charge) at C-2 of glucosamine [58]. This amino group forms an ionic bond with negative charges on the cell wall of gram-positive and gram-negative bacteria, inhibiting their growth. The MIC values of chitosan against bacteria and yeast are 0.9–3.0 mg mL^{-1}, whereas MICs of chitosan against *A. niger* is over 5 mg mL^{-1}. On the contrary, a recent study showed that the MICs of *Inula helenium* against *A. niger* were below 1.0 mg mL^{-1}. *Inula helenium* (Asteraceae) is a perennial plant, distributed in Korea, China and the European Continent. Although the active components of *Inula* are not known precisely, it has been shown that the extracts of its roots and stems possess preservative, anti-inflammatory and insecticidal properties [59].

A new system containing both chitosan powder and *Inula helenium* extract powder in the ratio 1 : 3, named CI mixture, with MICs against bacteria and fungi of 1.0–4.0 mg mL^{-1}, was evaluated for preservative efficacy. The mixture was incorporated at concentrations of 5–10% (w/w) in emulsion formulas, tonic skin lotion and pack. It was sufficient against *S. aureus*, *P. aeruginosa*, *C. albicans*, *A. niger* and *E. coli* in the O/W formulas and the tonic lotion but not effective for the preservation of the pack, possibly because of the binding of chitosan to the polymers of the formulation [60].

Totarol

Totarol or 14-isopropyl-8,11,13-podocarpatrien-13-ol (Compound **8**, Table I) is a diterpenoid phenol isolated from the heartwood of *Podocarpus nagi* (Podocarpaceae). Totarol possesses strong activity against gram-positive bacteria, such as *P. acnes*, *S. aureus*, *Streptococcus mutans* and *Mycobacterium tuberculosis* [60, 61]. It is also a potent antioxidant substance [62]. Although different mechanisms for the antibacterial activity of Totarol have been suggested, the mechanism of its action remains unclear. Haragushi *et al.* [63] have attributed the antimicrobial properties to the inhibition of oxygen consumption. Some studies claimed cell wall biosynthesis as a possible target. It has also been also indicated that Totarol may act by disrupting the phospholipid membrane of bacteria [64]. Because of its antimicrobial properties, Totarol has been incorporated in toothpastes and mouthwashes and in formulations for the treatment of acne and has been used in cosmetics as an alternative preservative as well [65, 66].

Usnic acid

Usnic acid or [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)]-dibenzo-furandione (Compound **9**, Table I) is a dibenzofuran derivative with the benzene nuclei bearing phenolic groups. It is extracted from lichen species, *Usnea barbata*

(Usneaceae), *Parmelia caperata* (Parmeliaceae), *Cladonia rangiferina* (Cladoniaceae) and others. It occurs in two enantiomeric forms, depending on the stereochemistry of the angular methyl group at the chiral 9b position. Both optical antipodes are active against gram-positive bacteria and mycobacteria. *Staphylococcus aureus*, *S. mutans*, *Mycobacterium aurum* and some *Enterococcus*, *Clostridium* and *Propionibacterium* species are sensitive to (+)- and (−)-usnic acid [67, 68].

However, (+)-usnic acid was found to exert superior activity in comparison with its enantiomer, against *S. mutans* isolated from human dental lesions [67–70]. (+)-Usnic acid has been effectively used in mouthwashes and toothpastes against dental caries and periodontal disease. On account of effects against gram-positive organisms mainly responsible for the development of body odour, usnic acid has been used in deodorant sprays [71]. The cosmetic additive named usnic acid multisolubilis which is the ethoxydiglycol extract from *Usnea barbata* and *Cladonia rangiferina* and contains 10% (w/w) usnic acid, has been tested for the preservation of a moisturizing O/W cream by Seifert and Bertram [72]. As shown in Fig. 4, addition of 2.5% usnic acid multisolubilis (w/w) (0.25% pure usnic acid) gave significant activity against gram-positive bacteria (*S. aureus* and *Streptococcus faecalis*), whereas gram-negative bacteria and fungi proved to be extremely resistant. Although the 5% level (i.e. 0.5% pure usnic acid) led to complete elimination of gram-positive bacteria and 40% reduction of surviving fungi, activity against gram-negative bacteria could not be detected. At 10% level (i.e. 1% pure usnic acid) good activity was exhibited against gram-negative bacteria (*P. aeruginosa* and *Providencia rettgeri*) and fungi (*A. niger* and *C. albicans*).

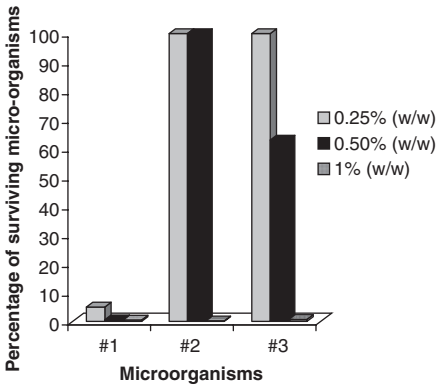


Figure 4 Comparison of the activity of 0.25% (w/w), 0.50% (w/w) and 1% (w/w) pure usnic acid at day 14 against #1: gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus faecalis*), #2: gram-negative bacteria (*Pseudomonas aeruginosa* and *Providencia rettgeri*) and #3: fungi (*Aspergillus niger* and *Candida albicans*) [73].

Fragrance ingredients

Ingredients of fragrances are more or less volatile compounds. The chemical composition of antimicrobial fragrances is not too much different from antimicrobial essential oils and extracts. Various aldehydes and alcohols i.e. aromatic, aliphatic or terpenes and organic acids are amongst the most active compounds [73]. In the past, a fragrance mixture mainly comprised of benzyl acetate, phenethyl alcohol and Linalool which was utilized in order to reduce the amount of parabens used in cosmetic formulations [74]. At this time, antimicrobial perfumes are commercially available, *p*-anisic acid (*p*-methoxy-benzoic acid) and levulinic acid (4-oxopentanoic acid) being the main components of them. *p*-Anisic acid is found in *Pimpinella anisum* and other herbs and levulinic acid has been found as byproduct in the production of diosgenin from wild yam (*Dioscorea villosa*) [15]. However, replacement of the chemical preservatives by fragrance ingredients will not necessarily ensure a reduction in the irritating effect of the formulation.

Conclusions

Studies that cast suspicion on some traditional/chemical preservatives i.e. parabens, formaldehyde releasers and isothiazolinones in combination with the increasing desire of consumers for 'natural' products has led the cosmetic industry to the development of new preservation methods. 'Hurdle Technology', a technology that has been used for the control of product safety in the food industry since 1970s, has been also utilized in the production of self-preserving cosmetics. The adherence to current GMP and the use of appropriate packaging in combination with the control of crucial factors for the growth of microorganisms i.e. water activity (a_w) and pH can remarkably decrease the amount of traditional/chemical preservatives needed for the stability of a cosmetic formulation. In self-preserving formulations, traditional preservatives have been replaced by other cosmetic

ingredients with antimicrobial properties i.e. caprylyl glycol, glyceryl caprylate, etc. and botanical essential oils and extracts. These materials which are used for their beneficial effect on the skin and coincidentally contribute to the preservation of the formulation are not listed in the Annex VI of the Commission Directive 76/768/EEC and the amending directives (2003/15/EC, 2007/17/EC and 2007/22/EC). However, the utilization of such alternative or natural substances does not ensure complete elimination of adverse events, irritating effects or sensitization. The 'ideal solution' that will replace traditional/chemical preservatives and will be absolutely safe, effective and compatible for all applications has not been found yet and probably never will.

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