



(86) Date de dépôt PCT/PCT Filing Date: 2004/07/15  
 (87) Date publication PCT/PCT Publication Date: 2005/02/03  
 (45) Date de délivrance/Issue Date: 2013/02/26  
 (85) Entrée phase nationale/National Entry: 2006/01/13  
 (86) N° demande PCT/PCT Application No.: US 2004/022505  
 (87) N° publication PCT/PCT Publication No.: 2005/009352  
 (30) Priorités/Priorities: 2003/07/17 (US60/488,349);  
 2003/12/18 (US60/530,864)

(51) Cl.Int./Int.Cl. *A61K 8/40* (2006.01),  
*A01N 33/12* (2006.01), *A61K 8/92* (2006.01),  
*A61Q 17/00* (2006.01)  
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(54) Titre : COMPOSITIONS ANTIMICROBIENNES CONTENANT DES COMBINAISONS SYNERGIQUES DE  
 COMPOSES D'AMMONIUM QUATERNAIRE ET D'HUILES ESSENTIELLES ET/OU DE LEURS CONSTITUANTS  
 (54) Title: ANTIMICROBIAL COMPOSITIONS CONTAINING SYNERGISTIC COMBINATIONS OF QUATERNARY  
 AMMONIUM COMPOUNDS AND ESSENTIAL OILS AND/OR CONSTITUENTS THEREOF

(57) **Abrégé/Abstract:**

The present invention relates to compositions comprising quaternary ammonium compounds and essential oils or individual constituents thereof which exhibit enhanced antimicrobial effects. Such combinations may be comprised in lotions, gels, creams, soaps, etc. for application to skin or mucous membranes. The invention is based, at least in part, on the observation that synergistic antimicrobial effects are achieved with combinations of essential oils or individual constituents thereof and low concentrations of quaternary ammonium compounds.

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
3 February 2005 (03.02.2005)

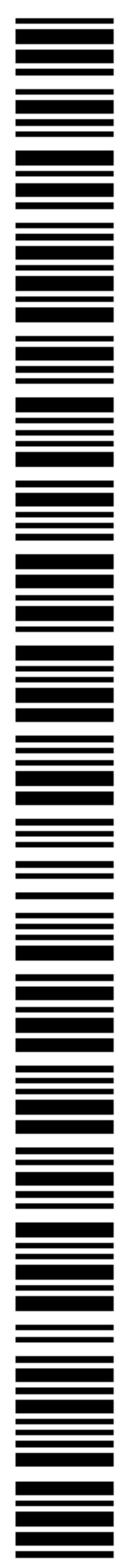
PCT

(10) International Publication Number  
**WO 2005/009352 A2**

- (51) International Patent Classification<sup>7</sup>: **A61K**
- (74) Agent: **KOLE, Lisa, B.**; Baker Botts L.L.P., 30 Rockefeller Plaza, New York, NY 10112-4498 (US).
- (21) International Application Number:  
PCT/US2004/022505
- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 15 July 2004 (15.07.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/488,349 17 July 2003 (17.07.2003) US  
60/530,864 18 December 2003 (18.12.2003) US
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
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- Published:**  
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: ANTIMICROBIAL COMPOSITONS CONTAINING SYNERGISTIC COMBINATIONS OF QUATERNARY AMMONIUM COMPOUNDS AND ESSENTIAL OILS AND/OR CONSTITUENTS THEREOF

(57) Abstract: The present invention relates to compositions comprising quaternary ammonium compounds and essential oils or individual constituents thereof which exhibit enhanced antimicrobial effects. Such combinations may be comprised in lotions, gels, creams, soaps, etc. for application to skin or mucous membranes. The invention is based, at least in part, on the observation that synergistic antimicrobial effects are achieved with combinations of essential oils or individual constituents thereof and low concentrations of quaternary ammonium compounds.



**WO 2005/009352 A2**

**ANTIMICROBIAL COMPOSITIONS CONTAINING SYNERGISTIC  
COMBINATIONS OF QUATERNARY AMMONIUM COMPOUNDS AND  
ESSENTIAL OILS AND/OR CONSTITUENTS THEREOF**

**SPECIFICATION**

**2. INTRODUCTION**

The present invention relates to compositions comprising combinations of quaternary ammonium compounds and essential oils and/or individual constituents thereof, and methods of using such compositions. It is based, at least in part, on the discovery that such combinations exhibit synergistically enhanced antimicrobial effects.

**3. BACKGROUND OF THE INVENTION**

Essential oils are volatile oils obtained from plant or animal sources and are composed of complex mixtures of several constituents, such as monoterpenes and sesquiterpene hydrocarbons, monoterpene and sesquiterpene alcohols, esters, ethers, aldehydes, ketones, oxides and the like. These essential oils and their isolated constituents are frequently utilized as fragrance and flavor agents, and have been widely used in folk medicine for wound healing properties.

Scientific research has corroborated the beneficial effects of essential oils. Essential oils of eucalyptus have been found to "possess central and peripheral analgesic effects as well as neutrophil-dependent and independent anti-inflammatory activities" (Silva et al., 2003, J. Ethnopharmacol. 89(2-3);277-283), and similar



activity has been observed in essential oils from *Lavendula angustifolia* Mill. (Hajhashemi et al., 2003, J. Ethnopharmacol. 89(1):67-71). Essential oils have been demonstrated to exhibit antibacterial (Bezic et al., 2003, Phytother. Res. 17(9):1037-1040; Goren et al., 2003, Z. Naturforsch. 58(9-10):687-690; de Abreu Gonzaga et al., 2003, Planta Med. 69(8):773-775; Valero and Salmera, 2003, Int. J. Food Microbiol. 85(1-2): 73-81) and antifungal (Paranagama et al., 2003, Lett. Appl. Microbiol. 37(1):86-90; Shin, 2003, Arch. Pharm. Res. 26(5):389-393; Velluti et al., 2003, Int. J. Food Microbiol. 89:145-154) activities. Virucidal activity of essential oils has also been observed, including direct virucidal effects against *Herpes simplex* viruses types 1 and 2 (Garcia et al., Phytother. Res. 17(9):1073-1075; Minami et al., 2003, Microbial Immunol. 47(a):681-684; Schuhmacher et al., 2003, Phytomedicine 10:504-510).

Quaternary ammonium compounds (“QAC”) are a group of ammonium salts in which organic radicals have been substituted for all four hydrogens of the original ammonium cation. They have a central nitrogen atom which is joined to four organic radicals and one acid radical. QACs have a tendency to distribute to the interface of two phases (liquid-liquid or solid-liquid) to introduce continuity between the two different phases. QACs are known to have potent antimicrobial activity, capable of disrupting bacterial cell processes. QACs have been used as antiseptics, disinfectants, preservatives, biocides, etc.

Johnson et al. (US 6,319,958 and US20020165130) relates to the use of sesquiterpenoids to promote uptake of exogenous antimicrobial compounds. Similarly, a related article discloses the use of sesquiterpenoids, such as nerolidol, farnesol, bisabolol and apritone, in enhancing bacterial permeability and susceptibility to exogenous antimicrobial compounds, suggesting that sesquiterpenoids have a non-specific and general effect (Brehm-Stecher et al. 2003, Antimicrobial Agents and Chemotherapy, 47(10):3357-3360). In particular, Brehm-Stecher *et al.* report that nerolidol, farnesol, bisabolol and apritone enhanced the susceptibility of *S. aureus* to the antibiotics erythromycin, gentamicin, vancomycin, ciproflaxin, clindamycin, and tetracycline. In addition, Brehm-Stecher *et al.* does not disclose the use of QACs as antimicrobial agents.

There is a continuing desire for an antimicrobial composition that is non-irritating, safe, and effective for repeated use in various professional and non-professional settings.

#### **4. SUMMARY OF THE INVENTION**

The present invention relates to antimicrobial compositions comprising combinations of quaternary ammonium compounds and essential oils and/or individual constituents thereof. Such combinations may be comprised in lotions, gels, creams, soaps, etc. for application to skin or mucous membranes. The invention is based, at least in part, on the observation that synergistic antimicrobial effects are achieved with combinations of essential oils and/or individual constituents thereof and low concentrations of quaternary ammonium compounds.

According to a first aspect, the invention provides for an antimicrobial composition for topical use, comprising between 0.01 and 0.5 percent (w/w) of a quaternary ammonium compound, between 0.1 and 2.0 percent (w/w) of a polyhexamethyl biguanide, two or more zinc salts, and an agent which is an essential oil or an individual constituent thereof, wherein the quaternary ammonium compound and the agent are present in amounts which exhibit synergistic antimicrobial activity and wherein the agent is present at a concentration of between 0.05 and 1.0 percent (w/w).

According to a second aspect, the invention provides for a use of a composition according to the invention and as described above, in the manufacture of a medicament for producing an antimicrobial effect on an area of skin of a subject.

According to a third aspect, the invention provides for a use of a composition according to the invention and as described above, in the manufacture of a medicament for producing an antimicrobial effect on an area of a mucous membrane of a subject.

According to a fourth aspect, the invention provides for a use of a composition according to the invention and as described above, for producing an antimicrobial effect on an area of skin of a subject.

According to a fifth aspect, the invention provides for a use of a composition according to the invention and as described above, for producing an antimicrobial effect on an area of a mucous membrane of the subject.



## **5. DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to compositions and methods for their use, wherein the antimicrobial activities of gels, creams, ointments, lotions or soaps is enhanced by the inclusion of synergistic amounts of quaternary ammonium compounds and essential oils and/or one or more individual constituent(s) thereof. The formulations of the instant invention comprise a synergistically effective amount of at least one quaternary ammonium compound and at least one essential oil and/or individual constituent(s) thereof.

The use of the terms, "synergistic" and "synergistically effective," are used in the present invention to mean a biological effect created from the application of two or more agents to produce a biological effect that is greater than the sum of the biological effects produced by the application of the individual agents.

Examples of quaternary ammonium compounds suitable for use in the instant invention include, but are not limited to, benzalkonium chloride ("BZK"), benzethonium chloride ("BZT"), other benzalkonium or benzethonium halides, including, but not limited to, benzalkonium or benzethonium bromide or fluoride, cetyl pyridinium chloride, alkylamidopropalkonium chloride, behenalkonium chloride, behentrimonium methosulphate, behenamidopropylethyldimonium ethosulphate, stearalkonium chloride, olealkonium chloride, cetrimonium chloride,

dequalinium chloride, N-myristyl-N-methyl-morpholinium methyl sulfate, poly[N-[3-(dimethylammonio)propyl]-N'-[3-(ethyleneoxyethelenedimethylammoinio)propyl]urea dichloride], alpha-4-[1-tris(2-hydroxyethyl)ammonium chloride-2-butenyl]-omega-tris(2-hydroxyethyl)ammonium chloride, poly[oxyethylene (dimethyliminio) ethylene (dimethyliminio)-ethylene dichloride].

The concentrations of quaternary ammonium compound may be between about 0.01 and 0.5 percent; preferably the quaternary ammonium compound is benzethonium chloride or benzalkonium chloride at a concentration between 0.05 and 0.3 percent, more preferably between 0.1 and 0.2 percent. These percentages, and other percentages herein, unless specified otherwise, are weight/weight.

Essential oils ("EOs"), as defined herein, are volatile oils obtained from plant or animal sources, or their synthetic equivalents, and are composed of complex mixtures of several constituents as monoterpenes and sesquiterpene hydrocarbons, monoterpene and sesquiterpene alcohols, esters, ethers, aldehydes, ketones, oxides and the like. Examples of EOs include, but are not limited to, bergamot oil, clary sage oil, ylang-ylang oil, neroli oil, sandalwood oil, frankincense oil, ginger oil, peppermint oil, lavender oil, jasmine absolute, geranium oil bourbon, spearmint oil, clove oil, patchouli oil, rosemary oil, rosewood oil, sandalwood oil, tea tree oil, vanilla oil, lemongrass oil, cedarwood oil, balsam oils, tangerine oil, Hinoki oil, Hiba oil, ginko oil, eucalyptus oil, lemon oil, orange oil, and sweet orange oil.

Individual constituents ("ICs") of essential oils may be isolated from the oil (natural) or entirely or partially synthetic, and include, but are not limited to, l-citronellol,  $\alpha$ -amylcinnamaldehyde, lylal, geraniol, farnesol, hydroxycitronellal, isoeugenol, eugenol, eucalyptol, linalool, citral, thymol, limonene and menthol.

Further examples of ICs include sesquiterpenoid compounds, which may be the active compounds in the essential oils. Sesquiterpenoid compounds, containing 15 carbons, are formed biosynthetically from three 5-carbon isoprene units. Sesquiterpenoid compounds include, but are not limited to, farnesol, nerolidol, bisabolol, apitone, chamazulene, santalol, zingiberol, carotol, and caryophyllen.

Mixtures of one or more EO, one or more IC, and one or more EO as well as one or more IC, are encompassed by the present invention.



The concentrations of EOs and ICs may be between about 0.01 and 10 percent; preferably between 0.05 and 1.0 percent or between 0.05 and 0.5 percent, and more preferably between 0.2 and 0.5 percent. In preferred embodiments, the EO is lemon oil and/or the IC is farnesol.

In certain specific, non-limiting embodiments, the present invention provides for formulations, including but not limited to gels, creams, lotions or ointments further comprising an amount of zinc that inhibits irritation of the skin or mucosa to which the formulation is applied. Zinc may be added to counteract the irritating effects of essential oils. The use of zinc in topical compositions is known in the art and disclosed in the following patents: U.S. Patent Nos. 5,708,023, 5,965,610, 5,985,918 and 6,037,386.

In a preferred embodiment of the invention, low concentrations of two or more water-soluble salts of zinc are used. The term "low concentration" means percentages of free zinc ions ( $Zn^{2+}$ ) in the gel or cream at less than 0.5% on a weight to weight (w/w) basis. Suitable zinc salts for use in these compositions include zinc acetate (molar solubility in water of 1.64 moles/l), zinc butyrate (molar solubility in water of 0.4 moles/l), zinc citrate (molar solubility in water of  $< 0.1$  moles/l), zinc gluconate (molar solubility in water of 0.28 moles/l), zinc glycerate (moderately water soluble), zinc glycolate (moderately water soluble), zinc formate (molar solubility in water of 0.33 moles/l), zinc lactate (molar solubility in water of 0.17 moles/l), zinc picolinate (moderately water soluble), zinc propionate (molar solubility in water of 1.51 moles/l), zinc salicylate (low water solubility), zinc tartrate (moderately water soluble) and zinc undecylenate (moderately water soluble). In particularly preferred embodiments, the zinc salts comprise a combination of effective amounts of two or more of the following: zinc acetate (0.05-2.0%), zinc citrate (0.05-2.0%), zinc gluconate (0.05-2.0%) and zinc lactate (0.05-2.0%). In preferred embodiments, the zinc salts are 0.2-0.6% zinc gluconate, 0.1-0.3% zinc acetate and 0.1-0.3% zinc lactate. In particularly preferred embodiments, the zinc salts are 0.3% zinc gluconate, 0.1% zinc acetate, and 0.1% zinc lactate, or 0.2% zinc zinc lactate and 0.2% zinc gluconate. Additional compositions that may comprise the synergistic combinations of the invention are described in International Patent Application No.



PCT/US03/03896, published on August 14, 2003, as WO03/066001.

The gels, ointments, lotions or creams of the invention may be applied topically to the skin or to the various mucous membranes of the body, including but not limited to those of the oral, nasal, vaginal or rectal cavities.

In preferred embodiments, the gel, lotion, ointment or cream may comprise a mixture of water, a gelling agent, a thickening agent, a hydrophilic or hydrophobic polymer, an emulsifying agent, an emollient, and/or alcohol, such as ethanol. In preferred embodiments, the presently claimed compositions comprise alcohol present at 10-90%w/w, water present at 15-70%w/w, thickeners and/or gelling agents present at 0.05-3.0% w/w, and emollients present at 0.1-3.0% w/w.

In preferred embodiments, if a thickener is present, it is not a polyacrylic acid-based thickener, such as but not limited to, carbomer, carbopol, or ultrez, as polyacrylic acid-based thickeners have been found to be incompatible with quaternary ammonium compounds. Without being bound by any particular theory, it is believed that anionic groups of such thickeners may interact with cationic groups of the quaternary ammonium compound. Preferably, if a gelling agent is used, it is not an anionic agent, but rather a non-ionic or cationic agent.

The compositions of the invention may optionally further include one or more additional antimicrobial agent such as, but not limited to, antiviral, antibacterial, or antifungal substances. Antimicrobial agents also include substances possessing any combination of virucidal or virustatic, bacteriocidal or bacteriostatic, or fungicidal or fungistatic properties. Antimicrobial agents are well known to those of ordinary skill in the art. Examples of antimicrobial agents include, but are not limited to, iodophors, iodine, benzoic acid, dehydroacetic acid, propionic acid, sorbic acid, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, cetrimide, chlorhexidine (free base and/or salts), other biguanides, such as polyhexamethyl biguanide (PHMB) and chlorhexidine gluconate (CHG), chloroeresol, chlorxylenol, benzyl alcohol, bronopol, chlorbutanol, ethanol, phenoxyethanol, phenylethyl alcohol, 2,4-dichlorobenzyl alcohol, thiomersal, clindamycin, erythromycin, benzoyl peroxide, mupirocin, bacitracin, polymyxin B, neomycin, triclosan, parachlorometaxylene, foscarnet, miconazole, fluconazole, itraconazole, ketoconazole, and pharmaceutically

acceptable salts thereof. These and further examples of antimicrobial agents useful in this invention can be found in such references as Goodman and Gilman's The Pharmacological Basis of Therapeutics (Goodman Gilman A, Rall TW, Nies AS, Taylor P, ed. (Pergamon Press; Elmsford, N.Y.: 1990)) .

In an embodiment, the compositions of the invention comprises a biguanide compound selected from the group consisting of chlorhexidine gluconate (CHG) and polyhexamethyl biguanide (PHMB). Preferably, the biguanide compound is present at a concentration of between 0.1 to 2.0% w/w.

Pharmaceutically acceptable chlorhexidine salts are well known to those of ordinary skill in the art and include, but are not limited to, chlorhexidine palmitate, chlorhexidine diphosphanilate, chlorhexidine digluconate ("CHG"), chlorhexidine diacetate, chlorhexidine dihydrochloride, chlorhexidine dichloride, chlorhexidine dihydroiodide, chlorhexidine diperchlorate, chlorhexidine dinitrate, chlorhexidine sulphate, chlorhexidine sulphite, chlorhexidine thiosulphate, chlorhexidine di-acid phosphate, chlorhexidine difluorophosphate, chlorhexidine diformate, chlorhexidine dipropionate, chlorhexidine di-iodobutyrate, chlorhexidine di-n-valerate, chlorhexidine dicaproate, chlorhexidine malonate, chlorhexidine succinate, chlorhexidine malate, chlorhexidine tartrate, chlorhexidine dimonoglycolate, chlorhexidine monodiglycolate, chlorhexidine dilactate, chlorhexidine di- $\alpha$ -hydroxyisobutyrate, chlorhexidine diglucoheptonate, chlorhexidine di-isothionate, chlorhexidine dibenzoate, chlorhexidine dicinnamate, chlorhexidine dimandelate, chlorhexidine di-isophthalate, chlorhexidine di-2-hydroxynaphthoate, and chlorhexidine embonate.

In formulating compositions of this invention, it is contemplated that the formulations may further comprise ingredients which, while not having the activity of the above-named ingredients, will aid in the formulation and use of the composition as a whole. Examples of such ingredients are well-known to those of ordinary skill in the art of producing formulations for biological purposes. Examples of these ingredients include such substances as binders, emollients, preservatives (such as methyl paraben), lubricants, colorants, perfumes, and the like. Accordingly, when the surface contemplated is skin, the composition of this invention may contain



ingredients which are added to known lotions or medicaments, which are physiologically acceptable to skin and which do not contain ingredients which will reverse or retard the action of the irritant-inactivating agent.

In certain non-limiting embodiments of the invention, the composition may be added to pre-existing formulations provided that the ingredients in those formulations do not prevent or retard the activity of the claimed composition. In a preferred embodiment, the claimed composition can be added to creams, ointments, gels or lotions which are commercially available. Examples of commercially available lubricants include, but are not limited to, those lubricants sold under the tradenames "KY JELLY," "ASTROGLIDE," and "PREVACARE." Examples of commercially available lotions include, but are not limited to, those lotions sold under the tradenames "SOFT-SENSE," "LOTION SOFT," "CUREL," and "KERI". SOFT-SENSE (Johnson & Son, Inc., Racine, Wis.) is known to contain purified water, glycerin USP, distearyldimonium chloride, petrolatum USP, isopropyl palmitate, 1-hexadecanol, tocopheryl acetate (vitamin E USP), dimethicone, titanium dioxide USP, methyl paraben, propyl paraben, sodium chloride, and fragrance. LOTION SOFT (Calgon Vestal, St. Louise, Mo.) is a nonionic moisturizing lotion which is known to contain mucopolysaccharide. CUREL (Bausch & Lomb Incorporated, Rochester, N.Y.) is known to contain deionized water, glycerin, quaternium-5, petrolatum, isopropyl palmitate, 1-hexadecanol, dimethicone, sodium chloride, fragrance, methyl paraben, and propyl paraben.

The claimed compositions may be used in anti-perspirants, aftershave lotions, hydroalcoholic skin disinfectants, and therapeutic creams, etc.

Certain preferred embodiments of the invention comprise, for example but not by way of limitation, one or more of the following: alcohol (10-90%w/w), which could include one or more of ethanol, n-propanol and iso-propanol; one or more zinc compound in an anti-irritant amount; one or more polymeric quaternary ammonium salts of hydroxyethyl cellulose reacted with trimethyl ammonium substituted epoxide (Polyquaternium), such as U-care polymer such as Ucare JR125, JR 400, JR 30M, LR 400, LR30M, or Ucare polymer LK; hydroxypropyl methyl cellulose such as the Methocel A,E,K, and 40 series products as Methocell K4MS, Methocel K100, Methocell 40-202, Methocel K15MS and others; one or more

quaternary ammonium compound such as BZK or BZT; cetyltrimethyl ammonium chloride ("CTAC"); cetyl trimethyl ammonium bromide ("CTAB"); olealkonium chloride; stearalkonium chloride; Incroquat BA 85 (babassuamidopropalkonium chloride); dibehenyldimonium methosulfate; IncroquatBES-35 S (Behenamidopropylethyldimonium Ethosulfate and stearyl alcohol); Incroquat B-65C (Behenalkonium chloride and cetyl alcohol); Incroquat Behenyl TMS (Behentrimonium methosulfate and cetearyl alcohol); and one or more emollient, such as Procetyl 10 PPG-10 cetyl ether, Procetyl 50 PPG-50 cetyl ether, Promyristyl PM-3 PPG-3Myristyl ether, PPG-3 benzyl ether myristate (Crodamol STS of Croda), PEG 20 Almond Glycerides, Probutyl DB-10, Glucam P20, Glucam E-10, Glucam P-10, Glucam E-20, Glucam P-20 distearate, glycerin, propylene glycol, cetyl acetate and acetylated lanolin alcohol (Acetulan), hydroxylated milk glycerides (Cremerol HMG); a silicone fluid such as Dow Corning Silicone Fluid 245, 244, 246, 344, 345, 556; an essential oil such as lemon oil, citronella oil, sandalwood oil, lemongrass oil, patchouli oil, clove oil, thyme oil, geranium oil, basil oil; an individual constituent of an essential oil, such as farnesol, citronellol, linalool, eugenol, citral, thymol, eucalyptol, menthol; and a biguanide such as chlorhexidine gluconate or polyhexamethyl biguanide.

The invention provides for methods of using the foregoing compositions to achieve an antimicrobial effect comprising applying an effective amount of the composition to the surface. An antimicrobial effect significantly diminishes the risk of infection or progression of existing infection by one or more pathogenic infectious agent. The risk of infection need not be reduced to zero, but preferably is reduced by at least 10, 20, 30, 40, 50, 60, 70, 80, or 90 percent. Examples of infectious agents against which protection may be afforded include, but are not limited to, *Staphylococcus* species such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Streptococcus* species such as *Streptococcus pneumoniae*, *Enterococcus* species, *Salmonella* species such as *Salmonella typh*, *Escherichia* species such as *Escherichia coli*, *Vibrio* species, *Neisseria* species, such as *Neisseria meningitidis* and *Neisseria gonnorrhoea*, Human Immunodeficiency



Virus (HIV), Human Papilloma Virus (HPV), Herpes Simplex Virus (HSV), *Chlamydia trachomatis*, , *Trichomonas vaginalis*, and *Candida albicans* .

In an embodiment of the invention, the compositions of the present invention do not contain antibiotics, including, but are not limited to, tetracycline, ampicillin, rifampin, vancomycin, amphotericin B, nystatin, and bacitracin.

The following are specific, non-limiting examples of formulations of the present invention with and without zinc salts.

### **FORMULATIONS WITHOUT ZINC SALTS**

#### **General Formula**

<b><u>Ingredients</u></b>	<b><u>Percent Range (w/w%)</u></b>
Ethanol	50-90
Water	15-35
U-care polymers	01.-0.5
Germall + <sup>TM</sup>	0.15-0.3
Quaternary ammonium compounds	0.05-0.2
Quaternary conditioners	0.2-1.0
Emollients	0.3-1.0
Phenoxyethanol (if present)	0.5-1.0
Silicone fluids	0.2-1.0
Essential oils/individual constituents	0.3-1.0
Biguanides' (if present)	0.05-2.0

#### **Specific Formulas**

##### **(A) Alcohol surgical hand prep-1**

<b><u>Ingredients</u></b>	<b><u>Percent (w/w)</u></b>
Ethanol	68
Babassuamidopropalkonium Chloride	0.5
Water	28.12
U-care JR 30	0.2
Panthenol	1.0
BZT	0.18
Silicone fluid (DC245)	0.5
Procetyl 10 (PPG-10, cetyl ether)	0.5
Farnesol	0.5
Phenoxyethanol	0.5

**(B) Alcohol surgical hand prep-2**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	68
Babassuamidopropalkonium Chloride	0.5
Water	27.97
U-care JR 30	0.2
Panthenol	1.0
BZT	0.18
Silicone fluid (DC245)	0.5
Procetyl 10 (PPG-10, cetyl ether)	0.5
Farnesol	0.5
Phenoxyethanol	0.5
PHMB	0.15

**(C) Alcohol surgical hand prep-3**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	68
Babassuamidopropalkonium Chloride	0.5
Water	28.12
U-care JR 30	0.2
Panthenol	1.0
BZT	0.18
Silicone fluid (DC245)	0.5
Procetyl 10 (PPG-10, cetyl ether)	0.5
Farnesol	0.25
Geranium oil	0.25
Phenoxyethanol	0.5

**(D) Pre-op skin disinfectant-1**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	60
Povidone iodine	7.5
Water	31.07
U-care JR 30	0.2
BZT	0.18
CHG	0.05
Farnesol	0.5
Phenoxyethanol	0.5



**FORMULATIONS WITH ZINC SALTS****General Formula**

<b><u>Ingredients</u></b>	<b><u>Percent range (w/w)</u></b>
Ethanol	50-90
Water	15-35
U-care polymers	01.-0.5
Germall +	0.15-0.3
Quaternary ammonium compounds	0.05-0.2
Quaternary conditioners	0.2-1.0
Emollients	0.3-2.0
Phenoxyethanol (if present)	0.5-1.0
Silicone fluids	0.0-1.0
Essential oils/individual ingredients	0.3-1.0
Biguanides (if present)	0.05-2.0
Zinc gluconate	0.3-0.6
Zinc acetate	0.1-0.3
Zinc lactate	0.1-0.3

**Specific formulas****(E) Alcohol hand disinfectant-1**

<b><u>Ingredients</u></b>	<b><u>Percent (w/w)</u></b>
Ethanol	60
Babassuamidopropalkonium Chloride	0.3
Water	36.23
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.3
Zinc lactate	0.1
Panthenol	1.0
Chlorhexidine gluconate (CHG)	0.05
BZK	0.12
Silicone fluid (DC245)	0.5
Procety-10 (PPG-10 cetyl ether)	0.5
Farnesol	0.5

**(F) Alcohol hand disinfectant-2**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	60
Babassuamidopropalkonium Chloride	0.3
Water	36.08
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.3
Zinc lactate	0.1
Panthenol	1.0
Chlorhexidine gluconate (CHG)	0.05
BZK	0.12
Silicone fluid (DC245)	0.5
Procetyl-10 (PPG-10 cetyl ether)	0.5
Farnesol	0.5
PHMB	0.15

**(G) Alcohol surgical hand prep-1**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Water	26.87
Zinc lactate	0.2
Zinc gluconate	0.2
Methocell K4MS	0.1
UcareJR 30	0.2
Panthenol 50W	1.0
Ethanol	68.0
CrodamolSTS	1.0
Babassuamidopropalkonium Chloride	0.3
Procetyl 10	0.5
PHMB	0.15
Farnesol	0.3
BZT	0.18
Propylene Glycol	1.0



**(H) Alcohol surgical hand prep-2**  
**Ingredients**

	<b><u>Percent (w/w)</u></b>
Ethanol	68
Babassuamidopropalkonium Chloride	0.5
Water	27.32
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.4
Zinc lactate	0.2
Panthenol	1.0
BZT	0.18
Silicone fluid (DC245)	0.5
Procety-10 (PPG-10 cetyl ether)	0.5
Farnesol	0.5
Phenoxyethanol	0.5

**(I) Alcohol surgical hand prep-3**  
**Ingredients**

	<b><u>Percent (w/w)</u></b>
Ethanol	68
Babassuamidopropalkonium Chloride	0.5
Water	27.02
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.4
Zinc lactate	0.2
Panthenol	1.0
BZT	0.18
Germall +	0.15
Silicone fluid (DC245)	0.5
Procety-10 (PPG-10 cetyl ether)	0.5
Farnesol	0.5
Phenoxyethanol	0.5
PHMB	0.15

**(J) Alcohol surgical hand prep-3**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	68
Babassuamidopropalkonium Chloride	0.5
Water	27.27
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.4
Zinc lactate	0.2
Panthenol	1.0
BZT	0.18
Silicone fluid (DC245)	0.5
Procety-10 (PPG-10 cetyl ether)	0.5
Farnesol	0.5
Phenoxyethanol	0.5
CHG	0.05

**(K) Anti-irritant Surgical Hand Prep-4**

<u>Ingredients</u>	<u>Percentage (w/w)</u>
Water	27.15
Zinc lactate	0.2
Zinc gluconate	0.2
Methocell K4MS	0.1
U Care-JR 30M	0.2
D,L-Panthenol	0.5
Benzethonium chloride	0.18
Ethanol	68.0
Crodamol STS	1.0
Procetyl	0.5
Babassuamidopropalkonium Chloride	0.3
Propylene Glycol	1.0
Farnesol	0.5
Polyhexamethylene biguanide	0.15
Fragrance	0.02



**(L) Anti-irritant Surgical Hand Prep-5**

<b><u>Ingredients</u></b>	<b>Percentage (w/w)</b>
Water	26.45
Zinc lactate	0.2
Zinc gluconate	0.2
Klucell MCS <sup>TM</sup>	0.8
U Care-JR 30M	0.2
D,L-Panthenol	0.5
Benzethonium chloride	0.18
Ethanol	68.0
Crodamol STS	1.0
Procetyl	0.5
Babassuamidopropalkonium Chloride	0.3
Propylene Glycol	1.0
Farnesol	0.5
Polyhexamethylene biguanide	0.15
Fragrance	0.02

**(M) Alcohol surgical hand prep containing Zinc gel**

The following formulation is advantageously used by persons who wear latex gloves, but who have a latex allergy.

<b><u>Ingredients</u></b>	<b><u>Percent ( w/w)</u></b>
Ethanol	68
Babassuamidopropalkonium Chloride	0.5
Water	22.93
U-care JR 30	0.6
Zinc acetate	0.2
Zinc gluconate	0.7
Zinc lactate	0.2
Zinc oxide	2.0
Panthenol	1.0
CHG	0.05
Incroquat TMS Behenyl	0.5
Silicone fluid (DC245)	0.5
Procety-10 (PPG-10 cetyl ether)	0.5
Glycerol	1.0
Farnesol	0.5
Germal Plus	0.2
Phenoxyethanol	0.5
Benzalkonium chloride	0.12

**(N) Alcohol based anti-irritant Surgical Prep**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Water	26.87
Zinc lactate	0.2
Zinc gluconate	0.2
Methocell K4MS	0.1
UcareJR 30	0.2
Panthenol 50W	1.0
Alcohol.SDA-40B	68.0
CrodamolSTS	1.0
Babassuamidopropalkonium Chloride	0.3
Procetyl 10	0.5
PHMB	0.15
Farnesol	0.3
BZT	0.18
Propylene Glycol	1.0

**(O) Topical cream-1**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Incroquat TMS Behenyl	0.8
Polawax NF <sup>TM</sup>	0.8
Petroleum Jelly	3.0
Crothix <sup>TM</sup>	1.0
Crodomol MM	1.0
Cremerol HMG <sup>TM</sup>	1.0
Propylene glycol	2.0
Glycerin	8.0
Water	78.22
UCare JR30-M	0.2
Germall Plus	0.2
BZT	0.18
Geranium oil	0.5
Zinc acetate	0.2
Zinc gluconate	0.7
Zinc lactate	0.2
Zinc oxide	2.0



**(P) Topical cream-2**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Incroquat TMS Behenyl	0.8
Polawax NF	0.8
Petroleum Jelly	3.0
Crothix	1.0
Crodomol MM	1.0
Cremerol HMG	1.0
Propylene glycol	2.0
Glycerin	8.0
Water	78.22
UCare JR30-M	0.2
Germall Plus	0.2
BZT	0.18
Basil Oil	0.5
Zinc acetate	0.2
Zinc gluconate	0.7
Zinc lactate	0.2
Zinc oxide	2.0

**(Q) Topical cream-3**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Incroquat TMS Behenyl	0.8
Polawax NF	0.8
Petroleum Jelly	3.0
Crothix	1.0
Crodomol MM	1.0
Cremerol HMG	1.0
Propylene glycol	2.0
Glycerin	8.0
Water	78.22
UCare JR30-M	0.2
Germall Plus	0.2
BZT	0.18
Sandalwood oil	0.25
Famesol	0.25
Zinc acetate	0.2
Zinc gluconate	0.7
Zinc lactate	0.2
Zinc oxide	2.0

**(R) Topical cream-4**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Incroquat TMS Behenyl	0.8
Polawax NF	0.8
Petroleum Jelly	3.0
Crothix	1.0
Crodomol MM	1.0
Cremerol HMG	1.0
Propylene glycol	2.0
Glycerin	8.0
Water	78.22
UCare JR30-M	0.2
Germall Plus	0.2
BZT	0.18
Thyme oil	0.5
Zinc acetate	0.2
Zinc gluconate	0.7
Zinc lactate	0.2
Zinc oxide	2.0

**(S) Pre-op skin disinfectant-1**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	60
Povidone iodine	7.5
Water	30.47
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.3
Zinc lactate	0.1
BZT	0.18
CHG	0.05
Farnesol	0.5
Phenoxyethanol	0.5



**(T) Pre-op skin disinfectant-2**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	60
Povidone iodine	2.0
Water	35.97
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.3
Zinc lactate	0.1
BZT	0.18
CHG	0.05
Farnesol	0.5
Phenoxyethanol	0.5

**(U) Pre-op skin disinfectant-3**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	60
Povidone iodine	2.0
Water	35.52
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.3
Zinc lactate	0.1
BZT	0.18
CHG	0.5
Farnesol	0.5
Phenoxyethanol	0.5

**(V) Pre-op skin disinfectant-4**

<u>Ingredients</u>	<u>Percent (w/w)</u>
Ethanol	60
Povidone iodine	2.0
Water	34.02
U-care JR 30	0.2
Zinc acetate	0.2
Zinc gluconate	0.3
Zinc lactate	0.1
BZT	0.18
CHG	2.0
Farnesol	0.5
Phenoxyethanol	0.5

**(W) Zinc gel hand wash #1**

<u>Ingredient</u>	<u>Percent (w/w)</u>
UCare JR30-M	0.05
Methocel K100	0.1
Water	36.08
Zinc gluconate	0.3
Zinc acetate	0.1
Zinc lactate	0.1
Germall Plus	0.2
Ethanol	58
Isopropanol	2.0
Silicone (Dimethicone)	0.2
Incroquat Behentyl TMS	0.7
Polawax A31	0.3
Glycerin	1.0
Cetyl ether (PPG10)	0.5
CHG	0.05
BZK	0.02
Farnesol	0.3

**(X) Zinc Gel Hand Wash#2**

<u>Ingredient</u>	<u>Percent (w/w)</u>
Water	33.45
Ethanol	62.0
Farnesol	0.3
Propylene glycol	1.0
Polyhexamethylene biguanide	0.15
Babassuamidopropalkonium Chloride	0.3
Procetyl-10 (PPG-10 Cetyl ether)	0.5
Crodamol STS	1.0
BZT	0.1
Zinc lactate	0.2
Zinc gluconate	0.2
D,L-Panthenol	0.5
UCare JR 30M	0.2
Methocel K4MS	0.1

**(Y) Zinc gel cream/lotion for under latex gloves**

<b><u>Ingredients</u></b>	<b><u>Percent (w/w)</u></b>
Ucare JR30-M	0.8
Methocel K100	0.3
Water	26.63
Zinc gluconate	0.6
Zinc acetate	0.2
Zinc lactate	0.2
Germall Plus	0.2
Zinc stearate	1.5
Zinc oxide	1.0
Glucate DO <sup>TM</sup>	5.0
Ethanol	55.0
Isopropanol	3.0
Silicone (Dimethicone)	0.5
Incroquat Behenyl TMS	1.0
Polawax A31	0.5
Glycerin	2.0
Cetyl ether (PPG10)	1.0
CHG	0.05
BZK	0.02
Farnesol	0.3
Vitamin E	0.2

**(Z) Anti-irritant disinfectant soap**

<b><u>Ingredients</u></b>	<b><u>Percent (w/w)</u></b>
Polyox WSR 205	0.1
UCare Jr30-M	0.2
Germall Plus	0.2
Water	86.93
Pluronic F87	2.0
Cocoamidopropylbetaine	1.0
Mirapol A-15	1.0
Propylene glycol	2.0
Polyquaternium-47(Merquat 3330)	3.0
Glycerin	2.0
CHG	0.05
BZK	0.12
Triclosan	0.3
Farnesol	0.3
Lemon oil	0.3
Zinc acetate	0.1
Zinc lactate	0.1
Zinc gluconate	0.3



## 6. WORKING EXAMPLES

### 6.1. EXAMPLE 1

Topical formulations containing zinc salts as anti-irritants were prepared in the presence and absence of EO and IC and in the presence and absence of antimicrobial compounds, such as benzalkonium chloride (BZK), chlorhexidine gluconate (CHG), zinc pyrithione (ZP) and triclosan (TC). The following EO and IC were evaluated: lemon oil as a representative essential oil, farnesol and linalool as representative terpene alcohols, and citral as a representative aldehyde. The resulting formulations then were evaluated for their antimicrobial activity.

#### 6.1.1. METHOD

##### **Preparation of alcohol based zinc gel**

An alcohol based gel was prepared as follows, and the antimicrobials, EOs and/or ICs were added to this base:

##### **BASE #1**

<u>Ingredient</u>	<u>% by weight</u>
UCare JR30-M	0.1
Methocel K100	0.1
Water	36.4
Zinc gluconate	0.3
Zinc acetate	0.1
Zinc lactate	0.1
Germall Plus	0.2
Ethanol	58
Isopropanol	2.0
Silicone (Dimethicone)	0.2
Incroquat Behenyl TMS	0.7
Polawax A31	0.3
Glycerin	1.0
Cetyl ether (PPG10)	0.5

##### **Evaluation of rapid antimicrobial activity in presence of serum.**

To determine the efficacy of the antimicrobial composition on the skin, which may be contaminated with blood or other proteinaceous fluids containing bacteria, the antimicrobial activity was evaluated in the presence of serum as follows. Briefly, 0.5 ml of  $10^8$  CFU of *S. aureus*/ml was added to 0.5 ml of bovine adult serum in a sterile culture tube and mixed. 0.5 ml of the test formulation was added to each tube and vortexed. After 15 seconds, it was further diluted 1:100 with drug inactivating media (LTSB) and 0.5 ml was plated on a TSA plate. The plates were incubated at  $37^{\circ}\text{C}$  for 24 hours and the colony count per ml of culture was determined.

#### 6.1.2. RESULTS

It was observed that farnesol, linanool, citral and lemon oil showed synergistic antimicrobial effects when combined with BZK. No such synergism was observed when farnesol was combined with other antimicrobial compounds, CHG, ZP or TC. See Table 1 below.

**Table 1.****Synergistic effect of EO and IC ingredients  
with antimicrobial agents*****Test organism: S. aureus***

<b>Group</b>	<b>CFU/mL</b>	<b>Log<sub>10</sub> reduction</b>
Control (Base)	$3.4 \times 10^7$	-
BZK	$5.4 \times 10^5$	1.8
CHG	$6.6 \times 10^5$	1.7
TC	$1.5 \times 10^6$	1.3
ZP	$1.4 \times 10^6$	1.4
F	$8.4 \times 10^6$	0.6
LI	$1.5 \times 10^6$	1.3
CI	$1.5 \times 10^6$	1.3
LO	$1.6 \times 10^6$	1.3
BZK + F	$3.0 \times 10^2$	5.0
BZK + CI	$2.0 \times 10^2$	5.2
BZK + LI	$6.0 \times 10^2$	4.7
BZK + LO	$1.4 \times 10^3$	4.4
CHG + BZK + F	$2.3 \times 10^2$	5.2
CHG + BZK + LO	$1.4 \times 10^3$	4.4
CHG + BZK + LI	$2.0 \times 10^2$	5.2
CHG + BZK + CI	$1.0 \times 10^2$	5.5
CHG + BZK + LO + TC	$1.4 \times 10^3$	4.4
CHG + BZK + F + TC	$1.5 \times 10^2$	5.3
CHG+BZK+F+ZP+LO	$2.0 \times 10^2$	5.2
CHG + LO	$1.6 \times 10^5$	2.3
TC + LO	$1.4 \times 10^6$	1.4
ZP + LO	$1.4 \times 10^6$	1.4
CHG + F	$6.8 \times 10^4$	2.7
TC + F	$1.2 \times 10^6$	1.4
ZP + F	$1.3 \times 10^6$	1.4

**Key:** BZK: Benzalkonium chloride (0.12%); PHMB: Polyhexamethylenebiguanide hydrochloride (0.15%); CHG: Chlorhexidene gluconate (0.05%); TC: Triclosan (0.3%); ZP: Zinc pyrithione (0.5%); F: Farnesol (0.3%); LO: Lemon oil (0.3%); LI : Linalool; (0.3%); CI : Citral (0.3%)



It can be seen from Table 1 that, among the antimicrobial compounds used, only the quaternary ammonium compound, benzalkonium chloride, exhibited significant synergistic activity in combination with EOs and ICs.

## 6.2. EXAMPLE 2

The present example shows the antimicrobial activity of compositions comprising a quaternary ammonium compound, benzethonium chloride (BZT) and farnesol in the presence and absence of zinc salts. The antimicrobial activity was evaluated as described in Example 1.

Farnesol and BZT were incorporated into Base #1 (containing zinc salts) and Base #2 (not containing zinc salts) shown below in proportions shown in Table 2.

### **BASE #2 (Without zinc salts)**

<u>Ingredient</u>	<u>% by weight</u>
UCare JR30-M	0.1
Methocel K100	0.1
Water	36.9
Germall Plus	0.2
Ethanol	58
Isopropanol	2.0
Silicone (Dimethicone)	0.2
Incroquat Behenyl TMS	0.7
Polawax A31	0.3
Glycerin	1.0
PPG-10 Cetyl ether (Procetyl-10)	0.5

**Table 2**Test organism: *S. aureus*

Quantity in Gel (% w/w )	Without zinc salts		With zinc salts	
	cfu/ml	log <sub>10</sub> reduction	cfu/ml	log <sub>10</sub> reduction
Control Gel	4.1 x 10 <sup>7</sup>	0.0	1.4 x 10 <sup>7</sup>	0.0
BZT (0.18%)	7.5 x 10 <sup>5</sup>	1.7	3.3 x 10 <sup>5</sup>	1.6
Farnesol (0.5%)	8.9 x 10 <sup>5</sup>	1.7	4.5 x 10 <sup>5</sup>	1.5
BZT (0.18%) + Farnesol (0.5%)	2.0 x 10 <sup>2</sup>	5.4	6.7 x 10 <sup>1</sup>	5.3

These results demonstrate the synergistic antimicrobial effects of BZT and farnesol, which occur both in the presence or absence of zinc salts.

### 6.3. EXAMPLE 3

The antimicrobial effects of varying proportions of farnesol and quaternary ammonium compounds, incorporated into Base #2, were evaluated using the same method described in Example 1. The results are shown in Table 3.

**Table 3**Test organism: *S. aureus*

Groups in Gel Base (% w/w )	cfu/ml	log <sub>10</sub> reduction
Control Gel	1.4 x 10 <sup>7</sup>	0.0
BZT (0.18%) (preservative level)	3.3 x 10 <sup>5</sup>	1.6
BZT (0.12%)	4.7 x 10 <sup>5</sup>	1.5
BZT (0.06%)	5.7 x 10 <sup>5</sup>	1.4
BZK (0.12%) (preservative level)	5.4 x 10 <sup>5</sup>	1.4
BZK (0.06%)	1.8 x 10 <sup>6</sup>	0.9
Farnesol (0.5%)	4.5 x 10 <sup>5</sup>	1.5
Farnesol (0.3%)	4.6 x 10 <sup>5</sup>	1.5
BZT (0.18%) + Farnesol (0.5%)	6.7 x 10 <sup>1</sup>	5.3
BZT (0.18%) + Farnesol (0.3%)	6.7 x 10 <sup>1</sup>	5.3

BZT (0.12%) + Farnesol (0.3%)	$1.0 \times 10^2$	5.1
BZT (0.06%) + Farnesol (0.3%)	$1.0 \times 10^2$	5.1
BZK (0.12%) + Farnesol (0.5%)	$2.0 \times 10^2$	4.8
BZK (0.12%) + Farnesol (0.3%)	$3.7 \times 10^3$	3.6
BZK (0.06%) + Farnesol (0.3%)	$7.9 \times 10^3$	3.2

These results demonstrate that both quaternary ammonium compounds, BZK and BZT, exhibit synergistic antimicrobial activity when used in combination with farnesol on *S. aureus* in a rapid serum-based assay.

#### 6.4. EXAMPLE 4

The antimicrobial effects of various EO and IC, in the presence or absence of quaternary ammonium compounds, BZK and BZT, incorporated into Base #2, were evaluated using the same assay described in Example 1. The amounts of various agents used are presented in Table 4. In addition to *S. aureus*, the rapid assay was also performed with *E. coli*. The results are shown in Table 4.

**Table 4**

Group	<i>S. aureus</i>		<i>E. coli</i>	
	cfu/ml	log <sub>10</sub> reduction	cfu/ml	log <sub>10</sub> reduction
Control (Base)	$1.4 \times 10^7$	0.0	$2.6 \times 10^7$	0.0
BZK	$3.3 \times 10^5$	1.6	$5.8 \times 10^5$	1.6
BZT	$5.3 \times 10^5$	1.4	$4.3 \times 10^5$	1.8
Farnesol	$4.5 \times 10^5$	1.5	$4.3 \times 10^5$	1.8
Patchouli oil	$2.0 \times 10^6$	0.8	$2.0 \times 10^6$	1.1
Basil oil	$1.4 \times 10^6$	1.0	$6.2 \times 10^4$	2.6
Eucalyptus oil	$1.6 \times 10^6$	0.9	$1.9 \times 10^6$	1.1
Thyme oil	$1.3 \times 10^6$	1.0	$4.9 \times 10^5$	1.7
Clove oil	$1.6 \times 10^6$	0.9	$1.4 \times 10^5$	2.3
Geranium oil	$3.8 \times 10^6$	0.6	$8.0 \times 10^4$	2.5
Orange oil	$6.9 \times 10^6$	0.3	$4.9 \times 10^5$	1.7
Mullein oil	$6.1 \times 10^5$	1.4	$6.2 \times 10^5$	1.6
Citronella oil	$4.7 \times 10^5$	1.5	$2.2 \times 10^5$	2.1
Sandalwood oil	$7.4 \times 10^5$	1.3	$4.9 \times 10^5$	1.7
Farnesol + BZK	$2.0 \times 10^2$	4.8	$3.4 \times 10^4$	2.9



Farnesol + BZT	$5.3 \times 10^2$	4.4	$6.8 \times 10^4$	2.6
Patchouli oil + BZT	$6.7 \times 10^1$	5.3	$1.1 \times 10^4$	3.4
Basil oil + BZT	$3.3 \times 10^1$	5.6	$6.7 \times 10^1$	5.6
Eucalyptus oil + BZT	$3.3 \times 10^1$	5.6	$4.2 \times 10^3$	3.8
Thyme oil + BZT	$3.3 \times 10^1$	5.6	$3.3 \times 10^1$	5.9
Clove oil + BZT	$3.3 \times 10^1$	5.6	$2.0 \times 10^2$	5.1
Geranium oil + BZT	$3.3 \times 10^1$	5.6	$3.3 \times 10^1$	5.9
Orange oil + BZT	$3.3 \times 10^3$	3.6	$3.5 \times 10^4$	2.9
Mullein oil + BZT	$2.4 \times 10^5$	1.8	$9.8 \times 10^4$	2.4
Citronella oil + BZT	$6.7 \times 10^2$	5.3	$2.8 \times 10^3$	4.0
Sandalwood oil + BZT	$2.0 \times 10^2$	4.8	$4.7 \times 10^3$	3.7

**Key:** BZK: Benzalkonium chloride (0.12% w/w: preservative level); BZT: Benzethonium chloride (0.18% w/w: preservative level); all other EO/IC (0.5% w/w).

These results demonstrate that various essential oils, when used in combination with quaternary ammonium compounds, exhibit rapid synergistic antimicrobial activity on *S. aureus* in a rapid serum-based assay. In particular, patchouli oil, basil oil, eucalyptus oil, thyme oil, clove oil, geranium oil, and citronella oil show pronounced antimicrobial effects.

### 6.5. EXAMPLE 5

The effect of the addition of various biguanide antimicrobial compounds to combinations of farnesol and quaternary ammonium compound, incorporated into Base #2 was tested using the assay described in Example 1. The results are shown in Table 5.

**Table 5.**

Group	<i>S. aureus</i>		<i>E. coli</i>	
	cfu/ml	log <sub>10</sub> reduction	cfu/ml	log <sub>10</sub> reduction
Gel base	1.4 x 10 <sup>7</sup>	0.0	2.6 x 10 <sup>7</sup>	0.0
CHG (0.05 %)	6.6 x 10 <sup>5</sup>	1.3	4.8 x 10 <sup>5</sup>	1.7
BZT (0.18%)	5.3 x 10 <sup>5</sup>	1.4	4.3 x 10 <sup>5</sup>	1.8
PHMB (0.15%)	4.3 x 10 <sup>4</sup>	2.9	6.0 x 10 <sup>5</sup>	1.6
Farnesol (0.5%)	4.5 x 10 <sup>5</sup>	1.5	4.3 x 10 <sup>5</sup>	1.8
Farnesol (0.5%) + CHG (0.05%)	6.8 x 10 <sup>4</sup>	2.3	7.2 x 10 <sup>4</sup>	2.5
Farnesol (0.5%) + PHMB (0.15%)	2.7 x 10 <sup>3</sup>	4.1	6.5 x 10 <sup>2</sup>	4.6
Farnesol (0.5%) + BZT (0.18%)	5.3 x 10 <sup>2</sup>	4.4	6.8 x 10 <sup>4</sup>	2.6
Farnesol (0.5%) + BZT (0.18%) + CHG (0.05%)	5.0 x 10 <sup>2</sup>	4.5	2.0 x 10 <sup>2</sup>	5.1
Farnesol (0.5%) + BZT (0.18%) + PHMB (0.15%)	3.0 x 10 <sup>2</sup>	5.0	1.0 x 10 <sup>2</sup>	5.4

PHMB appears to enhance the activity of the combination of BZT and farnesol.

#### 6.6. EXAMPLE 6

This example demonstrates the antimicrobial activity of gels comprising farnesol in combination with various antimicrobial agents. The concentrations of the antimicrobial agents were selected in keeping with the permissible levels of these ingredients in leave-on skin care formulations. The antimicrobial activity was assayed using the method described in Example 1 using Gel Base #1. The data has been presented in Table 6.

**Table 6**Test organism: *S. aureus*

Ingredients in the Gel (% w/w)	Recommended range as preservatives (%w/w)	log <sub>10</sub> reduction in colony count from the control <sup>a,b</sup>
Farnesol (0.3%)	-	0.6
CHG (0.05 %)	0.0-0.05	1.7
PHMB (0.15%)	0.1-0.3	2.9
TC (0.3%)	0.3-0.5	1.3
BZT (0.18%)	0.1-0.2	1.6
BZK (0.12%)	0.1-0.12	1.8
Farnesol (0.3%) + CHG (0.05%)	-	2.7
Farnesol (0.3%) + PHMB (0.15%)	-	3.4
Farnesol (0.3%) + TC (0.3%)	-	1.4
Farnesol (0.3%) + BZT (0.18%)	-	5.3
Farnesol (0.3%) + BZK (0.12%)	-	5.0

(a) The colony count of the Control Base experiment was  $3.4 \times 10^7$  cfu/ml.

(b) For Control, the same Gel Base as used for the Gels shown in Table 6, but without any antimicrobial agents.

The results show that farnesol's ability to enhance the activity of the antimicrobial agents ranges from compound to compound. Farnesol shows synergy with the quaternary ammonium compounds, benzalkonium chloride (BZT) and benzethonium chloride (BZK). Among biguanide antimicrobial compounds, it enhances the activity of polyhexamethylene biguanide hydrochloride (PHMB), but not that of chlorhexidine gluconate (CHG). Farnesol also does not show any synergy with triclosan, nor does it enhance triclosan's action. Therefore, the ability of farnesol to show synergy or enhance the action of biocides/preservatives is specific to the antimicrobial agents in question.

A similar study was performed using the same concentration of farnesol above in Table 6 (0.3% w/w, *i.e.* 1.35 mM per 100 g gel) and the various antimicrobial agents were added to the gel in the same molar concentration (0.06 mM per 100 g of gel). The data has been presented in Table 7 below.



**Table 7.**Test organism: *S. aureus*

Ingredients in the Gel (gram millimoles per 100 g Gel)	log <sub>10</sub> reduction in colony count from the control <sup>a,b</sup>
Farnesol (1.35 mM)	1.8
CHG (0.06 mM)	1.9
PHMB (0.06 mM)	2.1
TC (1.04mM) <sup>c</sup>	1.3
BZT (0.06 mM)	1.9
Farnesol (1.35 mM) + CHG (0.06 mM)	2.0
Farnesol (1.35 mM) + PHMB (0.06 mM)	3.2
Farnesol (1.35 mM) + TC (1.04 mM) <sup>c</sup>	1.4
Farnesol (1.35 mM) + BZT (0.06 mM)	5.5

(a) The colony count of the Control Base experiment was  $6.4 \times 10^7$  cfu/ml.

(b) For Control, the same Gel Base as used for the Gels shown in Table 7 but without any preservatives/biocides was used.

(c) Since Triclosan is not effective even at 1.04 mM concentration, experiment was not performed with 0.06 mM concentration.

Similar to the results shown in Table 6, farnesol has a varying effect on enhancement of the antimicrobial activity of the various antimicrobial agents. Farnesol appears to exhibit synergistic antimicrobial activity in combination with BZT, but not with triclosan. PHMB appears to enhance the activity of farnesol. The mechanism of farnesol to show synergy or enhance the activity of the antimicrobial agents varies for different compounds, but is the same for the same compound, irrespective of the concentration (whether the compound is taken in % w/w or in molar proportions).

CLAIMS

1. An antimicrobial composition for topical use, comprising between 0.01 and 0.5% (w/w) of a quaternary ammonium compound, between 0.1 and 2.0% (w/w) of a polyhexamethyl biguanide, two or more zinc salts, and an agent which is an essential oil or an individual constituent thereof, wherein the quaternary ammonium compound and the agent are present in amounts which exhibit synergistic antimicrobial activity and wherein the agent is present at a concentration of between 0.05 and 1.0% (w/w).
2. The composition of claim 1, wherein the quaternary ammonium compound is benzalkonium chloride or benzethonium chloride.
3. The composition of claim 1 or 2, wherein the agent is an essential oil which is bergamot oil, clary sage oil, ylang-ylang oil, neroli oil, sandalwood oil, frankincense oil, ginger oil, peppermint oil, lavender oil, jasmine absolute, geranium oil bourbon, spearmint oil, clove oil, patchouli oil, rosemary oil, rosewood oil, sandalwood oil, tea tree oil, vanilla oil, lemongrass oil, cedarwood oil, a balsam oil, tangerine oil, Hinoki oil, Hiba oil, ginko oil, eucalyptus oil, lemon oil, orange oil, or sweet orange oil.
4. The composition of claim 1 or 2, wherein the agent is an individual constituent of an essential oil, the individual constituent being 1-citronellol,  $\alpha$ -amylcinnamaldehyde, lylal, geraniol, farnesol, hydroxycitronellal, isoeugenol, eugenol, eucalyptol, linalool, or citral.
5. The composition of any one of claims 1 to 4, wherein the two or more zinc salts are present in an amount that reduces irritation, and further wherein the two or more zinc salts are each present at a concentration of between 0.1 and 0.5% (w/w).
6. The composition of any one of claims 1 to 5, wherein the two or more zinc salts are zinc acetate, zinc citrate, zinc gluconate, zinc glycerate, zinc glycolate, zinc formate, zinc lactate, zinc picolinate, zinc proprionate, zinc salicylate, zinc tartrate, zinc undecylenate, zinc oxide, or zinc stearate.
7. The composition of any one of claims 1 to 6, further comprising panthenol present at a concentration of between 0.5 and 2% (w/w).

8. The composition of any one of claims 1 to 7, further comprising alcohol present at a concentration of between 10 and 90% (w/w), water present at a concentration of between 15 and 70% (w/w), thickeners and/or gelling agents present at a concentration of between 0.05 and 3.0% (w/w), and emollients present at a concentration of between 0.1 and 3.0% (w/w).

9. Use of a composition as defined in any one of claims 1 to 8 in the manufacture of a medicament for producing an antimicrobial effect on an area of skin of a subject.

10. Use of a composition as defined in any one of claims 1 to 8 in the manufacture of a medicament for producing an antimicrobial effect on an area of a mucous membrane of a subject.

11. Use of a composition as defined in any one of claims 1 to 8 for producing an antimicrobial effect on an area of skin of a subject.

12. Use of a composition as defined in any one of claims 1 to 8 for producing an antimicrobial effect on an area of a mucous membrane of the subject.