

ANTIMICROBIAL ESSENTIAL OIL COMPOSITION, PRODUCT COMPRISING SAME, AND PREPARATION METHOD FOR PRODUCT THEREOF

Cross-Reference to Related Applications

[0001] This application claims the priority of the Chinese Invention Patent
5 Application No. 202110482092.1, entitled “Antimicrobial Essential Oil Composition,
Products Comprising The Same And Methods For Preparing The Same” filed on April
30, 2021, and the Chinese Invention Patent Application No. 202210059971.8 filed on
January 19, 2022, entitled “Antimicrobial Essential Oil Composition, Products
Comprising The Same And Methods For Preparing The Same”, both of which are
10 incorporated herein by reference in their entirety.

Technical Field

[0002] The present application relates generally to essential oil compositions, in
particular, to plant essential oil compositions with antimicrobial properties. The present
application also relates to products comprising the antimicrobial essential oil
15 compositions, as well as methods of preparing products comprising the antimicrobial
essential oil compositions.

Background

[0003] Many highly pathogenic microbial pathogens cause rapid onset and spread of
infectious diseases. Such infectious diseases can even spread across several provinces,
20 several countries, or even the whole world in a short period of time, that is, they can
rapidly develop into “pandemic”. For example, the coronavirus disease 2019 (COVID-
19, also called “novel coronavirus viral pneumonia”) outbreak began at the end of 2019
has been identified by WHO to meet the characteristics of “pandemic.”

[0004] During the pandemic, disinfection is an important task of epidemic prevention
25 and control. At present, synthetic chemical-based disinfectant products (e.g., ethanol,
peracetic acid, chlorine-containing disinfectants, phenolic disinfectants or the like),
ultraviolet light, heat, etc. are commonly used worldwide to inactivate pathogenic
microorganisms. For example, phenolic disinfectants were once one of the main

disinfectants in hospitals and played an important role in the prevention and control of the spreading of infectious diseases. At high concentrations, phenols can lyse and penetrate cell walls, and make bacterial proteins agglutinate and precipitate, thereby killing cells quickly; and at low concentrations, they can inactivate bacterial enzyme systems, thereby resulting in cells death. However, such disinfection measures have risks of inconvenient application, limited use, or easiness to cause secondary harmful pollution to human bodies and environment.

[0005] Natural plant-based essential oil products have traditionally been used in the treatment of infectious diseases and the epidemic prevention and control for thousands of years. In particular, phenolic essential oils have excellent antibacterial effects. However, this type of essential oils need to be used with caution in practical use due to their toxicity and irritation to skin and mucosal tissues. Some other plant essential oils have also been confirmed to have antimicrobial activity by many studies. Such plant essential oil products are harmless to human bodies, friendly to the environment, and in line with the trend of environmental friendliness and sustainable development.

[0006] Therefore, there is a need to provide plant essential oil products with antimicrobial activity.

[0007] Surprisingly, it is found that by using a combination of pure essential oils in cooperation with specific active antimicrobial ingredients, disinfectant products which have good antimicrobial properties and are not harmless to human bodies and friendly to environment can be obtained.

Summary of the Invention

[0008] In order to solve the above problems, embodiments of the present invention provide an antimicrobial essential oil composition, wherein the components of the essential oil composition are derived from natural plants, and the combination of these components enables the resultant essential oil composition to have good antimicrobial properties.

[0009] At least an embodiment of the present invention provides an antimicrobial essential oil composition comprising: (1) 0.0001% to 80% of one or more selected from

the group consisting of carvacrol and thymol; (2) 0.0001% to 99.9% of one or more selected from the group consisting of lemon oil, sweet orange oil, Citrus aurantium 'Daidai' peel oil, white lemon oil, and pomelo peel oil; (3) 0.0001% to 99.9% of one or more selected from the group consisting of Asian dementholized peppermint oil, Asian peppermint oil, Mentha Piperita Oil and pennyroyal oil; (4) 0.0001% to 20% of one or more selected from the group consisting of bay leaf oil and marjoram oil; (5) 0.0001% to 20% of one or more selected from the group consisting of patchouli oil, sandalwood oil and rosemary oil; (6) 0.0001% to 20% of one or more selected from the group consisting of geranium oil, pine needle oil and cedarwood oil; and (7) optionally, 0.0001% to 20% of one or more selected from the group consisting of perilla oil, Chrysanthemum morifolium Ramat oil, and Vitex cannabifolia leaf oil, based on the total weight of the composition.

[0010] In an aspect, the antimicrobial essential oil composition comprises: (1) 2% to 80% of one or more selected from the group consisting of carvacrol and thymol; (2) 2% to 50% of one or more selected from the group consisting of the group consisting of lemon oil, sweet orange oil, Citrus aurantium 'Daidai' peel oil, white lemon oil and pomelo peel oil; (3) 5% to 40% of one or more selected from the group consisting of Asian dementholized peppermint oil, Asian peppermint oil, Mentha Piperita Oil and pennyroyal oil; (4) 2% to 20% of one or more selected from the group consisting of bay leaf oil and marjoram oil; (5) 0.1% to 5% of one or more selected from the group consisting of patchouli oil, sandalwood oil and rosemary oil; (6) 2% to 20% of one or more selected from the group consisting of geranium oil, pine needle oil and cedarwood oil; and (7) optionally, 2% to 15% of one or more selected from the group consisting of perilla oil, Chrysanthemum morifolium Ramat oil and Vitex cannabifolia leaf oil, based on the total weight of the composition.

[0011] In another aspect, the antimicrobial essential oil composition further optionally comprises: (8) 0.0001% to 20% of honeysuckle oil; (9) 0.0001% to 20% of Chuanxiong Rhizome Oil; (10) 0.0001% to 20% of Siberian cocklebur achenia oil; and (11) 0.0001% to 20% of forsythia oil, based on the total weight of the composition.

[0012] In another aspect, the antimicrobial essential oil composition comprises: (1) 5%

of carvacrol and 5% of thymol; (2) 35% of lemon oil; (3) 30% of Asian dementholized peppermint oil; (4) 10% of bay leaf oil; (5) 0.5% of patchouli oil; (6) 5% of geranium oil; and (7) 9.5% of perilla oil, based on the total weight of the composition.

[0013] In another aspect, the antimicrobial essential oil composition comprises: (1)
5 0.005% of carvacrol and 0.005% of thymol; (2) 0.1% of lemon oil; (3) 99.58% of Asian dementholized peppermint oil; (4) 0.005% of bay leaf oil; (5) 0.25% of patchouli oil; (6) 0.005% of geranium oil; and (7) 0.05% of perilla oil, based on the total weight of the composition.

[0014] In another aspect, the antimicrobial essential oil composition comprises: (1)
10 0.005% of carvacrol and 0.005% of thymol; (2) 99.5% of lemon oil; (3) 0.25% of Asian dementholized peppermint oil; (4) 0.005% of bay leaf oil; (5) 0.18% of patchouli oil; (6) 0.005% of geranium oil; and (7) 0.05% of perilla oil, based on the total weight of the composition.

[0015] In another aspect, the antimicrobial essential oil composition comprises: (1)
15 51.25% of carvacrol; (2) 26.25% of thymol; (3) 8.75% of lemon oil; (4) 7.5% of Asian dementholized peppermint oil; (5) 2.5% of bay leaf oil; (6) 0.125% of patchouli oil; (7) 1.25% of geranium oil; and (8) 2.375% of perilla oil, based on the total weight of the composition.

[0016] In another aspect, the antimicrobial essential oil composition comprises: (1)
20 25% of carvacrol and 25% of thymol; (2) 2% of lemon oil; (3) 10% of Asian dementholized peppermint oil; (4) 20% of bay leaf oil; (5) 0.5% of patchouli oil; (6) 2% of geranium oil; (7) 5% of honeysuckle oil; (9) 4% of Chuanxiong Rhizome Oil; (10) 0.3% of Siberian cocklebur achenia oil; and (11) 0.2% of forsythia oil, based on the total weight of the composition.

25 [0017] In another aspect, the microorganism is selected from the group consisting of bacteria, fungi, viruses, mycoplasmas and combinations thereof.

[0018] In another aspect, the microorganism is one or more of novel coronavirus, influenza virus, rhinovirus, respiratory syncytial virus, enterovirus, adenovirus, herpes virus, Cocksackie virus, and poliovirus.

30 [0019] In another aspect, the microorganism is novel coronavirus.

[0020] In another aspect, the microorganism is one or more of *Diplococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus catarrhalis*, *Escherichia coli*, *Bacillus dysenteriae*, *Staphylococcus albus* and *Candida albicans*.

5 [0021] In another aspect, the microorganism is *Mycoplasma pneumoniae*.

[0022] At least an embodiment of the present invention further provides a product comprising an antimicrobial essential oil composition.

[0023] In one respect, the product comprises those capable of reducing or killing microorganisms on one or more of hard surfaces, textiles, skin, mucosa or hair.

10 [0024] In another aspect, the product is one or more selected from the group consisting of brittle capsules for mask, sanitizing wipes, air fresheners, oral sprays, liquid soaps for hand, shampoos, conditioners, body washes, cosmetics, skin care products, aerogels, antibacterial sprays, surface cleaners and detergents.

[0025] In another aspect, the product is a protective product.

15 [0026] In another aspect, the product is one or more selected from the group consisting of civilian masks, medical masks, protective gloves, and protective clothing.

[0027] In another aspect, the product is a brittle capsule-containing mask.

[0028] In another aspect, the product is food or health product.

20 [0029] In another aspect, the product is one or more selected from the group consisting of chewing gums, oral refreshers, brittle capsules, jelly candies, hard candies, beverages, and dietary supplements.

[0030] At least an embodiment of the present invention further provides a method of preparing a product comprising an antimicrobial essential oil composition, comprising: uniformly mixing all the components of the antimicrobial essential oil composition to
25 form essential oil masterbatch or microcapsule or brittle capsule, and combining the essential oil masterbatch or microcapsule or brittle capsule with other components of the product to give the product.

[0031] In one respect, the product is a civilian mask or medical mask, and the combining the essential oil masterbatch or microcapsule or brittle capsule with other
30 components of the product comprises adding the essential oil masterbatch or

microcapsule or brittle capsule into the civilian mask or medical mask.

Brief Description of Drawings

[0032] To better illustrate the technical solutions of the embodiments of the invention, the accompanying drawings of the embodiments are briefly introduced below. Clearly,
5 the accompanying drawings in the following description are not intended to limit the disclosure, but directed to only some embodiments of the disclosure.

[0033] FIG. 1 shows a comparison of inhibition rates of various compounded essential oils against H1N1, H3N, FluB, hRHV and RSV in accordance with at least an embodiment of the invention;

10 [0034] FIG. 2 shows minimum inhibitory concentrations of various compounded essential oils against Gram-positive bacteria in accordance with at least an embodiment of the invention; and

[0035] FIG. 3 shows minimum inhibitory concentrations of various compounded essential oils against Gram-negative bacteria in accordance with at least an embodiment
15 of the invention.

Detailed Description of the Invention

[0036] For the purpose of the detailed description below, it should be understood that various alternative variations and step orders may be adopted in the invention, unless expressly specified to the contrary. Except those in any operation example or otherwise
20 expressly stated, all values representing quantities of ingredients, etc. used in the specification and claims are to be construed as variable in all cases in accordance with the term "about". Therefore, unless stated to the contrary, the numerical parameters listed in the following specification and claims are approximations and can vary in accordance with the desired properties of the present invention. At the very least, not
25 to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be interpreted in terms of at least a significant number and by applying ordinary rounding techniques.

[0037] Notwithstanding that the numerical ranges and parameters representing the wide range of the present invention are approximations, the numerical values listed in

specific examples are recorded as precisely as possible. Any numerical value, however, inherently possesses certain errors, which is the inevitable result of the standard deviation obtained in their respective measurement methods.

[0038] In the present invention, any numerical range recited herein is intended to
5 comprise all subranges subsumed therein. For example, a range of “1 to 10” is intended to comprise all subranges between the recited minimum value of 1 and the recited maximum value of 10 (inclusive), i.e., all subranges having a minimum value equal to or greater than 1 and a maximum value equal to or less than 10.

[0039] In the present invention, unless expressly stated otherwise, the singular
10 includes the plural and the plural includes the singular in use. In the present invention, unless expressly stated otherwise, “or” is used to represent “and/or”, even though “and/or” can be explicitly used in some cases. Furthermore, in the present invention, the use of “one (a)” or “one (an)” means “at least one” unless expressly stated otherwise. For example, “a” polymer, “a” composition, etc., means any one or more of these
15 substances.

[0040] An embodiment of the present invention provides an antimicrobial essential oil composition. As used herein, the term “antimicrobial” means that the product as used has an activity of reducing/killing microorganisms. In the context of the invention, the “microorganism” can comprise bacteria, fungi, viruses, mycoplasmas and the like,
20 such as, but not limited to, coronaviruses (e.g. SARS-CoV (Severe Acute Respiratory Syndrome virus), MERS-CoV (Middle East Respiratory Syndrome virus), SARS-CoV-2 (Novel coronavirus), etc.), hepatitis viruses (e.g., hepatitis A, B, C viruses), influenza viruses (e.g., influenza A viruses, influenza B viruses, and avian influenza viruses), *Staphylococcus aureus*, *Bacillus pneumoniae*, *Salmonella typhi*, *Candida albicans*,
25 Mould, *Mycoplasma pneumoniae*, etc. In a preferred aspect, the viruses in the context of the invention can be novel coronaviruses.

[0041] As used herein, the term “plant essential oil” refers to a general term for volatile aromatic substances obtained from the processing and extraction of natural aromatic plants. Generally, plant essential oils are volatile aromatic substances
30 extracted from flowers, leaves, roots, seeds, fruits, barks, resins, heartwoods and other

parts of plants by means of steam distillation, cold pressing, enfleurage or solvent extraction. Plant essential oils tend to have specific biological activities; especially, some plant essential oils have good antimicrobial activities, such as, antibacterial, antifungal and/or antiviral activities, and can be used as effective environmentally friendly antimicrobials. Plant essential oils can be classified into pure essential oils and compounded essential oils. Among them, the compounded essential oils comprise a variety of effective essential oil components as compared with pure essential oils, and can achieve significantly better biological activities, such as antimicrobial activities, through the synergistic effect of various components. In the present applicaiton, the compounded essential oils are preferred.

[0042] At least an embodiment of the invention provides an antimicrobial essential oil composition, comprising:

(1) one ore more selected from the group consisting of carvacrol and thymol in amount of 0.0001% to 99.99%, preferably 2% to 80%, more preferably 2% to 60%, further preferably 6-16%;

(2) one ore more selected from the group consisting of lemon oil, sweet orange oil, Citrus aurantium 'Daidai' peel oil, white lemon oil and pomelo peel oil in amount of 0.0001% to 99.99%, preferably 2% to 80%, more preferably 2% to 50%, more preferably 10-50%, further preferably 32% to 38%;

(3) one ore more selected from the group consisting of Asian dementholized peppermint oil, Asian peppermint oil, Mentha Piperita Oil and pennyroyal oil in amount of 0.0001% to 99.99%, preferably 2% to 80%, more preferably 5% to 40%, further preferably 28-35%;

(4) one ore more selected from the group consisting of bay leaf oil and marjoram oil in amount of 0.0001% to 20%, preferably 2% to 20%, more preferably 2% to 18%, more preferably 2% to 15%, further preferably 8-12%;

(5) one ore more selected from the group consisting of patchouli oil, sandalwood oil and rosemary oil in amount of 0.0001% to 20%, preferably 0.1% to 18%, more preferably 0.1% to 5%, further preferably 0.3-1%;

(6) one ore more selected from the group consisting of geranium oil, pine needle oil

and cedarwood oil in amount of 0.0001% to 20%, preferably 2% to 20%, more preferably 2% to 18%, further preferably 3-8%;
based on the total weight of the composition.

[0043] In an aspect, the antimicrobial essential oil composition can further optionally
5 comprise: (7) one ore more selected from the group consisting of perilla oil, Chrysanthemum morifolium Ramat oil and Vitex cannabifolia leaf oil in amount of 0.0001% to 20%, preferably 2% to 18%, more preferably 2% to 15%, further preferably 8-12%, wherein the above percentage is based on the total weight of the composition.

[0044] In another aspect, the antimicrobial essential oil composition can further
10 optionally comprise: (8) 0.0001% to 20% of honeysuckle oil; (9) 0.0001% to 20% of Chuanxiong Rhizome Oil; (10) 0.0001% to 20% of Siberian cocklebur achenia oil; (11) 0.0001% to 20% of forsythia oil, based on the total weight of the composition.

[0045] For example, without limitation, a preferred example of the antimicrobial essential oil composition of the invention can comprise: (1) 5% of carvacrol and 5% of
15 thymol; (2) 35% of lemon oil; (3) 30% of Asian dementholized peppermint oil; (4) 10% of bay leaf oil; (5) 0.5% of patchouli oil; (6) 5% of geranium oil; and (7) 9.5% of perilla oil, based on the total weight of the composition.

[0046] For example, without limitation, another preferred example of the antimicrobial essential oil composition of the invention can comprise: (1) 0.005% of
20 carvacrol and 0.005% of thymol; (2) 0.1% of lemon oil; (3) 99.58% of Asian dementholized peppermint oil; (4) 0.005% of bay leaf oil; (5) 0.25% of patchouli oil; (6) 0.005% of geranium oil; and (7) 0.05% of perilla oil, based on the total weight of the composition.

[0047] For example, without limitation, still another preferred example of the antimicrobial essential oil composition of the invention can compose: (1) 0.005% of
25 carvacrol and 0.005% of thymol; (2) 99.5% of lemon oil; (3) 0.25% of Asian dementholized peppermint oil; (4) 0.005% of bay leaf oil; (5) 0.18% of patchouli oil; (6) 0.005% of geranium oil; and (7) 0.05% of perilla oil, based on the total weight of the composition.

30 [0048] For example, without limitation, still another example of the antimicrobial

essential oil composition of the invention can comprise: (1) 51.25% of carvacrol; (2) 26.25% of thymol; (3) 8.75% of lemon oil; (4) 7.5% of Asian dementholized peppermint oil; (5) 2.5% of bay leaf oil; (6) 0.125% of patchouli oil; (7) 1.25% of geranium oil; and (8) 2.375% of perilla oil, based on the total weight of the composition.

5 [0049] For example, without limitation, still another example of the antimicrobial essential oil composition of the invention can comprise: (1) 25% of carvacrol and 25% of thymol; (2) 2% of lemon oil; (3) 10% of Asian dementholized peppermint oil; (4) 20% of bay leaf oil; (5) 0.5% of patchouli oil; (6) 2% of geranium oil; (7) 5% of honeysuckle oil; (9) 4% of Chuanxiong Rhizome Oil; (10) 0.3% of Siberian cocklebur
10 achenia oil; and (11) 0.2% of forsythia oil.

[0050] The components of the antimicrobial essential oil composition as described in the invention are combined to achieve excellent antimicrobial properties by synergistic effect. For example, the antimicrobial essential oil composition as described in the invention can exhibit good killing or inhibitory effect against microorganisms selected
15 from the group consisting of bacteria, fungi, viruses, mycoplasmas, or combinations thereof. In one respect, examples of bacteria as described in the invention can comprise *Diplococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus catarrhalis*, *Escherichia coli*, *Bacillus dysenteriae*, *Staphylococcus albicans*, and *Candida albicans*. In another aspect, examples of viruses as described in
20 the invention can comprise one or more of novel coronavirus, influenza virus, rhinovirus, respiratory syncytial virus, enterovirus, adenovirus, herpes virus, Coxsackie virus, and poliovirus, e.g., novel coronavirus. In another aspect, the mycoplasmas as described in the invention can be *Mycoplasma pneumoniae*.

[0051] For example, when the essential oil compositions of the invention are used for
25 the *in vitro* disinfection of novel coronaviruses, the killing efficiencies can be greater than 75% after treatment for 3 min or 30 min, e.g., greater than 80%, 85%, 90%, 95%, 98% or even close to 100%. Without wishing to be bound by any theory, it is believed that the mechanism of the essential oil composition killing novel coronavirus can be based, at least in part, on the fact that novel coronaviruses have coating structure and
30 belong to lipophilic viruses, which are theoretically easy to be adsorbed and enriched

by natural plant essential oils, and then inactivated. In accordance with the applicant's research, it is found that the antiviral plant essential oils of the invention can "extract" novel coronavirus from the original solution of novel coronaviruses after contacting and mixing with the original solution, thereby inactivating the novel coronaviruses quickly and efficiently. There are complementation and addition of activity between essential oil components as selected in the invention, which achieve unexpected synergistic effect as compared with pure essential oils, and obtains significantly better antimicrobial properties. In addition, the essential oil compositions of the invention can further achieve other beneficial effects, including, without limitation, some components of the composition can block novel coronavirus from binding to the ACE2 receptor of respiratory epithelial cells (i.e., achieving blocking/protective effects); some components can be make breathing freely and improve nasal congestimany on discomfort caused by early viral upper respiratory tract infection or cold (i.e., smoothing effect). Moreover, the essential oil compositions of the invention are made from natural plant materials, smell fragrant, and have advantages like low irritation, low corrosion, non-toxicity and non-harm to human bodies, and environmental friendliness.

[0052] At least an embodiment of the present invention further provides a product comprising the antimicrobial essential oil composition. The product can comprise a product capable of disinfecting environment or human bodies for microorganisms. In an aspect, a product capable of disinfecting environment or human bodies for microorganisms in accordance with the present invention refers to those which are non-toxic, non-irritating, non-corrosive to human bodies, environmentally friendly and capable of reducing or killing harmful microorganisms in the environment or on human bodies. It can comprise, e.g., those capable of reducing or killing microorganisms on one or more of hard surfaces, textiles, skin, mucosa or hair. In the context of the invention, the term "hard surface" refers to an object surface in the environment in addition to textile surfaces and human body surfaces, including, without limitation, common surfaces of ceilings, walls, floors/tiles, elevators, stairs, handrails, furniture, tableware, vehicles and similar surfaces that are easily accessible in life; the term

"textile" comprises, without limitation, clothes, caps, towels, tablecloths, beddings, etc.; the term "skin" refers to human or animal skins; the term "mucosa" refers to oral, ocular, or rectal mucosa of human or animal; and the term "hair" refers to human hairs or body hairs, or animal hairs or feathers. In some examples, a product capable of reducing or killing microorganisms on one or more of hard surfaces, textiles, skin, mucosa, or hair can comprise one or more selected from the group consisting of brittle capsules for mask, sanitizing wipes, air fresheners (e.g., aerogel or spray, etc.), oral sprays, personal toilet articles (e.g., liquid soaps for hand, shampoos, conditioners, body washes, etc.), cosmetics, skin care products, aerogels, antibacterial sprays, surface cleaners (e.g., furniture cleaners, floor cleaners, kitchen cleaners, toilet cleaners, etc.) and detergents (e.g., clothing detergents, dishwashing detergents, etc.).

[0053] Alternatively, or optionally, the product of the invention can further comprise protective products. For example, the protective products can comprise one or more selected from the group consisting of daily clothings (e.g., underwears, outerwears or socks, etc.), masks (e.g., civilian masks, medical masks, etc.), protective gloves, and protective clothings, such as, brittle capsule-containing mask.

[0054] Alternatively, or optionally, the product of the invention can further comprise food or health products. For example, the food or health products can comprise one or more of chewing gum, oral refreshers, brittle capsules, jelly candies, hard candies, gummies, beverages, and dietary supplements.

[0055] At least an embodiment of the present invention further provides a method of preparing the antimicrobial essential oil composition as described above, comprising uniformly mixing all the components of the antimicrobial essential oil composition. In one respect, the mixing is carried out at room temperature and atmospheric pressure.

[0056] At least an embodiment of the present invention further provides a method of preparing a product comprising an antimicrobial essential oil composition, comprising uniformly mixing all the components of the antimicrobial essential oil composition to form an essential oil masterbatch or microcapsule or brittle capsule, and combining the essential oil masterbatch or microcapsule or brittle capsule with other components of the product to obtain the product. For example, the preparation method can comprise

uniformly mixing all the components of the antimicrobial essential oil composition to make an essential oil masterbatch or microcapsule or brittle capsule; and adding the resultant essential oil masterbatch or microcapsule or brittle capsule to a mask (e.g., civilian mask or medical mask). In use, the essential oil masterbatch or microcapsule or brittle capsule in the resultant mask (after being pressed to break) will slowly and continuously release the antimicrobial essential oil composition, thereby producing a disinfecting effect on the pathogenic microorganisms possibly existing in the gases (e.g., air) inhaled or expelled through the mask.

[0057] Hereinafter the present application is further explained in detail with reference to the examples below. However, those skilled in the art should understand that these examples are provided only for illustrative purposes and are not intended to limit the invention.

Examples

[0058] Hereinafter the embodiments of the present invention will be described in detail by reference to examples, but it should be understood for those skilled in the art that the following examples are used only to illustrate the present invention and should not be regarded as limiting the scope of the present invention. The examples in which specific conditions are not clearly indicated shall be carried out in accordance with common conditions or those recommended by the manufacturer. The reagents or instruments as used in which the manufacturer is not clearly indicated are common commercially available products. Unless otherwise indicated, all quantities as listed are described in parts by weight based on the total weight. The invention shall not be construed as being limited to the specific examples described.

Preparative Examples 1-13

[0059] According to the formulations in Table 1-2 below, various components were mixed at room temperature and atmospheric pressure to prepare the essential oil compositions of Examples 1-13, wherein Examples 1-7 involve the essential oil compositions in accordance with the invention, and Examples 8-13 involve control essential oil compositions.

Table 1.

	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Ex. 5	Ex. 6	Ex. 7
Carvacrol	5	51.25	25	20	25	0.05	67
Thymol	5	26.25	25	20	25	0.0001	0.0001
Bay Leaf Oil	10	2.5	20	10	20	0.0001	0.0001
Lemon Oil	35	8.75	2	5	5	0.65	0.21
Geranium Oil	5	1.25	2	5	5	0.05	0.016
Perilla Oil	9.5	2.4	-	1	3	5	1.65
Asian Dementholized Peppermint Oil	30	7.5	0.5	5	5	94	31
Patchouli Oil	0.5	0.125	10	20	0.5	0.25	0.08
Honeysuckle Oil	-	-	5	10	0.2	-	-
Chuanxiong Rhizome Oil	-	-	4	1	2	-	-
Siberian Cocklebur Achenia Oil	-	-	0.3	1	0.3	-	-
Forsythia Oil	-	-	0.2	2	2	-	-
Mentha Piperita Oil	-	-	-	-	5	-	-
Sandalwood Oil	-	-	-	-	1	-	-
Vitex Cannabifolia Leaf Oil	-	-	-	-	0.5	-	-
Chrysanthemum Morifolium Ramat Oil	-	-	-	-	0.5	-	-

[Note]:

1. The numbers in the table above represent percentages based on the total weight of the composition.
2. “-” indicates that it is free of the component .

Table 2.

	Ex.8	Ex.9	Ex.10	Ex.11	Ex.12	Ex.13
Carvacrol	20	50	-	20	5	-
Thymol	-	50	-	20	-	20
Bay Leaf Oil	-	-	20	20	-	-
Lemon Oil	-	-	70	20	30	-
Geranium Oil	-	-	10	20	-	-
Perilla Oil	-	-	-	-	15	30
Asian Dementholized Peppermint Oil	30	-	-	-	50	10
Patchouli Oil	-	-	-	-	-	-
Honeysuckle Oil	20	-	-	-	-	20
Chuanxiong Rhizome Oil	5	-	-	-	-	-
Siberian Cocklebur Achenia Oil	5	-	-	-	-	-
Forsythia Oil	10	-	-	-	-	20
Mentha Piperita Oil	-	-	-	-	-	-
Sandalwood Oil	10	-	-	-	-	-
Vitex Cannabifolia Leaf Oil	-	-	-	-	-	-
Chrysanthemum Morifolium Ramat Oil	5	-	-	-	-	-

[Note]:

1. The numbers in the table above represent percentages based on the total weight of the composition.
2. “-” indicates that it is free of the component .

5 Test Example 1

[0060] The essential oil composition of Example 1 (labeled as food-grade essential oil (FEO)) and the essential oil composition of Example 3 (labeled as cosmetic-grade essential oil (CEO)) were prepared, respectively.

[0061] According to the following test methods, the FEO and the CEO were tested for their cytotoxicity and killing effects on novel coronavirus (SARS-Cov-2).

Assay Equipments
☒ Biosafety cabinet (Manufacturer: NUAIRE LABGARD; Model: NU-437-600S) ☒ CO ₂ incubator (Manufacturer: Thermo Scientific; Model: 471) ☒ Inverted microscope (Manufacturer: EVOS; Model: XLCore)
Assay Reagents
☒ SARS-CoV-2 viruses (Vero E6 cell proliferation, passage generation: P7; titer: 4.0×10^7 PFU/mL) ☒ DMEM Basal Medium (brand: Gibco; item number: 11965092; batch number: 2122728) ☒ Vero E6 cells (source: Cell Bank, Chinese Academy of Sciences; passage generation: 8) ☒ Fetal bovine serum (brand: Gibco; item number: 16140071; batch number: 1624166) ☒ Penicillin-Streptomycin Liquid (brand: Bioind; item number: 03-031-1B; batch number: 1919555) ☒ 0.25% trypsin-EDTA (brand: Gibco; item number: 25200072; batch number: 2085552)
Experimental Operations
A. Inoculation of Cells: 1) Thawing Of Cryopreserved Vero E6 Cells: The cryopreserved cells were thawed in water bath at 37°C, centrifuged at 1000 rpm/min for 3 minutes, and then separated from the cryoprotectant. 20 mL of fresh medium (DMEM with 10% FBS) was added, and then the cells were transferred to a T75 cell culture flask for further culture in a CO₂ incubator at 37°C. After 24 hours, the cells were observed for their growth to confirm if the normal growth had been restored.

2) Passage of Cells:

The surface of Vero-E6 cells was washed once with 5 mL of PBS. The cells were digested with 3 mL of 0.25% trypsin-EDTA, and placed in an incubator. Once the cells were observed to become spherical and shedded, 6 mL of fresh medium was added to stop digestion. The digested cells were centrifuged at 1000 rpm/min for 3 minutes, and inoculated into a new culture flask at a ratio of 1:5.

3) Inoculation of Cells:

The Vero-E6 cells at the logarithmic growth stage were digested with 3 mL of 0.25% trypsin-EDTA, counted, and adjusted with fresh medium to a density of to 1×10^6 cells /mL. To 3 mL of the above cells was added 7 mL of fresh medium to prepare a cell suspension with cell density of 3×10^5 cells /mL. The suspension was inoculated into a 96-well plate with 100 μ L in each well, and each well contained 3×10^4 cells. The plate was placed in a CO₂ incubator at 37°C for continuous culture overnight.

B. Virus infection: 2021-3-29

4) Packaging of Viruses:

The virus stock solution has a titer of 4.0×10^7 PFU/mL. 600 μ L of the virus stock solution was added into six 1.5 mL EP tubes (with 100 μ L/ tube), respectively.

5) Pretreatment of Viruses:

100 μ L of the corresponding FEO, CEO, or ddH₂O were added into the respective tube of the six EP tubes containing the virus solution, mixed, and stood for 3 minutes or 30 minutes, respectively, which were labeled as "3-FEO", "30-FEO", "3-CEO", "30-CEO", "3-Ctrl", and "30-Ctrl".

6) Dilution of Virus:

The 6 viral solutions were subject to 10-fold serial dilution with a maintenance medium containing 2% FBS, respectively.

7) Infection of Viruses:

The upper medium of the adherent Vero E6 cells in the 96-well plate was discarded, and to each well was respectively added 100 μ L of the virus solution stored for different periods of time periods and mixed with different essential oils

or ddH₂O. Each sample had 4 replicates, which were continuously cultured in a CO₂ incubator at 37°C.

8) Observations for Cytopathic Effects:

After 48-72 hours, the cells were observed for cytopathic effect under microscope, and the TCID₅₀ value of the virus in the sample was calculated.

9) Calculation of Titer and Statistical Analysis of Results:

Karber method $\lg \text{TCID}_{50} = L + d(S - 0.5)$

L: The logarithm of the minimum dilution of the virus

d: The dilution factor, i.e., group interval

S: The sum of the cytopathic ratio (excluding the ratio of the minimum dilution cytopathy)

Assay Results

After 48-72 hours, the cells were observed for cytopathic effect under microscope. In all the four experimental groups of "3-FEO", "3-CEO", "30-CEO" and "30-FEO", it is found that the essential oil had cytotoxicity. Among them, in the "3-FEO" and "30-FEO" groups, the mixture of virus and essential oil (100 µL + 100 µL) was still cytotoxic after 10¹, 10², 10³-fold dilution, and a large number of cells died; and in the "3-CEO" and "30-CEO" groups, the mixture of virus and essential oil (100 µL + 100 µL) was still cytotoxic after 10¹, 10², 10³, 10⁴, 10⁵-fold dilution, and a large number of cells died. Moreover, the incubation time of essential oil with virus did not affect the cytotoxicity of essential oil. Therefore, in the treatment group with occurrence of cell death in this experiment, it cannot determine the virus killing status based on the degree of cytopathic effect. The specific results are shown in Annex 1.

After calculated, the infectious titers of the experiment control groups (i.e., "3-Ctrl" and "30-Ctrl") were 3.56 x 10⁷ TCID₅₀/mL. By contrast, the titers of the groups in which the virus was incubated with CEO for 3 minutes and 30 minutes was <632412 TCID₅₀/mL, and the titers of the groups in which the virus was incubated with FEO for 3 minutes and 30 minutes were <6324 TCID₅₀/mL.

Therefore, based on this experiment system, it can be concluded that the food-grade essential oil (FEO) and the cosmetic-grade essential oil (CEO) have different degrees

of killing effects on novel coronavirus, wherein the FEO has a killing efficiency of >99.82% after treatment for 3 and 30 minutes, and the CEO has a killing efficiency of >82.22% after treatment for 3 and 30 minutes.

Table 1. Effect of Infective Titers on Essential Oils Inactivating Novel Coronavirus

Group	Virus titer (TCID ₅₀ /mL)
3-Ctrl	3556313
30-Ctrl	<632412
3-CEO	<6324
3-FEO	3556313
30-CEO	<632412
30-FEO	<6324

Annex

1. Results of Cytopathy

Experimental Records of Inactivation Effect of Plant Essential Oils on Novel Coronavirus (SARS-Cov-2)

Annex 1: Results of Cytopathy

Plate 01			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸			
		1	2	3	4	5	6	7	8	9	10	11	12
3-Ctrl	A		+	+	+	+	+	-	-	-			
	B		+	+	+	+	+	+	-	-			
	C		+	+	+	+	+	+	-	-			
	D		+	+	+	+	+	+	-	-			
3-CEO	E		CC	CC	CC	CC	CC	-	-	-			
	F		CC	CC	CC	CC	CC	-	-	-			
	G		CC	CC	CC	CC	CC	-	-	-			
	H		CC	CC	CC	CC	CC	-	-	-			
Plate 02			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸			

		1	2	3	4	5	6	7	8	9	10	11	12
3-FEO	A		CC	CC	CC	-	-	-	-	-			
	B		CC	CC	CC	-	-	-	-	-			
	C		CC	CC	CC	-	-	-	-	-			
	D		CC	CC	CC	-	-	-	-	-			
30-Ctrl	E		+	+	+	+	+	-	-	-			
	F		+	+	+	+	+	+	-	-			
	G		+	+	+	+	+	+	-	-			
	H		+	+	+	+	+	+	-	-			
Plate 03			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸			
		1	2	3	4	5	6	7	8	9	10	11	12
30-CEO	A		CC	CC	CC	CC	CC	-	-	-			
	B		CC	CC	CC	CC	CC	-	-	-			
	C		CC	CC	CC	CC	CC	-	-	-			
	D		CC	CC	CC	CC	CC	-	-	-			
30-FEO	E		CC	CC	CC	-	-	-	-	-			
	F		CC	CC	CC	-	-	-	-	-			
	G		CC	CC	CC	-	-	-	-	-			
	H		CC	CC	CC	-	-	-	-	-			
CC: Cell Cytotoxicity +: with cytopathic effect -: without cytopathic effect													

[0062] It can be seen from the above, the essential oil compositions FEO and CEO of the invention have acceptable cytotoxicity and excellent killing effect on novel coronavirus.

Test Example 2

- 5 [0063] The essential oil compositions of the examples and the comparative examples were evaluated at cellular level for their effect against novel coronavirus (SARS-CoV-2) in accordance with the following method.

Materials

[0064] Cells: VeroE6 cells.

- 10 [0065] Viruses: SARS-CoV-2 (South African variant), with a titer of TCID₅₀ = 10⁻

⁵/100 μ L, using a virus titer of 100TCID₅₀.

[0066] Essential oils: the compounded essential oils were diluted with cell culture medium into 6 concentrations by double dilution.

Method

5 1. Antiviral Test of The Drugs to be Tested

[0067] (1) Drugs to be tested:

Table 2: Names, Experimental Concentrations and Grouping Of Cytotoxic Drugs Of Compounded Essential Oils

Grouping		Drug concentrations (μ L/L)
Test groups	compounded essential oils	600, 300, 150, 75, 37.5, 18.75
Cell control group	medium	

Table 3: Names, Experimental Concentrations and Grouping Of Cytotoxic Drugs Of Compounded Essential Oils to be Tested

Grouping		Drug concentration (μ L/L)
Test group	compounded essential oils	300, 150, 75, 37.5, 18.75, 9.375
Virus control group	SARS-CoV-2 virus liquor	100 TCID ₅₀ /well
Cell control group	medium	

[0068] (2) 100 μ L of VeroE6 cells were added into each well of a sterile 96-well culture plate at a concentration of 1×10^5 cells/mL, and cultured at 37°C for 24 hours with 5% CO₂;

[0069] (3) The virus liquor with 100 TCID₅₀ was added into the experimental groups and the control groups in the culture plate, with 100 μ L in each well at 37°C, and adsorbed in a 5% CO₂ incubator for 2 h;

[0070] (4) After 2 h, the cell culture in the 96-well culture plate was discarded; and the drug to be tested was diluted into various concentrations as listed in Table 1, with 3 duplicate wells for each concentration, and added at 100 μ L/well;

[0071] (5) At the same time, a cell control, a blank control (solvent control), a virus control (negative control), and a comparative drug control were set up;

[0072] (6) The cells were incubated at 37°C in a 5% CO₂ incubator for 3 days;

[0073] (7) The cells were observed under an optical microscope for their cytopathic effect (CPE), the degree of which was recorded in line with the following 6-grade criteria: "-" means that no cytopathic effect occurs; "±" means that the cytopathic effect is less than 10%; "+" means that the cytopathic effect is around 25%; "++" means that the cytopathic effect is around 50% of cytopathic effects; "+++" means that the cytopathic effect occurs in around 75% of the cells; and "++++" means that the cytopathic effect occurs in more than 75% of the cells. The median effective concentration (IC₅₀) was calculated by Reed-Muench method or GraphPad Prism5.0.

Criteria for determining the efficacy: A concentration with 50% inhibition of virus CPE is considered as an effective concentration.

[0074] (8) Experimental conditions: All the experimental operations were made in a BSL-3 laboratory.

Results

[0075] 1. Detection Of Cytotoxicity Of Compounded Essential Oils by MTS:

[0076] The VeroE6 cells were digested with 0.02% EDTA and 0.5% trypsin when they were in the best growth state. The cells were adjusted with a growth medium to a desired concentration, and added to a 96-well cell culture plate with 100 μ L in each well, and cultured to a dense cell monolayer at 37°C in a 5% CO₂ incubator. The compounded essential oil was diluted with medium to different concentrations, i.e., 600 μ L/L, 300 μ L/L, 150 μ L/L, 75 μ L/L, 37.5 μ L/L and 18.75 μ L/L, respectively, and added to the cell culture plate with 100 μ L in each well. Three parallel wells were set up for each dilution concentration, and normal cells were set as control. The cells were cultured at 37°C in a 5% CO₂ incubator, and detected by MTS method for their activity.

The specific results are shown in Attached Table 1. No compounded essential oil group exhibited toxicity to the VeroE6 cells at 300 μ L/L, which was thus selected as the highest experimental concentration in subsequent experiments.

[0077] 2. CPE Observation: 72h after the virus infection, the normal cell control group exhibited no CPE, while the virus control group exhibited a significant CPE; and the essential oil groups with different concentrations showed different inhibitory effects on the CPE. By observing the CPE and recording the experimental results, the median effective concentration (IC_{50}) was calculated by Reed-Muench method or GraphPad Prism5.0, and the specific results are shown in Attached Table 2. All the compounded essential oil groups have antiviral effect, and the compounded essential oils of the examples are significantly superior to those of the comparative examples.

Attached Table 1. Cytotoxicity RESULTS OF COMPOUNDED ESSENTIAL OILS

Conc. of essential oil ($\mu\text{L/L}$)	Inhibition rate (%)												
	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Ex. 5	Ex. 6	Ex. 7	Ex. 8	Ex. 9	Ex. 10	Ex. 11	Ex. 12	Ex. 13
600	24.38±0.27	21.77±0.21	26.45±2.22	32.54±0.71	32.90±0.71	27.94±1.64	25.85±1.38	26.15±1.46	26.42±2.11	28.59±1.35	24.48±1.45	26.40±0.67	27.69±0.84
300	9.16±0.72	7.93±0.58	8.90±0.73	7.46±0.50	7.36±0.70	11.42±0.90	8.56±0.56	10.68±1.00	9.80±0.51	0.57±0.30	2.49±0.85	11.45±1.04	6.61±0.24
150	1.42±1.43	4.70±0.40	5.04±0.66	1.82±1.17	3.55±0.31	1.43±0.40	3.77±0.34	5.40±1.39	2.00±0.37	2.90±0.83	6.16±0.45	5.32±1.40	5.83±0.27
75	1.71±0.38	2.14±0.23	1.12±0.55	2.38±0.30	1.34±0.87	1.42±0.49	0.66±0.43	2.19±0.35	1.75±0.72	1.53±0.78	1.54±0.87	1.35±0.71	2.07±0.36
37.5	0.25±0.28	-2.78±0.33	-5.96±0.42	-1.57±0.15	-5.17±0.39	-1.88±0.23	1.55±0.26	-3.81±0.49	-0.95±0.35	-5.63±0.13	-1.83±0.09	1.44±0.26	-2.35±0.42
18.75	-5.17±0.19	-5.87±0.23	-0.49±0.19	-0.34±0.21	-3.53±0.14	-6.47±0.14	-5.41±0.20	0.04±0.03	-6.17±0.18	-0.66±0.11	-3.60±0.09	-6.22±0.07	1.12±0.08

Attached Table 2: Results of anti-novel coronavirus of compounded essential oils

Conc. of essential oil(μL/L)	Inhibition Rate &												
	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Ex. 5	Ex. 6	Ex. 7	Ex. 8	Ex. 9	Ex. 10	Ex. 11	Ex. 12	Ex. 13
9.375	2.49 ±	0.13 ±	2.01 ±	-1.02 ±	-1.82 ±	1.50 ±	0.50 ±	-2.97 ±	-2.98 ±	-3.29 ±	-3.99 ±	-3.12 ±	-3.39 ±
	0.09 **	0.16 **	0.14 **	0.18 **	0.07 *	0.16 **	0.05 **	0.07	0.10	0.16	0.22	0.14	0.18
18.75	6.90 ±	5.01 ±	6.32 ±	3.48 ±	3.03 ±	5.69 ±	5.67 ±	1.87 ±	2.24 ±	1.71 ±	0.50 ±	2.18 ±	0.93 ±
	0.15 **	0.32 **	0.28 **	0.09*	0.13*	0.19 **	0.39 **	0.17	0.41	0.17	0.08	0.11	0.46
37.5	15.18 ±	10.92 ±	13.18 ±	10.00 ±	9.47 ±	12.46 ±	11.31 ±	4.96 ±	8.71 ±	7.14 ±	3.84 ±	8.53 ±	6.96 ±
	0.57 **	0.50 **	0.66 **	0.60*	0.33	0.82 **	0.26 **	0.27	0.53	0.89	0.53	0.08	0.45
75	35.47 ±	24.88 ±	31.85 ±	21.27 ±	20.14 ±	31.00 ±	27.26 ±	12.44	19.07	14.69	11.05 ±	17.43 ±	12.31 ±
	0.30 **	0.35 **	0.62 **	0.74*	0.93	0.53 **	0.93 **	± 0.67	± 0.82	± 0.22	0.85	1.44	0.44
150	56.31 ±	43.64 ±	50.48 ±	41.30 ±	40.49 ±	46.26 ±	45.57 ±	26.75	37.10	32.79	26.72	36.20 ±	29.50 ±
	0.49 **	1.35 **	0.54 **	1.23*	0.51	0.96 **	1.11 **	± 0.41	± 1.07	± 1.36	± 0.68	0.83	0.11
300	95.86 ±	79.80 ±	91.80 ±	79.44 ±	76.40 ±	86.04 ±	82.01 ±	55.82	73.68	60.96	52.27	67.19 ±	60.36 ±
	1.90 **	1.29 **	0.8 **1	2.40*	1.06 **	2.05 **	2.01 **	± 2.08	± 1.05	± 1.18	± 2.06	2.00*	0.36
IC ₅₀ (μL/L)	108.2	153.5	122.4	162.5	169.9	134.3	144.6	264.4	182.4	229.5	282.1	200.5	239.3

[0078] In comparison with Comparative Example 1, **p<0.01,*p<0.05

[0079] It can be seen from the above, the essential oil compositions of the invention are significantly better than the comparative example.

Test example 3

- 5 [0080] The essential oil compositions of the Examples and Comparative Examples were evaluated at cellular level for their antiviral effects *in vitro* against influenza viruses, rhinoviruses, and respiratory syncytial viruses in accordance with the following method.

Experimental material:

- 10 [0081] 1. Compositions to be tested: Compounded Essential Oils 1-10 were prepared in accordance with the formulations as listed in Table 4 below, wherein Compounded Essential Oil 8 is the essential oil composition of Example 2, Compounded Essential Oil 9 is the essential oil composition of Example 1, and Compounded Essential Oil 10 is the essential oil composition of Example 3.

15 Table 4.

No.	Composition
1	Carvacrol 50%, thymol 50%
2	Bay leaf oil 20%, lemon oil 70%, geranium oil 10%
3	Bay leaf oil 10%, perilla oil 9.5%, Asian dementholized peppermint oil 35%, patchouli oil 0.5%, lemon oil 35%, geranium oil 10%
4	Perilla oil 20%, Asian dementholized peppermint oil 20%, patchouli oil 5%, honeysuckle oil 15%, Chuanxiong Rhizome Oil 10%, Siberian cocklebur achenia oil 10%, forsythia oil 20%
5	Bay leaf oil 30%, Asian dementholized peppermint oil 20%, lemon oil 10%, geranium oil 10%, honeysuckle oil 10%, Chuanxiong Rhizome Oil 5%, patchouli oil 5%, Siberian cocklebur achenia oil 5%, forsythia oil 5%
6	Asian dementholized peppermint oil 94%, perilla oil 5%, patchouli oil 0.25%, lemon oil 0.65%, carvacrol 0.05%,

	Geranium oil 0.05%
7	Asian dementholized peppermint oil 31%, perilla oil 1.65%, patchouli oil 0.08%, lemon oil 0.21%, carvacrol 67%, Geranium oil 0.016%
8	Carvacrol 51.25%, thymol 26.25%, bay leaf oil 2.5%, perilla oil 2.4%, Asian dementholized peppermint oil 7.5%, patchouli oil 0.125%, lemon oil 8.75%, geranium oil 1.25%
9	Carvacrol 5.0%, thymol 5.0%, bay leaf oil 10.0%, perilla oil 9.5%, Asian dementholized peppermint oil 30.0%, patchouli oil 0.5%, lemon oil 35.0%, geranium oil 5.0%
10	Carvacrol 25%, thymol 25%, bay leaf oil 20%, Asian dementholized peppermint oil 10%, lemon oil 5%, geranium oil 5%, honeysuckle oil 5%, Chuanxiong Rhizome Oil 4%, patchouli oil 0.5%, Siberian cocklebur achenia oil 0.3%, forsythia oil 0.2%

[0082] 2. Cell: Vero-E6 cells.

[0083] 3. Viruses:

Strain name	Source
A/pr/8/34(h1n1)	ATCC
A/aichi/2/68(h3n2)	ATCC
B/Lee/1940(flub)	ATCC
Human rhinovirus (hRV)	ATCC
Respiratory syncytial virus (RSV)	ATCC

Experimental method

1. Cell culture and preparation:

5 1.1 Vero-E6 cell recovery:

[0084] The cryopreserved cells were rapidly thawed in water bath at 37°C, centrifuged at 800 rpm/min for 5 minutes, and then separated from the cryoprotectant. 5 mL of fresh medium (DMEM, 10% FBS) was added, and then transferred to a T25 cell culture flask

for further culture at 37°C in a CO₂ incubator. After 24 hours, the cells were observed for their growth status to confirm if normal growth had been restored.

1.2 Cell passage:

[0085] The surface of Vero-E6 cells was washed with 5mL PBS twice. The cells were digested with 1 mL 0.25% trypsin-EDTA, and placed in an incubator. Once the cells were observed to become spherical and shedded, 4 mL of fresh medium was added immediately to stop digestion. The digested cells were centrifuged at 800 pm/min for 5 min and inoculated into a new T75 culture flask at a ratio of 1:1.

1.3 Inoculated cells:

[0086] The Vero-E6 cells at logarithmic growth stage were digested with 3 mL of 0.25% trypsin-EDTA, centrifuged, re-suspended, plated onto a 96-well plate, and placed in a CO₂ incubator at 37°C for continuous culture.

2. Vero-E6 cytotoxicity test

2.1 Dilution of essential oil:

[0087] 60 µL of the essential oil to be tested was added into 60 µL of maintenance medium and mixed evenly. 100 µL of the mixture was subject to 10-fold serial dilution with the maintenance medium.

2.2 Cytotoxicity assay:

[0088] After about 20 hours, the Vero-E6 cells reached a density of 90%. The upper medium of Vero-E6 cells in the 96-well plate was discarded. The cells were washed twice with PBS, and the essential oil diluent was added to the 96-well plate. A cell control group was set up (N, wherein the essential oil was not added, but replaced with the maintenance medium). The cells were visually observed for their status after 72 hours.

3. Virus Treatment and Cell Infection

3.1 Mixed Treatment Of Virus and Essential Oil:

[0089] To EP tubes were added 200 µL of virus solution, with 2 EP tubes for each virus strain. To the 2 EP tubes containing the virus solution were added 200 µL of essential oil or DMEM solution, respectively, with a total of 400 µL of mixed solution

in each EP tube. After mixed, the mixed solutions stood at room temperature for 60 minutes, and were labeled as FEO (the essential oil mixed with the virus solution for 60 minutes) and Ctrl (the DMEM mixed with the virus solution for 60 minutes), respectively.

5 3.2 Dilution Of Virus Essential Oil Mixture:

[0090] The mixed solution stood at room temperature for 60 minutes. 100 μ L of the virus-essential oil solution was taken from each tube, and subject to 10-fold serial dilution with the diluted virus solution. It was repeated 3 times for each group.

3.3 Cell Infection:

10 [0091] To each well of the 96-well plate coated with the Vero-E6 cells was added 100 μ L of the diluted solution of essential oil - virus or DMEM-virus, with and 6 duplicate wells for each dilution, and virus positive control wells and normal cell wells were set up. The plate was further cultured at 37°C in the CO₂ incubator.

3.4 Cytopathic lesion observation:

15 [0092] After 72 hours, the cells were observed under the microscope for their cytopathic effect. The TCID₅₀ value of each group was calculated in accordance with the Reed-Muench formula, and the inhibition rate of the essential oil on the virus was compared.

Distance Ratio = (percentage of cytopathy above 50% - 50%) / (percentage of
20 cytopathy above 50% - percentage of lesions below 50%);

$\lg$ TCID₅₀ = logarithm of (distance ratio x dilution factor) + logarithm of
(dilution factor with cytopathy above 50%)

Inhibition Rate = (Titer of the control well virus – Titer of the experimental
well) / Titer of the control well virus x 100%

25 3.5 Experimental conditions:

[0093] All the experimental operations were m in a BSL-3 laboratory.

Experiment results

[0094] 1. Results of cytotoxicity of the compounded essential oils: At 72 hours, it was visually observed that the cell death occurred in the experimental wells when the essential was subject to 10^{-1} to 10^{-4} dilution. The cells effected by the essential oil undergoing 10^{-5} dilution exhibited normal morphology and no obvious difference as compared with the control cells. Therefore, it was visually observed that the maximum non-toxic concentration of the essential oils on the Vero-E6 cells is the 10^{-5} dilution of the essential oil stock.

[0095] 2. Results of virus inhibition by the essential oils: 72 hours after the virus infection, the normal cell control group exhibited no CPE, while the virus control group exhibited a significant CPE; and the essential oil groups with different concentrations exhibited different inhibitory effects on the CPE. The virus inhibition rates of the essential oils in various group was calculated by the virus titer of each group, and the specific results are shown in Tables 5-9. The inhibition rates of the compounded essential oils were summarized in Table 10 and Figure 1. The results show that all the compounded essential oils of various groups have certain anti-viral effect, wherein Compounded Essential Oils 9, 10 and 1 have comparable effects, which are better than Compounded Essential Oils 7 and 8; better than Compounded Essential Oils 3 and 6; and significantly better than Compounded Essential Oils 2, 4 and 5.

Table 5. Inhibition Effect Of Compounded Essential Oils On H1N1

Formulation of Essential oil	Logarithmic value of average virus titer $\lg\text{TCID}_{50}$	Average of total viruses $\text{TCID}_{50}/\text{ml}$	H1N1 virus inhibition rate %
1	3.57	3.72×10^3	98.68 ± 12.58
2	4.68	4.79×10^4	58.72 ± 7.41
3	4.54	3.47×10^4	79.12 ± 3.32
4	4.72	5.25×10^4	46.00 ± 6.45
5	4.60	3.98×10^4	64.84 ± 6.08
6	4.20	1.58×10^4	84.68 ± 5.43
7	4.28	1.91×10^4	83.12 ± 5.48

8	4.01	1.02×10^4	85.65±10.42
9	1.82	6.61×10^1	99.83±1.35
10	3.50	3.16×10^3	98.72±5.79

Table 6. Inhibition Effect Of Compounded Essential Oils On H3N2

Essential oil	Logarithmic value of average virus titer lgTCID ₅₀	Average of Total Viruses TCID ₅₀ /ml	H3N2 virus inhibition rate %
1	3.29	1.95×10^3	95.68±12.58
2	4.72	5.25×10^4	58.72±7.41
3	4.37	2.34×10^4	79.12±3.32
4	4.88	7.59×10^4	56.00±6.45
5	4.69	4.90×10^4	64.84±6.08
6	4.30	2.00×10^4	84.68±5.43
7	4.55	3.55×10^4	73.12±5.48
8	4.23	1.70×10^4	85.65±10.42
9	2.16	1.45×10^2	98.83±1.35
10	3.11	1.29×10^3	96.72±5.79

Table 7. Inhibition Effect Of Compounded Essential Oils On Flub

Essential oil	Logarithmic value of average virus titer lgTCID ₅₀	Average of Total Viruses TCID ₅₀ /ml	FluB virus inhibition rate %
1	1.55	3.55×10^1	99.12±3.80
2	4.62	4.17×10^4	59.66±6.64
3	3.55	3.55×10^3	88.84±12.19
4	4.55	3.55×10^4	61.55±11.48
5	4.15	1.41×10^4	63.66±6.68
6	3.77	5.89×10^3	84.80±7.21
7	3.65	4.47×10^3	86.05±3.90

8	2.38	2.40×10^2	92.72±5.62
9	2.02	1.05×10^2	98.67±4.16
10	2.13	1.35×10^2	97.97±2.11

Table 8. Inhibition effect of compounded essential oils on hRHV

Essential oil	Logarithmic value of average virus titer lgTCID ₅₀	Average of Total Viruses TCID ₅₀ /ml	hRHV virus inhibition rate %
1	2.98	9.55×10^2	95.28±3.39
2	4.67	4.68×10^4	54.30±5.93
3	4.01	1.02×10^4	71.09±10.87
3	4.78	6.03×10^4	52.51±5.96
5	4.34	2.19×10^4	55.99±10.24
6	3.61	4.07×10^3	76.72±6.43
7	3.87	7.41×10^3	74.27±3.48
8	3.54	3.47×10^3	78.25±5.01
9	2.13	1.35×10^2	98.49±4.28
10	2.34	2.19×10^2	96.13±2.04

Table 9. Inhibition effect of compounded essential oils on RSV

Essential oil	Logarithmic value of average virus titer lgTCID ₅₀	Average of Total Viruses TCID ₅₀ /ml	RSV virus inhibition rate %
1	2.33	2.14×10^2	98.07±3.65
2	4.02	1.05×10^4	53.15±6.64
3	3.91	8.13×10^3	76.11±11.9
4	4.32	2.09×10^4	46.50±6.52
5	4.15	1.41×10^4	50.31±11.2
6	3.82	6.61×10^3	78.24±9.97
7	3.65	4.47×10^3	83.58±9.61

8	3.46	2.88×10^3	83.88±8.19
9	2.45	2.82×10^2	97.98±6.67
10	2.67	4.68×10^2	97.45±5.49

Table 10. Comparison of the inhibition rates of compounded essential oils on H1N1, H3N, FluB, hRHV and RSV

Essential oil	H1N1	H3N2	FluB	hRHV	RSV
1	98.68±12.58	95.68±12.58	99.12±3.80	95.28±3.39	98.07±3.65
2	58.72±7.41	58.72±7.41	59.66±6.64	54.30±5.93	53.15±6.64
3	83.12±5.48	73.12±5.48	88.84±12.19	71.09±10.87	76.11±11.9
4	46.00±6.45	56.00±6.45	61.55±11.48	52.51±5.96	46.50±6.52
5	64.84±6.08	64.84±6.08	63.66±6.68	55.99±10.24	50.31±11.2
6	84.68±5.43	84.68±5.43	84.80±7.21	76.72±6.43	78.24±9.97
7	85.65±10.42	85.65±10.42	86.05±3.90	74.27±3.48	83.58±9.61
8	79.12±3.32	79.12±3.32	92.72±5.62	78.25±5.01	83.88±8.19
9	99.83±1.35	98.83±1.35	98.67±4.16	98.49±4.28	97.98±6.67
10	98.72±5.79	96.72±5.79	97.97±2.11	96.13±2.04	97.45±5.49

Test Example 4

[0096] The essential oil compositions of the Examples and comparative Examples were evaluated at cellular level for their *in vitro* inhibitory effects against a variety of Gram-positive and Gram-negative bacteria in accordance with the following method.

Experiment material:

[0097] 1. Composition to be tested: The compositions to be tested in this test example are the same as Compounded Essential Oils 1-10 in test example 3, wherein Compounded Essential Oil 8 is the essential oil composition of Example 2, Compounded Essential Oil 9 is the essential oil composition of Example 1, and Compounded Essential Oil 10 is the essential oil composition of Example 3.

[0098] 2. Standard strain:

Standard strain	Source
<i>Diplococcus pneumoniae</i> (No. 31001)	China Pharmaceutical and Biological Products Control Institute
<i>Staphylococcus aureus</i> (No. 26112)	China Pharmaceutical and Biological Products Control Institute
<i>Staphylococcus epidermidis</i> (No. 26487)	China Pharmaceutical and Biological Products Control Institute
<i>Micrococcus catarrhalis</i> (No. 29103)	China Pharmaceutical and Biological Products Control Institute
<i>Escherichia coli</i> (No. 44155)	China Pharmaceutical and Biological Products Control Institute
<i>Bacillus dysenteriae</i> (No. 51592)	China Pharmaceutical and Biological Products Control Institute

[0099] 3. Medium: ordinary broth medium, ordinary agar medium and serum agar medium, were prepared in accordance with conventional methods.

Experiment method

[0100] The compounded essential oils were observed for their *in vitro* inhibitory effect on standard strains using test tube method, wherein three Gram-positive strains (*Diplococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and three Gram-negative strains (*Micrococcus catarrhalis*, *Escherichia coli*, *Bacillus dysenteriae*) were selected as the standard strains.

[0101] (1) Dilution of Essential oils: 1 ml of the essential oil stock was diluted five times to an essential oil concentration of 200 $\mu\text{L}/\text{ml}$, and then subject to doubling dilution to give a total of 9 essential oil liquors with final concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 $\mu\text{L}/\text{ml}$, respectively.

[0102] (2) Preparation of bacterial solution: The cultures of *Diplococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus catarrhalis* and *Escherichia coli* and *Bacillus dysenteriae* were cultured for 18 h and diluted with sodium chlorine-peptone buffer to 10^{-7} to 10^{-5} at pH 7.0.

[0103] (3) Test method: The air-dried drug-sensitive paper containing 1000 CFU bacterial solution was soaked in 8 compounded essential oils with different concentrations for 1 h, and then cultured in 10 ml of TSB at 37°C for 48 h to observe if the TSB was turbid. Meanwhile, it was inoculated on strain-isolation agar plates, and
 5 cultured at 37°C for 24 h to observe if any colony grew. Meanwhile, positive controls without essential oil liquors and negative controls with essential oil but without strain were set up, with three duplicates for each sample. The minimum essential oil concentration without growth of bacteria was used as the minimum inhibitory concentration (MIC) for this strain.

10 Experiment result

[0104] Under the conditions of this test, the compounded essential oils showed different degrees of inhibition against common Gram-positive bacteria (*Diplococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative bacteria (*Micrococcus catarrhalis*, *Escherichia coli*, *Bacillus dysenteriae*). As seen
 15 from the results of minimum inhibitory concentrations in Table 11, Figure 2, and Figure 3, the essential oils of Examples 9, 10, and 1 have comparable effects, better than those of Examples 3, 6, 7, and 8, and significantly better than those of 2, 4, and 5.

Table 11. The minimum inhibitory concentration of compounded essential oil against standard strains *in vitro*(MIC, μ L/ml)

Essential oil	<i>D. pneumoniae</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>M. catarrhalis</i>	<i>E. coli</i>	<i>B. dysenteriae</i>
1	3.13	6.25	3.13	25	6.25	12.5
2	100	25	50	50	100	50
3	25	50	12.5	12.5	50	50
4	50	100	25	25	100	50
5	50	100	12.5	100	25	50
6	12.5	50	12.5	25	25	50
7	12.5	12.5	25	25	50	25
8	12.5	25	12.5	12.5	50	25

9	6.25	3.13	1.56	6.25	6.25	12.5
10	3.13	6.25	6.25	3.13	12.5	12.5

Test example 5

[0105] According to the method used in the simulated field test of the air disinfection effect of the Technical Specification for Disinfection, 2002 - 2.1.3.4, the essential oil composition of Example 1 (labeled as the food-grade Essential oil (FEO)) was tested for its air disinfection effect against *Staphylococcus albus*.

[0106] 200 mL FEO was added to an atomizer. After atomization at the maximum power for 1 h, a sample was taken by a BY-300 air microorganism sampler at the pumping air rate of 28.3 L/min. The sampling time of the control group was 30s, the sampling time of the experiment group was 5 min, and the experimental chamber size was 20 m³. The results of the killing rate experiment were processed to eliminate the influence of natural death factors of microorganisms in the air. The results are shown in Table 12 below.

Table 12.

Time	Test Microorganisms	No.	Total bacteria in air	Killing rate
0 (CK)	<i>Staphylococcus albicans</i> (<i>Staphylococcus albus</i>) 8032	1	2.3 x 10 ⁵	-
		2	1.6 x 10 ⁵	-
		3	1.4 x 10 ⁵	-
1 h	<i>Staphylococcus albicans</i> (<i>Staphylococcus albus</i>) 8032	1	1.8 x 10 ⁴	86.58
		2	1.2 x 10 ⁴	89.50
		3	1.2 x 10 ⁴	87.23

[0107] It can be seen that the essential oil composition of the invention has a good disinfection effect against white *Staphylococcus albus* in the air.

Test example 6

[0108] According to the virus killing test method of the Technical Specification for Disinfection, 2002 - 2.1.1.10.7, the essential oil composition of Example 1 (labeled as

the food-grade essential oil (FEO)) was tested for its virus killing effect against enterovirus 71, adenovirus, herpes virus, Cocksackie virus, influenza A virus H1N1, and poliovirus. The results show:

- 5 - After treating enterovirus 71 (ATCC VR-1432, host name: Vero cell) with the FEO stock solution for 30 min, the mean killing logarithm (KL) was 1.36, and the virus killing rate was 95.69%;
- After treating human herpes virus A5C strain (ATCC VR-2019, host name: Vero cell) with the FEO stock solution for 30 min, the mean killing logarithm (KL) was > 4.37, and the virus killing rate was >99.99%;
- 10 - After treating adenovirus type 3 (ATCC VR-3, host name: A454 cell) with the FEO stock solution for 30 min, the mean killing logarithm (KL) was 1.61, and the virus killing rate was 97.56%;
- After treating Cocksackie virus A21 (ATCC VR-850, host name: Vero cell) with THE FEO stock solution for 30 min, the mean killing logarithm (KL) was 1.41, and the virus
- 15 killing rate was 95.97%;
- After treating influenza A virus H1N1: A/PR/8/34 (ATCC VR-1469, host name: MDCK cell) with the FEO stock solution for 30 min, the mean killing logarithm (KL) was >4.37, and the virus killing rate was > 99.99%;
- After treating poliovirus type I vaccine strain (host name: Vero cell) with the FEO
- 20 stock solution 30 min, the mean killing logarithm (KL) was 1.24, and the killing rate was 94.23%;
- The negative control group exhibited good cell growth, and the experimental results met all the requirements specified in the evaluation specification.

[0109] It can be seen from the above results that the applied essential oil composition

25 has good virus killing against enterovirus 71, adenovirus, herpes virus, Cocksackie virus, influenza A virus H1N1, poliovirus.

Test example 7

[0110] According to the bacteria inhibition detection method of the Technical Specification for Disinfection (2002)-2.1.11.3, the essential oil composition of

Example 1 (labeled as the food-grade essential oil (FEO)) was tested for its bacteria inhibition against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The results show that by treating *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* with the FEO stock solution for 2 min, the bacteria inhibition was >99.9%,
 5 which met the requirements of the Technical Specification for Disinfection (2002)-2.1.11.3.

[0111] It can be seen that the essential oil composition of the invention exhibits good bacteriostatic effect on *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.

Test example 8

10 [0112] According to the mycoplasma culture method and the detection method of the Technical Specifications for Disinfection, 2002, 2.1.1.7 and 2.1.1.9, the essential oil composition of Example 1 (labeled as the food-grade essential oil (FEO)) was tested for its inhibition of mycoplasma growth against *Mycoplasma pneumoniae*. The results show that under the specified experimental conditions, after treating *Mycoplasma pneumoniae*
 15 *pneumoniae* (ATCC 15531) with FEO stock solution at 25°C for 30 min, the logarithm of the live bacteria concentration decreased by >3, and the killing rate was >99%, indicating that it has a killing effect on *Mycoplasma pneumoniae*.

[0113] It can be seen that the essential oil composition of the invention has a good killing effect on *Mycoplasma pneumoniae*.

What is claimed is:

1. An antimicrobial essential oil composition, comprising:

(1) 0.0001% to 80% of one or more selected from the group consisting of carvacrol and thymol;

5 (2) 0.0001% to 99.9% of one or more selected from the group consisting of lemon oil, sweet orange oil, Citrus aurantium 'Daidai' peel oil, white lemon oil and pomelo peel oil;

10 (3) 0.0001% to 99.9% of one or more selected from the group consisting of Asian dementholized peppermint oil, Asian peppermint oil, Mentha Piperita Oil and pennyroyal oil;

(4) 0.0001% to 20% of one or more selected from the group consisting of bay leaf oil and marjoram oil;

(5) 0.0001% to 20% of one or more selected from the group consisting of patchouli oil, sandalwood oil and rosemary oil;

15 (6) 0.0001% to 20% of one or more selected from the group consisting of geranium oil, pine needle oil and cedarwood oil; and

(7) optionally, 0.0001% to 20% of one or more selected from the group consisting of perilla oil, Chrysanthemum morifolium Ramat oil and Vitex cannabifolia leaf oil, based on the total weight of the composition.

20 2. The antimicrobial essential oil composition of claim 1, comprising:

(1) 2% to 80% of one or more selected from the group consisting of carvacrol and thymol;

25 (2) 2% to 50% of one or more selected from the group consisting of lemon oil, sweet orange oil, Citrus aurantium 'Daidai' peel oil, white lemon oil and pomelo peel oil;

(3) 5% to 40% of one or more selected from the group consisting of Asian dementholized peppermint oil, Asian peppermint oil, Mentha Piperita Oil and pennyroyal oil;

(4) 2% to 20% of one or more selected from the group consisting of bay leaf oil

and marjoram oil;

(5) 0.1% to 5% of one or more selected from the group consisting of patchouli oil, sandalwood oil and rosemary oil;

5 (6) 2% to 20% of one or more selected from the group consisting of geranium oil, pine needle oil and cedarwood oil; and

(7) optionally, 2% to 15% of one or more selected from the group consisting of perilla oil, Chrysanthemum morifolium Ramat oil and Vitex cannabifolia leaf oil, based on the total weight of the composition.

10 3. The antimicrobial essential oil composition of claim 1 or 2, further comprising optionally one or more of

(8) 0.0001% to 20% of honeysuckle oil;

(9) 0.0001% to 20% of Chuanxiong Rhizome Oil;

(10) 0.0001% to 20% of Siberian cocklebur achenia oil; and

(11) 0.0001% to 20% of forsythia oil,

15 based on the total weight of the composition.

4. The antimicrobial essential oil composition of any one of claims 1-3, comprising:

(1) 5% of carvacrol and 5% of thymol;

(2) 35% of lemon oil;

(3) 30% of Asian dementholized peppermint oil;

20 (4) 10% of bay leaf oil;

(5) 0.5% of patchouli oil;

(6) 5% of geranium oil; and

(7) 9.5% of perilla oil,

based on the total weight of the composition.

25 5. The antimicrobial essential oil composition of any one of claims 1-3, comprising:

(1) 0.005% of carvacrol and 0.005% of thymol;

(2) 0.1% of lemon oil;

(3) 99.58% of Asian dementholized peppermint oil;

- (4) 0.005% of bay leaf oil;
- (5) 0.25% of patchouli oil;
- (6) 0.005% of geranium oil; and
- (7) 0.05% of perilla oil,

5 based on the total weight of the composition.

6. The antimicrobial essential oil composition of any one of claims 1-3, comprising:

- (1) 0.005% of carvacrol and 0.005% of thymol;
- (2) 99.5% of lemon oil;
- (3) 0.25% of Asian dementholized peppermint oil;
- (4) 0.005% of bay leaf oil;
- (5) 0.18% of patchouli oil;
- (6) 0.005% of geranium oil; and
- (7) 0.05% of perilla oil,

10

based on the total weight of the composition.

15

7. The antimicrobial essential oil composition of claim 1, comprising:

- (1) 51.25% of carvacrol;
- (2) 26.25% of thymol;
- (3) 8.75% of lemon oil;
- (4) 7.5% of Asian dementholized peppermint oil;
- (5) 2.5% of bay leaf oil;
- (6) 0.125% of patchouli oil;
- (7) 1.25% of geranium oil; and
- (8) 2.375% of perilla oil,

20

based on the total weight of the composition.

25

8. The antimicrobial essential oil composition of any one of claims 1-3, comprising:

- (1) 25% of carvacrol and 25% of thymol;
- (2) 2% of lemon oil;
- (3) 10% of Asian dementholized peppermint oil;

- (4) 20% of bay leaf oil;
- (5) 0.5% of patchouli oil;
- (6) 2% of geranium oil;
- (7) 5% of honeysuckle oil;

- 5 (9) 4% of Chuanxiong Rhizome Oil;
- (10) 0.3% of Siberian cocklebur achenia oil; and
- (11) 0.2% of forsythia oil,
- based on the total weight of the composition.

9. The antimicrobial essential oil composition of any one of claims 1-8, wherein
- 10 the microorganism is selected from the group consisting of bacteria, fungi, viruses, mycoplasmas and their combinations.

10. The antimicrobial essential oil composition of any one of claims 1-9, wherein the microorganism is one or more of novel coronavirus, influenza virus, rhinovirus, respiratory syncytial virus, enterovirus, adenovirus, herpes virus, Coxsackie virus,
- 15 poliovirus.

11. The antimicrobial essential oil composition of claim 10, wherein the microorganism is novel coronavirus.

12. The antimicrobial essential oil composition of any one of claims 1-9, wherein the microorganism is one or more of *Diplococcus pneumoniae*, *Staphylococcus aureus*,
- 20 *Staphylococcus epidermidis*, *Micrococcus catarrhalis*, *Escherichia coli*, *Bacillus dysenteriae*, *Staphylococcus albus* and *Candida albicans*.

13. The antimicrobial essential oil composition of any one of claims 1-9, wherein the microorganism is *Mycoplasma pneumoniae*.

14. A product comprising the antimicrobial essential oil composition of any one of
- 25 claims 1-13.

15. The product of claim 14, wherein the product comprises a product capable of

reducing or killing microorganisms on one or more of hard surfaces, textiles, skin, mucosa or hair.

16. The product of claim 14 or 15, wherein the product comprises one or more selected from the group consisting of brittle capsules for mask, sanitizing wipes, air
5 fresheners, oral sprays, liquid soaps for hand, shampoos, conditioners, body washes, cosmetics, skin care products, aerogels, antibacterial sprays for skin, surface cleaners and detergents.

17. The product of claim 14, wherein the product is a protective product.

18. The product of claim 14 or 17, wherein the product is one or more selected
10 from the group consisting of civilian masks, medical masks, protective gloves, and protective clothing.

19. The product of any one of claims 14, 17 and 18, wherein the product is a brittle capsule-containing mask.

20. The product of claim 14, wherein the product is food or health care products.

15 21. The product of claim 14 or 20, wherein the product is one or more selected from the group consisting of chewing gums, oral refreshers, brittle capsules, jelly candies, hard candies, beverages, and dietary supplements.

22. A method of preparing the product of any one of claims 14-21, comprising:
uniformly mixing all components of the antimicrobial essential oil composition to
20 make an essential oil masterbatch or microcapsule or brittle capsule, and
combining the essential oil masterbatch or microcapsule or brittle capsule with
other components of the product to obtain the product.

23. The method of claim 22, wherein the product is a civilian mask or medical
mask, and
25 the combining the essential oil masterbatch or microcapsule or brittle capsule with
the other components of the product comprises adding the essential oil masterbatch or

microcapsule or brittle capsule into the civilian masks or medical masks.