

USE OF ANTIMICROBIAL AGENT COMBINATION IN PREPARING COMPOSITION FOR VAGINA, AND COMPOSITION FOR VAGINA

TECHNICAL FIELD

5 **[0001]** The present disclosure relates to a use of an antimicrobial agent combination in preparing vaginal composition. The combination of antimicrobial agents can be used for preparing various therapeutic or non-therapeutic compositions for vagina. The present disclosure also relates to a composition for vagina, which can be used for vaginal health care, and prevention and treatment of vaginal infection.

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BACKGROUND ART

[0002] Gram-positive bacilli referred to as “normal vaginal flora”, dominate on vaginal mucosa surface in the healthy woman. Such dominating Gram-positive bacilli, the main type of which is lactobacillus, can metabolize glycogen in vaginal mucosa epithelial cells to produce acid, thereby keep the pH value of the vaginal in a range
15 from 3.5 to 4.3, which is essential for the female genital tract to resist bacterial infections.

[0003] The vaginal flora may be disturbed by various factors so to decrease the vagina’s resistance to infections. As a result, vaginal infections are very common, such as bacterial vaginosis, aerobic vaginitis, and Candidal vaginitis, etc.. These
20 infectious disorders frequently result from the increase of bacteria such as Escherichia coli, Staphylococcus aureus, group B Streptococcus, Gardnerella vaginalis, Prevotella, and Candida. Therefore it is of great clinical significance to genital tract infection prevention and treatment to avoid inhibiting or destroying beneficial lactobacilli in the course of antimicrobial treatment against pathogenic bacteria and fungi

[0004] However currently, there is still lack of effective technology or method which may not only selectively inhibit bacteria and fungi such as *Escherichia coli* and *Staphylococcus aureus*, but also protect and promote beneficial lactobacilli in the vagina.

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SUMMARY

[0005] The object of the present disclosure is to provide an antimicrobial agent composition that has a strong inhibition effect on *Escherichia coli*, *Staphylococcus aureus*, and *Candida*. When being used in the vagina, the composition may promote the restoration of beneficial Lactobacilli and increase vaginal acidity.

10 **[0006]** The development of technologies for selectively inhibiting pathogenic bacteria and fungi while promoting beneficial lactobacilli remains a frontier topic in the field of gynecologic infections. According to the researches by the inventor, any one of 2-phenylethanol, benzoic acid and/or its sodium salt, and propionic acid and/or its salt could selectively inhibit the growth of *Lactococcus lactis* while having no
15 significant inhibition effect against *Lactobacillus*. The inhibition effect of other antimicrobial agents such as acetic acid, dehydroacetic acid, and caprylic acid separately against *Lactococcus lactis* as well as *Lactobacillus*, is not selective.

[0007] It has been found out by the research that 2-phenylethanol and/or phenoxyethanol, combined with benzoic acid and/or its sodium salt and/or
20 p-methoxybenzoic acid and/or its sodium salt and/or esters of p-hydroxybenzoate, or 2-phenylethanol and/or phenoxyethanol, combined with propionic acid and/or its salt and/or levulinic acid and/or its salt and/or acetic acid and/or its sodium salt, exhibits a synergistic inhibition effect against *Escherichia coli* and/or *Staphylococcus aureus*. Further, it gets stronger the inhibiting action against *Escherichia coli* and/or
25 *Staphylococcus aureus* and/or *Candida* when the following three types of agents are combined: (1) 2-phenylethanol and/or phenoxyethanol; (2) benzoic acid and/or its sodium salt, and/or p-methoxybenzoic acid and/or its sodium salt, and/or esters of p-hydroxybenzoate; (3) propionic acid and/or its salt, and/or levulinic acid and/or its

salt, and/or acetic acid and/or its sodium salt.

[0008] However, the combination of 2-phenylethanol and/or phenoxyethanol with some other antimicrobial agents such as dehydroacetic acid and/or its sodium salt, or sorbic acid and/or its potassium salt, is less effective than the combination as mentioned above.

[0009] The present disclosure provides a use of an antimicrobial agent combination in preparing a composition for vagina, the composition includes the following antimicrobial agents: (1) 2-phenylethanol and/or phenoxyethanol, total content of which is calculated as 0.05 to 0.60 percent(weight/volume) of 2-phenylethanol; (2) propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate, total content of which is calculated as 0.25 to 0.70 percent(weight/volume) of sodium propionate; and/or benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or ester of p-hydroxybenzoate, total content of which is calculated as 0.05 percent to 0.15 percent(weight/volume) of sodium benzoate.

[0010] The propionic acid and its homologous together with acetic acid and its homologous according to the present disclosure belong to the short-chain fatty acid preservatives. The preferred is propionic acid and/or its sodium salt. The esters of p-hydroxybenzoic acid include methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl p-hydroxybenzoate, and butylparaben, and the ethyl 4-hydroxybenzoate is preferred.

[0011] According to the present disclosure, the content of phenoxyethanol calculated as 2-phenylethanol refers to that the content of phenoxyethanol is converted to that of 2-phenylethanol, by the method of multiplying the content of phenoxyethanol with the molecular weight ratio of 2-phenylethanol to phenoxyethanol, and the result obtained is the content of 2-phenylethanol converted from the content of phenoxyethanol. For example, the molecular weight of 2-phenylethanol is 122; the molecular weight of phenoxyethanol is 138. 0.034 percent (weight/volume) of phenoxyethanol can be converted to 0.030 percent of

2-phenylethanol (weight/volume). On the contrary, the content of 2-phenylethanol converted from 0.034 percent (weight/volume) of phenoxyethanol is 0.030 percent (weight/volume). And so on and vice versa.

[0012] According to the present disclosure, the content of benzoic acid, p-methoxybenzoic acid and/or its sodium salt, esters of p-hydroxybenzoate and its homologous calculated as sodium benzoate refers to that the content of benzoic acid, etc. is converted to that of sodium benzoate. The method is to multiply the content of benzoic acid, etc. with the molecular weight ratio of sodium benzoate to benzoic acid, etc., and the result obtained is the content of sodium benzoate converted from the content of benzoic acid, etc. For example, the molecular weight of sodium benzoate is 144. The molecular weight of benzoic acid is 122, the molecular weight of p-methoxybenzoic acid is 152, the molecular weight of sodium p-methoxybenzoate is 174, and the molecular weight of ethyl 4-hydroxybenzoate is 150. Then 0.1 percent (weight/volume) of benzoic acid, 0.127 percent (weight/volume) of p-methoxybenzoic acid, 0.145 percent (weight/volume) of sodium p-methoxybenzoate, and 0.125 percent (weight/volume) of ethyl 4-hydroxybenzoate thereof, are respectively converted into 0.12 percent (weight/volume) of sodium benzoate. And so on and vice versa.

[0013] According to the present disclosure, the content of propionic acid and/or its calcium salt, levulinic acid and/or its sodium salt, or acetic acid and/or its sodium salt calculated as sodium propionate refers to that the content of propionic acid, etc. is converted to that of sodium propionate. The method is to multiply the content of propionic acid, etc. with the molecular ratio of sodium propionate to propionic acid, etc., and the result obtained is the content of sodium propionate converted from the content of propionic acid, etc. For example, the molecular weight of sodium propionate is 96, the molecular weight of propionic acid is 74, the molecular weight of levulinic acid is 116, the molecular weight of sodium levulinate is 138, the molecular weight of acetic acid is 60, and the molecular weight of sodium acetate is 82. Then 0.31 percent (weight/volume) of propionic acid, 0.48 percent (weight/volume) of levulinic acid, 0.575 percent (weight/volume) of sodium

levulinate, 0.25 percent of acetic acid (weight/volume), and 0.341 percent of sodium acetate (weight/volume) are respectively converted into 0.4 percent (weight/volume) of sodium propionate. And so on and vice versa.

[0014] Preferably, the total content of 2-phenylethanol and/or phenoxyethanol calculated as that of 2-phenylethanol is 0.10 to 0.40 percent (weight/volume); the total content of propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate calculated as that of sodium propionate is 0.525 to 0.575 percent (weight/volume); the total content of benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate calculated as that of sodium benzoate is 0.065 to 0.090 percent (weight/volume).

[0015] Preferably, the composition includes (1) 2-phenylethanol and/or phenoxyethanol; (2) propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate; (3) benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate.

[0016] Preferably, the antimicrobial agents are 2-phenylethanol, propionic acid and/or sodium propionate, and benzoic acid and/or sodium benzoate.

[0017] Preferably, the composition further includes one or more antimicrobial agents selected from a group consisting of dehydroacetic acid, sodium dehydroacetate, sorbic acid, potassium sorbate, sodium sorbate, diacetic acid, sodium diacetate, caprylic acid, sodium caprylate, capric acid, sodium caprate, undecylenic acid, sodium undecylenate, lauric acid, sodium laurate, natamycin, lactoferrin, lactoferrin peptide, lysozyme, antibacterial protein, antibacterial peptide, lichenic acid, bergenin, tropolone, chlorogenic acid, palmatine, benzyl alcohol, propylene phenoxyethanol, 1,2 - pentanediol, 1,2 - hexanediol, 1,6 - hexanediol, 1,2 - octanediol, 1,2 - decanediol, 2-methyl-1,3-propanediol, ethylhexylglycerin, and benzoylperoxide. The antimicrobial agents are used to further enhance the antimicrobial effect of the vaginal composition of the present disclosure against *Staphylococcus aureus*, *Escherichia coli*, *Candida*, as well as against other pathogenic microorganisms, such as virus HIV, HPV,

etc.

[0018] Preferably, the composition further includes one or more saccharides selected from the following group consisting of glucose, fructose, mannose, sucrose, isomaltulose, 1-kestose, nistose trihydrate, 1F-fructofuranosylmaltose, maltose, 5 isomaltose, isomaltotriose, isomaltotetraose, isomaltopentaose, trehalose, cellobiose, melibiose, gentiobiose, gentiooligosaccharide, raffinose, panose, maltooligosaccharide, isomaltooligosaccharide, fructooligosaccharide, glucomannan, dextrin, starch, and glycogen, with a total content of 1.0 to 9.0 percent (weight/volume). The saccharide-contained vaginal composition of the present disclosure can modulate 10 vaginal flora biphasically, that is, it can selectively inhibit *Escherichia coli* and/or *Staphylococcus aureus* and/or *Candida*, etc. It can also selectively promote beneficial *Lactobacilli* in the vagina. Compared with the prior art, the vaginal composition of the present disclosure has the advantages of modulating vaginal flora, enhancing the vaginal acidity, and maintaining vaginal beneficial *Lactobacilli*. Thus it can enhance 15 the vaginal resistance to infections and can be used for prevention and/or treatment of vaginal *Lactobacilli* reduction, or vaginal dysbacteriosis, or bacterial vaginosis, or for restoring normal vaginal flora after antibacterial treatment of genital tract infection, or as an adjuvant treatment of genital tract infection. It has also been found out by the inventor that when the total content of saccharides is less than 1 percent 20 (weight/volume), the composition is weak in promoting *Lactobacilli* growth and acid production; and when the total content of saccharides is higher than 9 percent (weight/volume), it may be irritative to vaginal mucosa.

[0019] Preferably, the composition for vagina further includes one or more amino acids selected from a group consisting of glutamic acid, glutamine, aspartic acid, 25 asparagine, isoleucine, phenylalanine, valine, leucine, proline, and threonine, with a total content of 0.1 to 10.0 percent (weight/volume). The amino acids contained composition for vagina of the present disclosure can reduce acid production by vaginal flora, and can be used to treat or assist in the treatment of cytolytic vaginosis, *Lactobacillosis*, and *Candidal vaginitis*. It is also found out by the inventor that when 30 the total content of amino acid is less than 0.1 percent (weight/volume), the

composition is weak in decreasing acid production by vaginal flora; and when the total content of amino acids is higher than 10 percent (weight/volume), it may cause abnormal change of vaginal flora.

[0020] Preferably, the composition further includes one or more estrogen and/or phytoestrogen agents selected from a group consisting of diethylstilbestrol, estradiol, estriol, daidzein, aglycone of daidzein, genistin, genistein, glycitein, aglycone of glycitein, biochanin, coumestrol, and formononetin, with a total content of 0.001 to 1.0 percent (weight/volume). The vaginal composition of the present disclosure that contains estrogen and/or phytoestrogen can promote the vaginal mucosa epithelial cells to synthesize glycogen, maintain or restore the normal vaginal microenvironment, and promote the growth of beneficial lactobacillus to restore the balance of micro-ecosystem.

[0021] Preferably, the composition further includes one or more substances selected from a group consisting of 0.01 to 5.0 percent (weight/volume) of aloe extract, 0.01 to 5.0 percent (weight/volume) of lavender extract, 0.001 to 1.0 percent (weight/volume) of vitamin E, 0.001 to 1.0 percent (weight/volume) of vitamin A, 0.001 to 1.0 percent (weight/volume) of vitamin D, and 0.001 to 1.0 percent (weight/volume) of vitamin C. The composition for vagina prepared by the present disclosure that includes the above substances may be used for protecting vaginal mucosa and promoting the repair of damaged vaginal mucosa.

[0022] Preferably, the dosage form of the composition is a solution, a water-soluble gel, or an emulsive ointment, in which the pH value of the composition is within a range from 3.0 to 4.3.

[0023] According to the use of the present disclosure, the composition for vagina use may be a non-therapeutic vaginal health product, a vaginal use commodity, a vaginal health care product, a vaginal nursing product, a vaginal cosmetic, a vaginal sanitary product, or a vaginal cleaning product; or the composition for vagina use may be a therapeutic vaginal health product, a vaginal medical device, a vaginal disinfection device, a vaginal drug-device, or a vaginal drug; or the composition for vagina may be a lotion, a detergent, a curing agent, an odor removing agent, or an

antipruritic agent, a refreshing agent, a wetting agent, a lubricant, a disinfectant, a fungicide, an antimicrobial agent, a mucosal surface microbicide, a microecological modulator, or a microbial modulator, for vagina.

[0024] The present disclosure also provides a composition for vagina, the composition includes: (1) a total content of 2-phenylethanol and/or phenoxyethanol calculated as that of 2-phenylethanol is from 0.05 to 0.6 percent (weight/volume) (2) a total content of propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate calculated as that of sodium propionate is from 0.25 to 0.7 percent (weight/volume); and/or a total content of benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate calculated as that of sodium benzoate is 0.05 to 0.15 percent (weight/volume).

[0025] The dosage form of the composition is a solution, a water-soluble gel, or an emulsive ointment, in which the pH value of the composition is within a range from 3.0 to 4.3.

[0026] Preferably, a total content of 2-phenylethanol and/or phenoxyethanol calculated as that of 2-phenylethanol is from 0.10 to 0.40 percent (weight/volume); a total content of propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate calculated as that of sodium propionate is from 0.525 to 0.575 percent (weight/volume); and/or a total content of benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate calculated as that of sodium benzoate is from 0.065 to 0.090 percent (weight/volume).

[0027] Preferably, the composition includes: (1) 2-phenylethanol and/or phenoxyethanol; (2) propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate thereof; (3) benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of

p-hydroxybenzoate thereof.

[0028] Preferably, the antimicrobial agents are 2-phenylethanol, propionic acid and/or sodium propionate, and benzoic acid and/or sodium benzoate.

[0029] Preferably, the composition further includes one or more antimicrobial
5 agents selected from a group consisting of dehydroacetic acid, sodium dehydroacetate, sorbic acid, potassium sorbate, sodium sorbate, diacetic acid, sodium diacetate, caprylic acid, sodium caprylate, capric acid, sodium caprate, undecylenic acid, sodium undecylenate, lauric acid, sodium laurate, natamycin, lactoferrin, lactoferrin peptide, lysozyme, antibacterial protein, antibacterial peptide, lichenic acid, bergenin,
10 tropolone, chlorogenic acid, palmatine, benzyl alcohol, propylene phenoxyethanol, 1,2-pentanediol, 1,2-hexanediol, 1,6-hexanediol, 1,2-octanediol, 1,2-decanediol, 2-methyl-1,3-propanediol, ethylhexylglycerin, and benzoyl peroxide.

[0030] Preferably, the composition also includes one or more estrogen and/or phytoestrogen agents selected from a group consisting of diethylstilbestrol, estradiol,
15 estriol, daidzein, aglycone of daidzein, genistin, genistein, glycitein, aglycone of glycitein, biochanin, coumestrol, and formononetin, with a total content of 0.001 to 1.0 percent (weight/volume).

[0031] Preferably, the composition also includes one or more saccharides selected from a group consisting of glucose, fructose, mannose, sucrose, isomaltulose,
20 1-kestose, nistose trihydrate, 1F-fructofuranosylnystose, maltose, isomaltose, isomaltotriose, isomaltotetraose, isomaltopentaose, trehalose, cellobiose, melibiose, gentiobiose, gentiooligosaccharide, raffinose, panose, maltooligosaccharide, isomaltooligosaccharide, fructooligosaccharide, glucomannan, dextrin, starch, and glycogen, with a total content of 1.0 to 9.0 percent (weight/volume).

[0032] Preferably, the saccharide is one or more saccharides selected from the group consisting of glucose, fructose, mannose, sucrose, maltose, isomaltulose, isomaltose, trehalose, and maltooligosaccharide, with a total content of 2.0 to 6.5 percent (weight/volume).

[0033] Preferably, the composition further includes one or more substances
30 selected from a group consisting of 0.01 to 5.0 percent (weight/volume) of aloe

extract, 0.01 to 5.0 percent (weight/volume) of lavender extract, 0.001 to 1.0 percent (weight/volume) of vitamin E, 0.001 to 1.0 percent (weight/volume) of vitamin A, 0.001 to 1.0 percent (weight/volume) of vitamin D, and 0.001 to 1.0 percent (weight/volume) of vitamin C.

5 **[0034]** Preferably, the composition further includes one or more amino acids and/or physiologically acceptable salts thereof, which is selected from a group consisting of glutamic acid, glutamine, aspartic acid, asparagine, isoleucine, phenylalanine, valine, leucine, proline, and threonine, with a content of 0.1 to 10.0 percent (weight/volume).

10 **[0035]** Preferably, the total content of amino acid is from 1.0 to 5.0 percent (weight/volume), and the amino acid is glutamic acid and/or aspartic acid.

[0036] The composition of the present disclosure may be in various packages, including but not limited to a sealed and sterilized single dose package or the composition is packaged in a vaginal device or a disposable vaginal applicator. Sealed
15 and sterilized single dose package is preferable. The producing processes, method, and the choice of adjuvant are all known to those skilled in the art based on the disclosure of the present disclosure.

[0037] The composition of the present disclosure may optionally comprise a low-dose antimicrobial drug such as metronidazole or tinidazole, etc. The content of
20 metronidazole or tinidazole in single unit dosage or single unit package or unit dosage form such as a tablet, a suppository, or a single dosage ointment is from 0.001 to 0.5 milligrams, preferably 0.01 to 0.25 milligrams. The composition of the present disclosure including low dosage metronidazole or tinidazole has the effects of nursing and/or modulating vaginal micro-ecosystem, inhibiting anaerobic bacteria, and
25 keeping vaginal micro-ecosystem in balance.

[0038] The present disclosure also provides a method for manufacturing a vaginal composition, which includes the following steps: adding the following agents into water, and/or water-soluble gel matrix, and/or emulsive ointment matrix: (1) a total content of 2-phenylethanol and/or phenoxyethanol calculated as that of
30 2-phenylethanol is 0.05 to 0.6 percent (weight/volume); (2) a total content of

propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate calculated as that of sodium propionate is 0.25 to 0.7 percent (weight/volume); and/or a total content of benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate calculated as that of sodium benzoate is 0.05 to 0.15 percent (weight/volume).

[0039] For example, viscous, non-flowable, water-soluble gel matrixes can be used in the preparation of the gel composition, such as Carbomer, and/or methylcellulose (MC), and/or glucomannan, and/or tragacanth gum, and/or west tragacanth gum, and/or Xanthan gum, among which Xanthan gum is preferred. The gel composition can be manufactured according to a method known to those skilled in the art. For example, the following agents will be weighed proportionally according to the technological process: (1) 2-phenylethanol and/or phenoxyethanol; (2) propionic acid, and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate; and/or benzoic acid and/or sodium benzoate and/or p-methoxybenzoic acid and/or sodium p-methoxybenzoate and/or esters of p-hydroxybenzoate; (3) other components, and (4) Xanthan gum. Homogeneously mixing the above components. Quantitatively adding distilled water and stirring to dissolve the components and to swell Xanthan gum so to form a homogenous gel. Adjusting the pH value of the composition to a range from 3.0 to 4.3 with acid and/or alkali. Further sterilization can be carried out. There are a variety of sterilization processes to choose from: radiation sterilization, intermittent sterilization (such as sterilization at 60°C for 15 minutes, then at 36 °C for 6 to 12 hours, then at 60°C for 15 minutes, then at 36 °C for 6 to 12 hours, and finally at 60 °C for 15 minutes). Or the components such as 2-phenylethanol, sodium propionate, and sodium benzoate are separately prepared into solution, filtered and sterilized, and then added to the sterilized water-soluble colloidal matrix.

[0040] For example, when preparing an emulsive ointment, a matrix substance known to those skilled in the art can be chosen, such as glyceryl monostearate, and/or paraffin wax, and/or beeswax, and/or white vaseline, and/or sodium lauryl sulfate,

and/or polysorbate, etc.

[0041] For example, when preparing a solution, the above-mentioned components other than a water-soluble colloidal matrix or an emulsive ointment matrix can be mixed, watered, dissolved, sterilized, and reserved. Cotton balls or tampons can be soaked with the solution and used vaginally. Or can be made into a vaginal lotion.

[0042] According to the technical proposals of the present disclosure, the composition of the present disclosure can be used to eliminate or alleviate vaginal discomfort such as vulvovaginal pruritus, pain, and/or sexual intercourse pain, and/or to improve leucorrhea properties, and/or eliminate leucorrhea smell, and/or clean and care vagina.

[0043] Accordingly, the present disclosure also relates to a method for preventing and/or treating vaginal infectious diseases; the method includes administering an effective amount of the composition of the present disclosure into the vagina of a woman in need. The vaginal infectious diseases include, but are not limited to, Candidal vaginitis, cytolytic vaginosis, Lactobacillosis, vaginal dysbacteriosis, senile vaginitis, aerobic vaginitis, and bacterial vaginosis. According to the technical proposal of the present disclosure, the composition is applied to the vagina of a woman in need. For example, the composition of the water-soluble gel dosage form of the present disclosure is applied into the vagina with an injector, or the composition of solution dosage form is soaked by a vaginal tampon or a cotton ball or a vaginal plug, and then placed into the vagina.

DETAILED DESCRIPTION

[0044] Composition Examples

[0045] Example 1:

[0046] The water-soluble gel composition of the present disclosure can be prepared by the following method: homogeneously mixing 0.05grams sodium benzoate, 0.25grams sodium propionate, and 1.0gram Xanthan gum, adding 100 milliliters distilled water and stirring to dissolve sodium benzoate and sodium

propionate and to swell the Xanthan gum into a uniform viscous gel. Then adding 0.05 grams 2-phenylethanol, adjusting the pH value of the solution to 3.0, and finally heating at 60°C for 15 minutes.

[0047] Example 2:

5 **[0048]** The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following ratio:

	2-Phenylethanol	0.60 percent (weight/volume)
	Sodium propionate	0.70 percent (weight/volume)
10	Glucose	1.00 percent (weight/volume)
	Xanthan gum	2.15 percent (weight/volume)
	Distilled water	100 milliliters
	pH	4.0

[0049] Example 3:

15 **[0050]** The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following ratio:

	2-Phenylethanol	0.06 percent (weight/volume)
	Sodium propionate	0.55 percent (weight/volume)
20	Sodium benzoate	0.12 percent (weight/volume)
	Maltose	6.50 percent (weight/volume)
	Xanthan gum	2.0 percent (weight/volume)
	Distilled water	100 milliliters
	pH	3.2

25 **[0051]** Example 4:

[0052] The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following ratio:

	2-Phenylethanol	0.50 percent (weight/volume)
30	Sodium benzoate	0.075 percent (weight/volume)

Distilled water 100 milliliters

pH 3.5

[0053] Example 5:

[0054] The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following ratio:

2-phenylethanol 0.45 percent (weight/volume)

Sodium propionate 0.60 percent (weight/volume)

Distilled water 100 milliliters

10 pH 4.0

[0055] Example 6:

[0056] The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following ratio:

15 2-Phenylethanol 0.35 percent (weight/volume)

Sodium propionate 0.55 percent (weight/volume)

Glutamic acid 3.0 percent

Aspartic acid 3.0 percent

Xanthan gum 1.5 percent(weight/volume)

20 Distilled water 100 milliliters

pH 4.0

[0057] Example 7:

[0058] The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following ratio:

2-Phenylethanol 0.20 percent (weight/volume)

Sodium propionate 0.575 percent (weight/volume)

Sodium benzoate 0.075 percent (weight/volume)

Maltose 5.00 percent (weight/volume)

30 Xanthan gum 1.00 percent (weight/volume)

Distilled water 100 milliliters

pH 3.5

[0059] Example 8:

[0060] The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following substances:

2-Phenylethanol 0.3 grams, sodium propionate 0.5 grams, sodium benzoate 0.06 grams, and lactoferrin 0.5 grams;

Glutamic acid 0.5 grams, glutamine 0.25 grams, aspartic acid 0.5 grams, asparagine 0.25 grams, isoleucine 0.25 grams, methionine 0.25 grams, phenylalanine 0.25 grams, valine 0.25 grams, leucine 0.25 grams, and proline 0.25 grams;

Xanthan gum 1.5 grams, distilled water 100 milliliters, the pH was adjusted to 3.5.

[0061] Example 9:

[0062] The 100 milliliters composition can be prepared basically according to the technological processes of Example 1 by weighing the ingredients according to the following ratio:

Phenoxyethanol 0.05 percent (weight/volume)

Sodium propionate 0.50 percent (weight/volume)

Sodium benzoate 0.15 percent (weight/volume)

Xanthan gum 1.2 percent (weight/volume)

Distilled water 100 milliliters

pH 4.3

[0063] Example 10:

[0064] The following solution composition can be prepared basically according to the conventional technological processes in the field:

2-Phenylethanol 0.60 percent (weight/volume)

Sodium benzoate 0.15percent (weight/volume)

Sodium propionate 0.70percent (weight/volume)

Distilled water 100 milliliters

pH 4.0

[0065] Experimental Example

[0066] Experimental Example 1

[0067] 1. Experimental objective: to observe the inhibition effects of each of the following agents in a specific concentration range against *Lactococcus lactis* and *Lactobacillus*: 2-phenylethanol, propionic acid and/or its salt, benzoic acid and/or its sodium salt, acetic acid and/or its salt, dehydroacetic acid and/or its salt, and caprylic acid and/or its salt.

[0068] 2. Experimental method:

10 **[0069]** 2.1 Experimental strains: one *Lactococcus lactis* strain and one *Lactobacillus jensenii* strain, both were clinical isolates.

[0070] 2.2 Preparation of base gel: 1.0 percent Xanthan gum, 1.5 percent isomaltulose, and 10 percent calf serum were added into MRS liquid medium. The pH value was adjusted to 5.0.

15 **[0071]** 2.3 preparing based on the base gel the test gels containing different concentrations of antimicrobial agents, as shown in Tables 1 to 6, then dividing the test gels into tubes, 5ml per tube and reserved.

[0072] 2.4 Experimental procedure: the test gel tubes containing different concentrations of antimicrobial agents, as shown in Tables 1 to 6 were inoculated with 100 microliters of *Lactobacillus lactis* suspension and 100 microliters of *Lactobacillus jensenii* suspension at the same time. The turbidities of both suspensions were 0.5 MCF. Then the tubes were cultivated at 37°C and under 5 percent CO₂ for 32 hours. Then the changes of the pH values of gels were tested. The gels were smeared, Gram stained, and microscopically examined for the morphology and quantity of bacteria.

25 **[0073]** 3. Experimental result:

[0074] 3.1 As shown in table 1, the 0.35 to 0.45 percent of 2-phenylethanol had a stronger inhibition effect on the growth of *Lactococcus lactis* and a weaker inhibition effect on the growth of *Lactobacillus*. However, the pH values were in the range from 4.1 to 4.6 and did not decrease to 3.8, which indicated that acid production was inhibited.

30

Table 1. Inhibitory effect of 2-phenylethanol on the growth of *Lactococcus lactis* and *Lactobacillus jensenii*

Concentration (%)	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
pH value	<3.8	<3.8	<3.8	<3.8	3.8 -4.1	4.4	4.6	4.8
Smear	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c+~++	G ⁺ c+	G ⁺ c0~+	G ⁺ c0~+
	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+++	G ⁺ b+++	G ⁺ b++	G ⁺ b+

[0075] 3.2 As shown in table 2, the 0.4 to 0.6 percent of sodium propionate had a stronger inhibition effect on the growth of *Lactococcus lactis* and a weaker inhibition effect on the growth of *Lactobacillus*, which indicated that the antimicrobial action was selective. However, the pH value was in the range from 4.4 to 4.6, did not decrease to 3.8, which indicated that acid production was inhibited.

Table 2. Inhibitory effect of sodium propionate on the growth of *Lactococcus lactis* and *Lactobacillus jensenii*

Concentration (%)	0.1	0.2	0.3	0.4	0.5	0.6	0.7
pH value	3.8	3.8	3.8	4.4	4.4 -4.6	4.6	4.8
	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c+	G ⁺ c+	G ⁺ c0~+	G ⁺ c0~+
Smear	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+++	G ⁺ b+++	G ⁺ b++	G ⁺ b+

[0076] 3.3 As shown in table 3, the 0.065 to 0.1 percent of sodium benzoate had a stronger inhibition effect on the growth of *Lactococcus lactis* and a weaker inhibition effect on the growth of *Lactobacillus*, which indicated that the antimicrobial effect was selective. However, the pH values were in the range of 4.1 to 4.6 and did not decrease to 3.8, which indicated that acid production was inhibited.

Table 3. Inhibitory effect of sodium benzoate on the growth of *Lactococcus*

lactis and Lactobacillus jensenii				
Concentration	0.035	0.05	0.065	0.10
(%)				
pH value	<3.8	<3.8	4.1	4.6
Smear	G ⁺ c++++	G ⁺ c++++	G ⁺ c++	G ⁺ c+
	G ⁺ b+	G ⁺ b+	G ⁺ b++	G ⁺ b+++

[0077] 3.4 As shown in table 4, 0.1 to 0.8 percent of sodium acetate had little inhibition effect on the growth of Lactococcus lactis. Lactococcus lactis grew more, while Lactobacillus grew less.

5 Table 4. Inhibitory effect of sodium acetate on the growth of lactococcus lactis and Lactobacillus jensenii

Concentration	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
(%)								
pH value	<3.8	<3.8	<3.8	<3.8	<3.8	3.8	3.8	3.8-4.1
Smear	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++
	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+

[0078] 3.5 As shown in table 5, 0.005 to 0.035 percent of sodium dehydroacetate had little inhibition effect on the growth of Lactococcus lactis. Lactococcus lactis grew more, while Lactobacillus grew less.

10 Table 5. Inhibitory effect of sodium dehydroacetate on growth of Lactococcus lactis and Lactobacillus jensenii

Concentration	0.005	0.010	0.015	0.020	0.025	0.030	0.035
(%)							
pH value	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Smear	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++	G ⁺ c++++
	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+	G ⁺ b+

[0079] 3.6 As shown in table 6, 0.010 to 0.020 percent of sodium caprylate had little inhibition effect on the growth of *Lactococcus lactis*. *Lactococcus lactis* grew more, while *Lactobacillus* grew less. However, 0.05 percent of sodium caprylate had a stronger inhibition effect on both of the *Lactococcus lactis* and *Lactobacillus*.

5 Table 6. Inhibitory effects of sodium octanoate on the growth of *Lactococcus lactis* and *Lactobacillus jensenii*

Concentration (%)	0.000	0.010	0.015	0.020	0.050
pH value	<3.8	<3.8	<3.8	3.8	4.8
	G ^{+c} ++++	G ^{+c} ++++	G ^{+c} ++++	G ^{+c} ++++	G ^{+c} 0~+
Smear	G ^{+b} +	G ^{+b} +	G ^{+b} +	G ^{+b} +	G ^{+b} 0~+

[0080] 4. Conclusion: each one of 2-phenylethanol, propionic acid and/or its salt, and benzoic acid and/or its sodium salt in a specific concentration range had a strong inhibition effect on *Lactococcus lactis* and a weak inhibition effect on *Lactobacillus jensenii*, which indicated that the antimicrobial effect was selective. Similar selectivity of acetic acid and/or its salt, dehydroacetic acid and/or its salt, and caprylic acid and/or its salt in inhibiting *Lactococcus lactis* and *Lactobacillus* had not been observed in a specific concentration range.

[0081] Experimental example 2

15 **[0082]** 1. Experimental objective: to observe the inhibition effects of solution compositions containing any two agents selected from a group consisting of 2-phenylethanol, sodium benzoate, sodium propionate, and sodium dehydroacetate.

[0083] 2. Experimental method:

[0084] A. Experimental strains: *Escherichia coli* (ATCC25922) and
20 *Staphylococcus aureus* (ATCC6538).

[0085] B. Experimental grouping: grouping the antimicrobial solution compositions into group-a and the group-b.

[0086] Group-a: all of the antimicrobial solutions in group-a contained water and antimicrobial agents. Specific compatibilities of antimicrobial agents were shown in

Table 7A; all of the antimicrobial solutions had a pH value of 4.3.

[0087] Group-b: all of the antimicrobial solutions in group-b contained water, antimicrobial agents, and 6.5 percent (weight/volume) of maltose. Specific compatibilities of antimicrobial agents in group-b were referred to Table 7B, which
5 were correspondingly identical to that of group-a. All of the antimicrobial solutions had pH values of 4.3.

[0088] C. Experimental method: the experiment was implemented basically according to the method in appendix C of GB15979-2002.

[0089] 3. Experimental result: as shown in Tables 7A and 7B, the inhibition ratio
10 of each antimicrobial solution with a pH value of 4.3 was presented.

Table 7A. Antimicrobial effects of the compatibility of two antimicrobial agents against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
		2-Phenylethanol	Sodium propionate	Sodium benzoate	Sodium dehydroacetate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	4.3	0.35	0	0	0	49	0.00%	32	31.25%
Antimicrobial solution 2	4.3	0	0.5	0	0	47	0.00%	37	18.75%
Antimicrobial solution 3	4.3	0	0	0.06	0	54	0.00%	33	28.13%
Antimicrobial solution 4	4.3	0	0	0	0.02	32	17.95%	48	0.00%
Antimicrobial solution 5	4.3	0.35	0.5	0	0	7	82.05%	13	71.74%

Antimicrobial solution 6	4.3	0.35	0	0.06	0	2	94.12%	1	96.77%
Antimicrobial solution 7	4.3	0.35	0	0	0.02	32	17.65%	37	19.36%
Antimicrobial solution 8	4.3	0	0.5	0.06	0	31	20.0%	24	46.88%
Antimicrobial solution 9	4.3	0	0.5	0	0.02	23	41.18%	48	0.00%
Antimicrobial solution 10	4.3	0	0	0.06	0.02	42	0.00%	37	19.35%
Positive Control	7.0	0	0	0	0	39	0.00%	46	0.00%
Negative control	7.0	0	0	0	0	0	0.00%	0	0.00%

Table 7B. Antimicrobial effects of the compatibility of two antimicrobial agent against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Maltose (%)	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
			2-Phenylethanol	Sodium propionate	Sodium benzoate	Sodium dehydroacetate	Colony count after 20 minutes	Antimicrobial rate	Colony count after 20 minutes	Antimicrobial rate
Antimicrobial solution 1	4.3	6.5	0.35	0	0	0	53	0.00%	44	8.70%
Antimicrobial solution 2	4.3	6.5	0	0.5	0	0	56	0.00%	41	15.22%
Antimicrobial solution 3	4.3	6.5	0	0	0.06	0	61	0.00%	40	17.39%
Antimicrobial solution 4	4.3	6.5	0	0	0	0.02	45	12.82%	50	0.00%
Antimicrobial	4.3	6.5	0.35	0.5	0	0	12	76.92%	16	67.39%

solution 5										
Antimicrobial solution 6	4.3	6.5	0.35	0	0.06	0	4	92.31%	2	95.65%
Antimicrobial solution 7	4.3	6.5	0.35	0	0	0.02	43	17.95%	41	15.22%
Antimicrobial solution 8	4.3	6.5	0	0.5	0.06	0	40	23.08%	26	45.65%
Antimicrobial solution 9	4.3	6.5	0	0.5	0	0.02	27	48.72%	51	0.00%
Antimicrobial solution 10	4.3	6.5	0	0	0.06	0.02	53	0.00%	38	21.74%
Positive Control	7	6.5	0	0	0	0	52	0.00%	48	0.00%
Negative control	7	0	0	0	0	0	0	0.00%	0	0.00%

[0090] 4. Conclusion:

[0091] 4.1 There was no significant difference between the inhibition ratio of two compatibilities that were identical in the content of antimicrobial agents and pH values, despite whether the 6.5 percent of maltose was contained or not.

- 5 **[0092]** 4.2 When the pH value of the solution was 4.3, the compatibility of 0.35 percent (weight/volume) of 2-phenylethanol and 0.06 percent (weight/volume) of sodium benzoate had a synergistic antimicrobial effect against Escherichia coli and Staphylococcus aureus, the inhibition ratio thereof exceeded 50 percent. The compatibility of 0.35 percent (weight/volume) of 2-phenylethanol and 0.5 percent (weight/volume) of sodium propionate had a synergistic antimicrobial effect against Escherichia coli and Staphylococcus aureus, and the inhibition ratio thereof exceeded 50 percent.

- 15 **[0093]** 4.3 When the pH value of the solution was 4.3, the compatibility of 0.35 percent (weight/volume) of 2-phenylethanol and 0.02 percent (weight/volume) of sodium dehydroacetate, the compatibility of 0.06 percent (weight/volume) of sodium

benzoate and 0.02 percent (weight/volume) of sodium dehydroacetate, the compatibility of 0.5 percent (weight/volume) of sodium propionate and 0.02 percent (weight/volume) of sodium dehydroacetate, and the combination of 0.06 percent (weight/volume) of sodium benzoate and 0.5 percent (weight/volume) of sodium propionate thereof, exhibited a weaker antimicrobial effect respectively..

[0094] 4.4 In conclusion, when the pH values of antimicrobial solution compositions were 4.3, the compatibility of 2-phenylethanol with sodium benzoate in specific concentration had a synergistic antimicrobial effect against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538). The compatibility of 2-phenylethanol with sodium propionate in specific concentration had a synergistic antimicrobial effect against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538).

[0095] However, any of the following compatibilities, i.e., 2-phenylethanol with sodium dehydroacetate, sodium propionate with sodium dehydroacetate, sodium propionate with sodium benzoate, and sodium dehydroacetate with sodium benzoate thereof, was less effective in inhibiting *Escherichia coli* and *Staphylococcus aureus* than that of the compatibility of 2-phenylethanol with sodium benzoate or with sodium propionate.

[0096] Experimental example 3

[0097] 1. Experimental objective: to observe the impact of pH on the inhibition effects of solution compositions containing any two agents selected from a group consisting of 2-phenylethanol, sodium benzoate, sodium propionate, and sodium dehydroacetate.

[0098] 2. Experimental method:

[0099] A. Experimental strains: *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC 6538).

[00100] B. Experimental groups: as shown in Tables 8 and 9, each antimicrobial combination solution contained water and antimicrobial agents.

[00101] C. Experimental procedure: the experiment was implemented basically according to the method in appendix C of GB15979-2002.

[00102] 3. Experimental result: as shown in Tables 8 and 9, the inhibition ratio of antimicrobial solutions with a pH value of 4.1 and the antimicrobial solutions with a pH value of 4.5 were presented:

5 Table 8. The inhibitory effects of solutions containing two antimicrobial agents with pH value of 4.1 against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
		2-Phenyl ethanol	Sodium propionate	Sodium benzoate	Sodium dehydroacetate	colony count after 20 minutes	Inhibition ratio	colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	4.1	0.35	0.5	0	0	6	95.45%	20	92.34%
Antimicrobial solution 2	4.1	0.35	0	0.06	0	1	99.24%	1	99.62%
Antimicrobial solution 3	4.1	0.35	0	0	0.02	94	28.79%	133	49.04%
Antimicrobial solution 4	4.1	0	0.5	0.06	0	122	7.58%	184	29.50%
Antimicrobial solution 5	4.1	0	0.5	0	0.02	68	48.48%	245	6.13%
Antimicrobial solution 6	4.1	0	0	0.06	0.02	73	44.70%	220	15.71%
Positive Control	7.0	0	0	0	0	132	0.00%	261	0.00%
Negative control	7.0	0	0	0	0	0	0	0	0

Table 9. The inhibitory effects of solutions containing two antimicrobial agents with a pH value of 4.5 against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
		2-Phenylethanol	Sodium m	Sodium m	Sodium m	Colony count	Inhibition ratio	Colony count	Inhibition ratio

		nol	propio nate	benzo ate	dehyd roacet ate	after 20 minutes		after 20 minutes	
Antimicrob ial solution 1	4.5	0.35	0.5	0	0	30	34.78%	61	12.86%
Antimicrob ial solution 2	4.5	0.35	0	0.06	0	39	15.22%	61	12.86%
Antimicrob ial solution 3	4.5	0.35	0	0	0.02	53	0.00%	84	0.00%
Antimicrob ial solution 4	4.5	0	0.5	0.06	0	69	0.00%	75	0.00%
Antimicrob ial solution 5	4.5	0	0.5	0	0.02	41	10.87%	48	31.43%
Antimicrob ial solution 6	4.5	0	0	0.06	0.02	78	0.00%	77	0.00%
Positive Control	7.0	0	0	0	0	46	0.00%	70	0.00%
Negative control	7.0	0	0	0	0	0	0	0	0

[00103] 4. Conclusion:

[00104] Compared with the previous experimental example 2, the pH values of the antimicrobial solutions of this experimental example 3 were adjusted to 4.1 and 4.5, while the concentration of each antimicrobial agent was not changed, so as to observe the impact of pH on the inhibition effects of solution compositions.

[00105] When the pH value of the solution compositions was 4.1, the combination solution of 0.35 percent (weight/volume) of 2-phenylethanol and 0.5 percent (weight/volume) of sodium propionate, and the combination solution of 0.35 percent (weight/volume) of 2-phenylethanol and 0.06 percent (weight/volume) of sodium benzoate, respectively had a stronger antimicrobial effect against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538), and the inhibition ratio of these combination solutions were higher than that of the corresponding solutions with pH value of 4.3. However, when the pH value of the solution was 4.5, the

combination solution of 0.35 percent (weight/volume) of 2-phenylethanol and 0.5 percent (weight/volume) of sodium propionate, and the combination solution of 0.35 percent (weight/volume) of 2-phenylethanol and 0.06 percent (weight/volume) of sodium benzoate respectively had a weaker antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*.

[00106] However, even if the pH value of the solution was 4.1, the inhibition effects of all the combinations such as the combination of 2-phenylethanol and sodium dehydroacetate, the combination of sodium benzoate and sodium dehydroacetate, the combination of sodium propionate and sodium dehydroacetate, and the combination of sodium benzoate and sodium propionate, was still weak, and the inhibition ratio of these combinations were lower than 50 percent respectively.

[00107] To sum up and combined with the results of experimental example 2, it was shown that the combination of 2-phenylethanol and sodium benzoate, and the combination of 2-phenylethanol and sodium propionate of specific concentration were synergistic in inhibiting against *Escherichia coli* and *Staphylococcus aureus*. The pH value had significant influence on the inhibition effects of the combination solution of 2-phenylethanol and sodium benzoate, and the combination solution of 2-phenylethanol and sodium propionate. In the range from 4.1 to 4.5, the solution with a lower pH value had a stronger synergistic inhibition effect, and the solution with a higher pH value had a weaker synergistic inhibition effect.

[00108] Experimental example 4

[00109] 1. Experimental objective: to observe the inhibition effects of the antimicrobial gels containing any two agents selected from a group consisting of 2-phenylethanol, sodium benzoate, sodium propionate, and sodium dehydroacetate..

[00110] 2. Experimental method:

[00111] A. Experimental strains: *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC 6538).

[00112] B. Experimental groups: as shown in Table 10, each antimicrobial combination gel contained water, antimicrobial agent, and xanthan gum,.

[00113] C. Experimental procedure: the experiment was implemented basically

according to the method in appendix C of GB15979-2002.

[00114] 3. Experimental result: as shown in Table 10, the inhibition ratio of each antimicrobial gel with pH value 4.3 was presented:

Table 10. The inhibitory effects of the gels containing two antimicrobial agents
5 against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Xanthan gum(%)	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
			2-Phenylethanol	Sodium propionate	Sodium benzoate	Sodium dehydroacetate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	4.3	1.0	0.375	0	0	0	127	0.00%	95	16.67%
Antimicrobial solution 2	4.3	1.0	0	0.55	0	0	150	0.00%	67	41.23%
Antimicrobial solution 3	4.3	1.0	0	0	0.06	0	100	10.71%	130	0.00%
Antimicrobial solution 4	4.3	1.0	0	0	0	0.02	100	10.71%	138	0.00%
Antimicrobial solution 5	4.3	1.0	0.375	0.55	0	0	29	74.11%	33	71.05%
Antimicrobial solution 6	4.3	1.0	0.375	0	0.06	0	66	41.07%	56	50.88%
Antimicrobial solution 7	4.3	1.0	0.375	0	0	0.02	80	28.57%	114	0.00%
Antimicrobial solution 8	4.3	1.0	0	0.55	0.06	0	86	23.21%	116	0.00%
Antimicrobial solution 9	4.3	1.0	0	0.55	0	0.02	92	17.86%	110	3.51%

Antimicrobial solution 10	4.3	1.0	0	0	0.06	0.02	78	30.36%	161	0.00%
Positive Control	7	1.0	0	0	0	0	112	0.00%	114	0.00%
Negative control	7	0	0	0	0	0	0	0.00%	0	0.00%

[00115] 4. Conclusion:

[00116] 4.1 When the pH value of the antimicrobial gel was 4.3, 0.375 percent (weight/volume) of 2-phenylethanol combined with 0.06 percent (weight/volume) of sodium benzoate had a synergistic antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*, and the inhibition ratio of the combination against *Staphylococcus aureus* exceeded 50 percent. 0.375 percent (weight/volume) of 2-phenylethanol combined with 0.55 percent (weight/volume) of sodium propionate had a synergistic antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*, and both of the inhibition ratio against *Escherichia coli* and on *Staphylococcus aureus* exceeded 50 percent.

[00117] 4.2 When the pH value of antimicrobial gel was 4.3, the antimicrobial effect of the following combinations was not as good as that of the combination of 2-phenylethanol with sodium benzoate or with sodium propionate: the combination of 0.375 percent (weight/volume) of 2-phenylethanol and 0.02 percent (weight/volume) of sodium dehydroacetate, the combination of 0.06 percent (weight/volume) of sodium benzoate and 0.02 percent (weight/volume) of sodium dehydroacetate, the combination of 0.55 percent (weight/volume) of sodium propionate and 0.02 percent (weight/volume) of sodium dehydroacetate, and the combination of 0.06 percent (weight/volume) of sodium benzoate and 0.55 percent (weight/volume) of sodium propionate.

[00118] In conclusion, the inhibition effects of the antimicrobial gels as revealed in the experimental example further demonstrated that the combination of 2-phenylethanol and sodium propionate, and the combination of 2-phenylethanol and sodium benzoate had a strong synergistic antimicrobial effect against *Escherichia coli*

and/or *Staphylococcus aureus*.

[00119] Experimental example 5

[00120] 1. Experimental objective: to observe the inhibition effects of the combination of 2-phenylethanol and sodium acetate, the combination of 2-phenylethanol and potassium sorbate, the combination of 2-phenylethanol and ethyl p-hydroxybenzoate, and the combination of 2-phenylethanol and sodium para-methoxybenzoate, against *Escherichia coli* and *Staphylococcus aureus*.

[00121] 2. Experimental method:

[00122] A. Experimental strains: *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC 6538).

[00123] B. Experimental groups: as shown in Table 11, each antimicrobial combination solution contained water and antimicrobial agents..

[00124] C. Experimental procedure: the experiment was implemented basically according to the method in appendix C of GB15979-2002.

[00125] 3. Experimental result: the inhibition effects of the combination solutions of 2-phenylethanol and sodium acetate were shown in Table 11. The inhibition effects of the combination solutions of 2-phenylethanol and potassium sorbate were shown in Table 12. The inhibition effects of the combination solutions of 2-phenylethanol and ethyl p-hydroxybenzoate were shown in Table 13. The inhibition effects of the combination solutions of 2-phenylethanol and sodium para-methoxybenzoate were shown in Table 14.

Table 11. The inhibitory effects of the combination of 2-phenylethanol and sodium acetate against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		2-Phenylethanol	Sodium acetate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	3.5	0.35	0	117	13.97%	161	13.44%
Antimicrobial solution 2	3.5	0.4	0	101	25.74%	168	9.68%

Antimicrobial solution 2							
Antimicrobial solution 3	3.5	0	0.6	113	16.91%	163	12.37%
Antimicrobial solution 4	3.5	0	0.8	122	10.29%	168	9.68%
Antimicrobial solution 5	3.5	0.35	0.6	24	82.35%	150	19.35%
Antimicrobial solution 6	3.5	0.4	0.6	0	100.00%	167	10.22%
Antimicrobial solution 7	3.5	0.35	0.8	0	100.00%	164	11.83%
Antimicrobial solution 8	3.5	0.4	0.8	0	100.00%	161	13.44%
Positive Control	7.0	0	0	136	0.00%	186	0.00%
Negative control	7.0	0	0	0	0.00%	0	0.00%

Table 12. The inhibitory effects of the combination of 2-phenylethanol and potassium sorbate against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		2-phenyl ethanol	Potassium sorbate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	3.5	0.35	0	125	19.35%	203	10.96%
Antimicrobial solution 2	3.5	0.4	0	110	29.03%	188	17.54%
Antimicrobial solution 3	3.5	0	0.06	155	0.00%	233	0.00%
Antimicrobial	3.5	0	0.09	125	19.35%	179	21.49%

solution 4							
Antimicrobial solution 5	3.5	0.35	0.06	70	54.84%	180	21.05%
Antimicrobial solution 6	3.5	0.4	0.06	7	95.48%	150	34.21%
Antimicrobial solution 7	3.5	0.35	0.09	2	98.71%	89	60.96%
Antimicrobial solution 8	3.5	0.4	0.09	0	100.00%	78	65.79%
Positive Control	7.0	0	0	155	0.00%	228	0.00%
Negative control	7.0	0	0	0	0.00%	0	0.00%

Table 13. The inhibitory effects of the combination of 2-phenylethanol and ethyl Benzoate against Escherichia coli and Staphylococcus aureus

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		2-Phenylethanol	Ethyl p-hydroxybenzoate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	3.5	0.2	0	84	18.45%	201	0.00%
Antimicrobial solution 2	3.5	0.3	0	65	36.89%	122	33.70%
Antimicrobial solution 3	3.5	0	0.025	94	8.74%	185	0.00%
Antimicrobial solution 4	3.5	0	0.05	79	23.30%	113	38.59%
Antimicrobial solution 5	3.5	0.2	0.025	74	28.16%	105	42.93%
Antimicrobial solution 6	3.5	0.3	0.025	44	57.28%	50	72.83%

Antimicrobial solution 7	3.5	0.2	0.05	32	68.93%	44	76.09%
Antimicrobial solution 8	3.5	0.3	0.05	0	100.00%	40	78.26%
Positive Control	7.0	0	0	103	0.00%	184	0.00%
negative control	7.0	0	0	0	0.00%	0	0.00%

Table 14. Inhibitory effects of the combination of 2-phenylethanol and sodium anisate against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		2-phenyl ethanol	sodium anisate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	3.5	0.35	0	86	31.75%	144	15.79%
Antimicrobial solution 2	3.5	0.4	0	83	34.13%	139	18.71%
Antimicrobial solution 3	3.5	0	0.08	107	15.08%	136	20.47%
Antimicrobial solution 4	3.5	0	0.1	93	26.19%	132	22.81%
Antimicrobial solution 5	3.5	0.35	0.08	48	61.90%	136	20.47%
Antimicrobial solution 6	3.5	0.4	0.08	8	93.65%	116	32.16%
Antimicrobial solution 7	3.5	0.35	0.1	24	80.95%	150	12.28%
Antimicrobial	3.5	0.4	0.1	6	95.24%	128	25.15%

solution 8							
Positive Control	7.0	0	0	126	0.00%	171	0.00%
Negative control	7.0	0	0	0	0.00%	0	0.00%

[00126] 4. Conclusion:

[00127] It has been found out that at a low pH (pH 3.5), the combination solution of 0.35 to 0.4 percent (weight/volume) of 2-phenylethanol and 0.6 to 0.8 percent (weight/volume) of sodium acetate had a synergistic antimicrobial effect against Escherichia coli. No synergistic antimicrobial effect against Staphylococcus aureus was observed.

[00128] At a low pH (pH 3.5), the combination solution of 0.35 to 0.4 percent (weight/volume) of 2-phenylethanol and 0.09 percent (weight/volume) of potassium sorbate have a synergistic antimicrobial effect against Escherichia coli and Staphylococcus aureus. But the inhibition ratio against Staphylococcus aureus was significant lower than that of the combination solution of 0.35 percent (weight/volume) of 2-phenylethanol and 0.05 percent (weight/volume) of sodium benzoate (pH 4.1), of which the inhibition ratio was higher than 99 percent.

[00129] At a low pH (pH 3.5), the combination solution of 0.3 percent (weight/volume) of 2-phenylethanol and 0.025 percent (weight/volume) of ethyl p-hydroxybenzoate had a synergistic antimicrobial effect against Escherichia coli and Staphylococcus aureus. The combination of 0.2 to 0.3 percent (weight/volume) of 2-phenylethanol and 0.050 percent (weight/volume) of ethyl p-hydroxybenzoate had a synergistic antimicrobial effect against Escherichia coli and Staphylococcus aureus, However, the inhibition ratio against Staphylococcus aureus was lower than that of the combination solution (pH 4.1) of 0.35 percent (weight/volume) of 2-phenylethanol and 0.05 percent (weight/volume) of sodium benzoate against Staphylococcus aureus, of which the inhibition ratio was higher than 99 percent.

[00130] At a low pH (pH 3.5), the combination of 0.35 to 0.4 percent (weight/volume) of 2-phenylethanol and 0.08 to 0.1 percent (weight/volume) of sodium anisate had a synergistic antimicrobial effect against Escherichia coli,

However, no synergistic antimicrobial effect of the combination against *Staphylococcus aureus* was observed.

[00131] Experimental example 6

[00132] 1. Experimental objective: to observe inhibition effects of solution compositions containing three antimicrobial agents.

[00133] 2. Experimental method:

[00134] A. Experimental strains: *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC 6538).

[00135] B. Experimental groups: as shown in Table 15, each antimicrobial solution contained water and antimicrobial agents,.

[00136] C. Experimental procedure: the experiment was implemented basically according to the method in appendix C of GB15979-2002.

[00137] 3. Experimental result: as shown in Table 15, the inhibition effects of antimicrobial solutions with pH value of 4.3 were presented:

Table 15. The inhibitory effects of the combination solutions containing three antimicrobial agents against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
		2-phenylethanol	Sodium propionate	Sodium benzoate	Sodium dehydroacetate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	4.3	0.35	0.5	0.05	0.02	0	100.00%	0	100.00%
Antimicrobial solution 2	4.3	0	0.5	0.05	0.02	23	54.00%	27	0.00%
Antimicrobial solution 3	4.3	0.35	0	0.05	0.02	3	94.00%	10	62.96%
Antimicrobial solution 4	4.3	0.35	0.5	0.05	0	0	100.00%	0	100.00%

Antimicrobial solution 5	4.3	0.35	0.5	0	0.02	0	100.00%	14	48.15%
Positive Control	7.0	0	0	0	0	50	0.00%	27	0.00%
Negative control	7.0	0	0	0	0	0	0	0	0

[00138] 4. Conclusion:

[00139] In the present experimental example, there were four combination solutions containing three agents, among which the combination solution (pH 4.3) containing “0.35 percent (weight/volume) of 2-phenylethanol, 0.5 percent (weight/volume) of sodium propionate, and 0.05 percent (weight/volume) of sodium benzoate” was most effective. Its inhibition ratio against *Escherichia coli* and *Staphylococcus aureus* were 100 percent respectively, which is significantly higher than that of the combination solution of 0.35 percent (weight/volume) of 2-phenylethanol and 0.5 percent (weight/volume) of sodium propionate, and the combination of 0.35 percent (weight/volume) of 2-phenylethanol and 0.05 percent (weight/volume) of sodium benzoates (pH 4.3). However, it was comparable to that of the combination solution containing 0.35 percent (weight/volume) of 2-phenylethanol, 0.5 percent (weight/volume) of sodium propionate, 0.05 percent (weight/volume) of sodium benzoate, and 0.02% sodium dehydroacetate.

[00140] Experimental example 7

[00141] 1. Experimental objective: to observe the antimicrobial effect of the combination solutions of lower pH value, which contained 2-phenylethanol, sodium propionate, and sodium benzoate.

[00142] 2. Experimental method:

[00143] A. Experimental strains: *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC 6538).

[00144] B. Experiment groups: as shown in Table 16, each antimicrobial solution contained water and antimicrobial agents,.

[00145] C. Experimental procedure: the experiment was implemented basically according to the method in appendix C of GB15979-2002.

[00146] 3. Experimental result: as shown in Table 16, the inhibition effects of the antimicrobial solutions (pH 4.1) containing different components were presented.

Table 16. The inhibitory effects of combinations solutions containing three antimicrobial agents against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
		2-Phenylethanol	Sodium propionate	Sodium benzoate	Sodium dehydroacetate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	4.1	0.35	0.5	0.05	0.02	0	100.00%	0	100.00%
Antimicrobial solution 2	4.1	0	0.5	0.05	0.02	56	0.00%	21	59.62%
Antimicrobial solution 3	4.1	0.35	0	0.05	0.02	5	86.49%	0	100.00%
Antimicrobial solution 4	4.1	0.35	0.5	0.05	0	0	100.00%	0	100.00%
Antimicrobial solution 5	4.1	0.35	0.5	0	0.02	17	54.05%	0	100.00%
Positive Control	7.0	0	0	0	0	37	0.00%	52	0.00%
Negative control	7.0	0	0	0	0	0	0	0	0

5 **[00147]** 4. Conclusion:

[00148] The antimicrobial effect against *Staphylococcus aureus* of combination solutions containing three antimicrobial agents (pH 4.1) were stronger than that of solutions containing identical antimicrobial agents but with a pH value of 4.3. The combination solution containing :0.35 percent (weight/volume) of 2-phenylethanol,
10 0.5 percent (weight/volume) of sodium propionate, and 0.05 percent (weight/volume) of sodium benzoate showed the strongest inhibitory action, of which the inhibition

ratio were 100 percent for against *Escherichia coli* as well as for against *Staphylococcus aureus*.

[00149] The inhibition ratio of the combination solution containing 2-phenylethanol, sodium propionate, and sodium benzoate was also significantly higher than that of the combination solution containing 0.35 percent (weight/volume) of 2-phenylethanol and 0.5 percent (weight/volume) of sodium propionate, and the combination of 0.35 percent (weight/volume) of 2-phenylethanol and 0.05 percent (weight/volume) of sodium benzoates (pH 4.1). It was comparable to that of the combination solution containing 0.35 percent (weight/volume) of 2-phenylethanol, 0.5 percent (weight/volume) of sodium propionate, 0.05 percent (weight/volume) of sodium benzoate, and 0.02% sodium dehydroacetate (pH 4.1).

[00150] The antimicrobial effect of the combination solution containing 0.5 percent (weight/volume) of sodium propionate, 0.05 percent (weight/volume) of sodium benzoate, and 0.02 percent (weight/volume) of sodium dehydroacetate was the weakest. It had little antimicrobial effect against *Escherichia coli* while the inhibition ratio against *Staphylococcus aureus* was 59.62 percent.

[00151] Experimental Example 8

[00152] 1. Experimental objective: to observe inhibition effects of gel compositions containing three antimicrobial agents.

[00153] 2. Experimental method:

[00154] A. Experimental strains: *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC 6538).

[00155] B. Experimental groups: as shown in Table 17, each antimicrobial gel group contained water, antimicrobial agents, and xanthan gum.

[00156] C. Experimental procedure: the experiment was implemented basically according to the method in appendix C of GB15979-2002.

[00157] 3. Experimental result: as shown in Table 17, the inhibition effects of the antimicrobial gels containing different antimicrobial agents (pH 4.1) were presented.

Table 17. The inhibitory effects of combination gels containing three

antimicrobial agents against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Xanthan gum (%)	Antimicrobial agent (%)				Escherichia coli		Staphylococcus aureus	
			2-Phenylethanol	Sodium propionate	Sodium benzoate	Sodium dehydroacetate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	4.1	1.0	0.4	0.55	0.07	0.01	32	89.08%	28	89.86%
Antimicrobial solution 2	4.1	1.0	0.4	0.55	0.07	0	1	99.66%	24	91.30%
Antimicrobial solution 3	4.1	1.0	0.4	0.55	0	0.01	147	49.83%	51	81.52%
Antimicrobial solution 4	4.1	1.0	0.4	0	0.07	0.01	1	99.66%	30	89.13%
Antimicrobial solution 5	4.1	1.0	0	0.55	0.07	0.01	220	24.91%	208	24.64%
Positive Control	7	1.0	0	0	0	0	293	0.00%	276	0.00%
Negative control	7	1.0	0	0	0	0	0	0.00%	0	0.00%

[00158] 4. Conclusion:

[00159] Among the combination gels (pH 4.1), the gel containing 0.4 percent (weight/volume) of 2-phenylethanol, 0.55 percent (weight/volume) of sodium propionate, and 0.07 percent (weight/volume) of sodium benzoate showed the strongest inhibition effects. The inhibition ratio against *Escherichia coli* and *Staphylococcus aureus* were 99.66 percent and 91.30 percent respectively. The antimicrobial effect of the gel containing sodium propionate, sodium benzoate, and sodium dehydroacetate, which did not contain 2-phenylethanol, was the weakest, and the inhibition ratio against *Escherichia coli* and *Staphylococcus aureus* were both less than 50 percent.

[00160] Experimental Example 9

[00161] 1. Experimental objective: to observe the inhibition effects of the combination solution of phenoxyethanol and sodium benzoate, the combination solution of phenoxyethanol and sodium propionate, the combination solution of phenoxyethanol and sodium acetate, and the combination solution of phenoxyethanol and sodium dehydroacetate.

[00162] 2. Experimental method:

[00163] A. Experimental strains: *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC 6538).

10 **[00164]** B. Experimental groups: as shown in Tables 18, 19, 20 and 21, each antimicrobial solution group contained water and antimicrobial agent,.

[00165] C. Experimental procedure: the experiment was implemented basically according to the method in appendix C of GB15979-2002.

15 **[00166]** 3. Experimental result: the inhibition effects of the combination solution of phenoxyethanol and sodium benzoate were shown in Table 18. The inhibition effects of the combination solutions of phenoxyethanol and sodium propionate were shown in Table 19. The inhibition effects of the combination solutions of phenoxyethanol and sodium acetate were shown in Table 20. The inhibition effects of the combined solutions of phenoxyethanol and sodium dehydroacetate were shown in Table 21.

20 Table 18. The inhibitory effects of the combination of phenoxyethanol and sodium benzoate against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		Phenoxyethanol	Sodium benzoate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	3.5	0.3	0	103	0.96%	215	2.71%
Antimicrobial solution 2	3.5	0.4	0	90	13.46%	220	0.45%
Antimicrobial solution 3	3.5	0	0.06	101	2.88%	221	0.00%

Antimicrobial solution 3							
Antimicrobial solution 4	3.5	0	0.08	91	12.50%	171	22.62%
Antimicrobial solution 5	3.5	0	0.1	60	42.31%	153	30.77%
Antimicrobial solution 6	3.5	0.3	0.06	26	75.00%	102	53.85%
Antimicrobial solution 7	3.5	0.4	0.06	3	97.12%	58	73.76%
Antimicrobial solution 8	3.5	0.3	0.08	1	99.04%	74	66.52%
Antimicrobial solution 9	3.5	0.4	0.08	0	100.00%	8	96.38%
Antimicrobial solution 10	3.5	0.3	0.1	0	100.00%	26	88.24%
Antimicrobial solution 11	3.5	0.4	0.1	0	100.00%	0	100.00%
Positive Control	7.0	0	0	104	0.00%	221	0.00%
Negative control	7.0	0	0	0	0.00%	0	0.00%

Table 19. The inhibitory effects of the combination of phenoxyethanol and sodium propionate against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		Phenoxyethanol	Sodium propionate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial	3.5	0.3	0	116	3.33%	179	0.00%

Antimicrobial solution 1							
Antimicrobial solution 2	3.5	0.4	0	101	15.83%	153	14.53%
Antimicrobial solution 3	3.5	0	0.4	68	43.33%	129	27.93%
Antimicrobial solution 4	3.5	0	0.5	66	45.00%	169	5.59%
Antimicrobial solution 5	3.5	0	0.55	91	24.17%	162	9.50%
Antimicrobial solution 6	3.5	0.3	0.4	109	9.17%	138	22.91%
Bacteriostatic solution 7	3.5	0.4	0.4	60	50.00%	84	53.07%
Antimicrobial solution 8	3.5	0.3	0.5	86	28.33%	126	29.61%
Antimicrobial solution 9	3.5	0.4	0.5	67	44.17%	70	60.89%
Antimicrobial solution 10	3.5	0.3	0.55	87	27.50%	109	39.11%
Antimicrobial solution 11	3.5	0.4	0.55	64	46.67%	102	43.02%
Positive Control	7.0	0	0	120	0.00%	179	0.00%
Negative control	7.0	0	0	0	0.00%	0	0.00%

Table 20. The inhibitory effects of the combination of phenoxyethanol and sodium acetate against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		Phenoxyethanol	Sodium acetate	Colony count after 20 minutes	Inhibition ratio	Colony count after 20 minutes	Inhibition ratio
Antimicrobial solution 1	3.5	0.3	0	95	0.00%	154	8.33%
Antimicrobial solution 2	3.5	0.4	0	97	14.91%	109	35.12%
Antimicrobial solution 3	3.5	0	0.4	60	47.37%	117	30.36%
Antimicrobial solution 4	3.5	0	0.5	55	51.75%	110	34.52%
Antimicrobial solution 5	3.5	0	0.55	37	67.54%	109	35.12%
Antimicrobial solution 6	3.5	0.3	0.4	32	71.93%	126	25.00%
Antimicrobial solution 7	3.5	0.4	0.4	30	73.68%	68	59.52%
Antimicrobial solution 8	3.5	0.3	0.5	42	63.16%	88	47.62%
Antimicrobial solution 9	3.5	0.4	0.5	27	76.32%	30	82.14%
Antimicrobial solution 10	3.5	0.3	0.55	31	72.81%	119	29.17%
Antimicrobial solution 11	3.5	0.4	0.55	21	81.58%	16	90.48%

Positive Control	7.0	0	0	114	0.00%	168	0.00%
Negative control	7.0	0	0	0	0.00%	0	0.00%

Table 21. The inhibitory effects of the combination of phenoxyethanol and sodium dehydroacetate against *Escherichia coli* and *Staphylococcus aureus*

Groups	pH	Antimicrobial agent (%)		Escherichia coli		Staphylococcus aureus	
		phen oxyet hanol	Sodium dehydroa cetate	Colony count after 20 minutes	Inhibitio n ratio	Colony count after 20 minutes	Inhibitio n ratio
Antimicro bial solution 1	3.5	0.3	0	95	0.00%	154	8.33%
Antimicro bial solution 2	3.5	0.4	0	97	15.65%	109	35.12%
Antimicro bial solution 3	3.5	0	0.015	102	11.30%	147	12.50%
Antimicro bial solution 4	3.5	0	0.02	98	14.78%	147	12.50%
Antimicro bial solution 5	3.5	0	0.025	96	16.52%	140	16.67%
Antimicro bial solution 6	3.5	0.3	0.015	72	37.39%	119	29.17%
Antimicro bial solution 7	3.5	0.4	0.015	41	64.35%	94	44.05%
Antimicro bial solution 8	3.5	0.3	0.02	75	34.78%	114	32.14%
Antimicro bial solution 9	3.5	0.4	0.02	71	38.26%	71	57.74%
Antimicro bial	3.5	0.3	0.025	67	41.74%	107	36.31%

solution 10							
Antimicrobial solution 11	3.5	0.4	0.025	70	39.13%	86	48.81%
Positive Control	7.0	0	0	115	0.00%	168	0.00%
Negative control	7.0	0	0	0	0.00%	0	0.00%

[00167] 4. Conclusion:

[00168] The combination of 0.3 percent to 0.4 percent (weight/volume) of phenoxyethanol and 0.06 percent to 0.10 percent (weight/volume) of sodium benzoate had a synergistic antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*.

[00169] The combination solution of 0.4 percent (weight/volume) of phenoxyethanol and 0.4 percent to 0.55 percent (weight/volume) of sodium propionate had a synergistic antimicrobial effect, of which the inhibition ratio were from 40 to 60 percent against *Escherichia coli* and *Staphylococcus aureus*. But the antimicrobial effect was weaker than that of the combination solution of phenoxyethanol and sodium benzoate mentioned above.

[00170] The combination solution of 0.4 percent (weight/volume) of phenoxyethanol and 0.4 percent to 0.55 percent (weight/volume) of sodium acetate had a synergistic antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*.

[00171] The combination solution of 0.4 percent (weight/volume) of phenoxyethanol and 0.015 percent to 0.025 percent (weight/volume) of sodium dehydroacetate had a synergistic antimicrobial effect, of which the inhibition ratio were from 40 percent to 65 percent against *Escherichia coli* and *Staphylococcus aureus*. But the antimicrobial effect was weaker than that of the combination solution of phenoxyethanol and sodium benzoate mentioned above.

[00172] Experimental example 10

[00173] 1. Experimental objective: to observe the inhibition effects of the

antimicrobial agent combination of the present disclosure against the growth of *Escherichia coli* and *Staphylococcus aureus*.

[00174] 2. Experimental method:

[00175] (1). Experimental material:

5 **[00176]** a. Experimental strains, three strains of *Escherichia coli* and three strains of *Staphylococcus aureus*, which were all isolated from the vaginal secretion of patients with vaginitis.

[00177] b. Enrichment broth, which contained 1 percent of yeast extract powder, 9 percent of sucrose, 0.025 percent of manganese sulfate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$), 0.058 percent
10 of magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), and 0.9 percent of lactic acid, the pH value of the broth was adjusted to 4.8, and the enrichment broth was sterilized for later use;

[00178] (2). Experimental groups: the requirements for aseptic operation technique were strictly followed to prepare the sterile test tubes:

[00179] a. sodium benzoate group: each test tube contained 5 milliliters of
15 enrichment broth, and was added in 0.05 percent (weight/volume) sodium benzoate ;

[00180] b. 2-phenylethanol group: each test tube contained 5 milliliters of enrichment broth, and was added in 0.05 percent (weight/volume) of 2-phenylethanol;

[00181] c. sodium propionate group: each test tube contained 5 milliliters of enrichment broth, and was added in 0.25 percent (weight/volume) of sodium
20 propionate;

[00182] d. “a group of the combinations of sodium benzoate and sodium propionate”: each test tube contained 5 milliliters of enrichment broth, and was added in 0.05 percent (weight/volume) of sodium benzoate and 0.25 percent (weight/volume) of sodium propionate;

25 **[00183]** e. “a group of the combinations of sodium benzoate and 2-phenylethanol”: each test tube contained 5 milliliters of enrichment broth, and was added in 0.05 percent (weight/volume) of sodium benzoate and 0.05 percent (weight/volume) of 2-phenylethanol;

[00184] f. “a group of the combinations of sodium propionate and
30 2-phenylethanol”: each test tube contained 5 milliliters of enrichment broth, and was

added in 0.25 percent (weight/volume) of sodium propionate and 0.05 percent (weight/volume) of 2-phenylethanol;

5 [00185] g. "a group of the combinations of sodium benzoate, sodium propionate and 2-phenylethanol": each test tube contained 5 milliliters of enrichment broth, and was added in 0.05 percent (weight/volume) of sodium benzoate, 0.25 percent (weight/volume) of sodium propionate, and 0.05 percent (weight/volume) of 2-phenylethanol;

10 [00186] h. positive control: one tube that contained 5 milliliters of enrichment broth without adding in any of the 2-phenylethanol, sodium benzoate, and sodium propionate, but inoculated the suspension of *Escherichia coli* or *Staphylococcus aureus*.

15 [00187] i. negative control: one tube that contained 5 milliliters of enrichment broth without adding in any of the 2-phenylethanol, sodium benzoate, and sodium propionate, and not inoculated the suspension of *Escherichia coli* or *Staphylococcus aureus*.

[00188] (3). Experimental procedure:

20 [00189] The requirements for aseptic operation technique were strictly followed to prepare the bacterial suspensions. The colonies of *Escherichia coli* and *Staphylococcus aureus* were diluted with physiological saline respectively to prepare bacterial suspension. The turbidities of the *Escherichia coli* suspension and the *Staphylococcus aureus* suspension were adjusted to 0.5MCF (about 10^8 CFU/ml) by a turbidimeter. 1 milliliter of the 0.5MCF bacterial suspensions were added into 9 milliliters of the enrichment broth respectively, so each bacterial solution was diluted for ten times to contain 10^7 CFU/ml of bacterial organisms.

25 [00190] The requirements for aseptic operation technique were strictly followed to take 100 microliters of the prepared bacterial suspensions respectively, and to add the bacterial suspensions into the aforesaid test tubes from a to h as well as positive control tube. No bacterial suspension was added into the negative control tube i.

30 [00191] The above tubes were cultured in a biochemical incubator at 37°C for 24 hours, then turbidity changes of the test tube solutions were observed and recorded.

[00192] 3. Experimental result:

[00193] As shown in table 22A:

Table 22A. Effects of antimicrobial agent solutions against the growth of
Escherichia coli and Staphylococcus aureus

Bacterial strain	Escherichia coli			Staphylococcus aureus		
	1	2	3	1	2	3
a. 0.05 % sodium benzoate	+	+	+	+	+	+
b. 0.05% 2-phenylethanol	+	+	+	+	+	+
c. 0.25% sodium propionate	+	+	+	+	+	+
d. 0.05% sodium benzoate and 0.25% sodium propionate	+	+	+	+	+	+
e. 0.05% sodium benzoate and 0.05% 2-phenylethanol	-	+	+	+	+	+
f. 0.25% sodium propionate and 0.05% 2-phenylethanol	-	+	+	+	-	+
g. 0.05% sodium benzoate and 0.25% sodium propionate and 0.05% 2-phenylethanol	-	+	-	+	-	-
h. positive control	+	+	+	+	+	+
i. negative control	-	-	-	-	-	-

5 [00194] Remark: “+” means the growth of bacteria; “-” means no growth of bacteria.

[00195] 4. Conclusion:

[00196] (1) In the experimental example, no one of the three antimicrobial agents, i.e., 0.05 percent (weight/volume) of sodium benzoate, 0.05 percent (weight/volume) of 2-phenylethanol, and 0.25 percent (weight/volume) of sodium propionate, could inhibit the growth of Escherichia coli and Staphylococcus aureus respectively.

[00197] (2) In the experimental example, the combination of “0.05 percent (weight/volume) of sodium benzoate and 0.25 percent (weight/volume) of sodium propionate” had no significant inhibition effect against the growth of Escherichia coli and Staphylococcus aureus.

[00198] (3) In the experimental example, the growth of one-third of the Escherichia

coli strains were inhibited by the combination of “0.05 percent (weight/volume) of sodium benzoate and 0.05 percent (weight/volume) of 2-phenylethanol”; but the growth of Staphylococcus aureus strains were not inhibited by the combination of “0.05 percent (weight/volume) of sodium benzoate and 0.05 percent (weight/volume) of 2-phenylethanol”. The growth of one-third of the Escherichia coli strains and one-third of the Staphylococcus aureus strains were inhibited by the combination of “0.25 percent (weight/volume) of sodium propionate and 0.05 percent (weight/volume) of 2-phenylethanol”.

[00199] (4) In the experimental example, the growth of two-thirds Escherichia coli strains and two-thirds Staphylococcus aureus strains were inhibited by the combination of “0.05 percent (weight/volume) of 2-phenylethanol, 0.05 percent (weight/volume) of sodium benzoate, and 0.25 percent (weight/volume) of sodium propionate”. It was suggested that the combination of the three antimicrobial agents had a synergistic antimicrobial effect against Escherichia coli and Staphylococcus aureus.

[00200] Experimental example 11

[00201] 1. Experimental objective: to observe the inhibition effect of the antimicrobial agents combination solution of the present disclosure on Candida albicans.

[00202] 2. Experimental method:

[00203] (1). Experimental material:

[00204] a. Experimental strains: nine strains of Candida albicans which were clinical strains isolated from vaginal secretion of patients with fungal vaginitis, and all of them could grow in enrichment broth containing 0.025 percent (weight/volume) of sodium benzoate.

[00205] b. Enrichment broth: which contained 1 percent of yeast extract powder, 9 percent of sucrose, 0.025 percent of manganese sulfate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$), 0.058 percent of magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), and 0.9 percent of lactic acid, the pH value of the broth was adjusted to 4.5, and the enrichment broth was sterilized for later use;

[00206] (2) Experimental groups: the requirements of aseptic operation technique

were strictly followed so to prepare the sterile test tubes;

[00207] a. sodium benzoate group: each test tube contained 5 milliliters enrichment broth, and was added in 0.025 percent (weight/volume) of sodium benzoate;

[00208] b. 2-phenylethanol group: each test tube contained 5 milliliters enrichment
5 broth, and was added in 0.050 percent (weight/volume) of 2-phenylethanol;

[00209] c. sodium propionate group: each test tube contained 5 milliliters enrichment broth, and was added in 0.125 percent (weight/volume) of sodium propionate;

[00210] d. “a group of the combinations of sodium benzoate and sodium
10 propionate”: each test tube contained 5 milliliters enrichment broth, and was added in 0.025 percent (weight/volume) of sodium benzoate and 0.125 percent (weight/volume) of sodium propionate;

[00211] e. “a group of the combinations of sodium benzoate and 2-phenylethanol”:
each test tube contained 5 milliliters enrichment broth, and was added in 0.025
15 percent (weight/volume) of sodium benzoate and 0.050 percent (weight/volume) of 2-phenylethanol;

[00212] f. “a group of the combinations of sodium propionate and
2-phenylethanol”: each test tube contained 5 milliliters enrichment broth, and was
added in 0.125 percent (weight/volume) of sodium propionate and 0.050 percent
20 (weight/volume) of 2-phenylethanol;

[00213] g. “a group of the combinations of sodium benzoate, sodium propionate
and 2-phenylethanol”: each test tube contained 5 milliliters enrichment broth, and was
added in 0.025 percent (weight/volume) of sodium benzoate, 0.125 percent
(weight/volume) of sodium propionate and 0.050 percent (weight/volume) of
25 2-phenylethanol;

[00214] h. positive control: the control tube of enrichment broth without adding in 2-phenylethanol, sodium benzoate, and sodium propionate; but inoculated the suspension of *Candida albicans*;

[00215] i. negative control: the control tube of enrichment broth without adding in
30 2-phenylethanol, sodium benzoate, and sodium propionate; and without inoculating

Candida albicans;

[00216] (3). Experimental procedure:

[00217] The requirements for aseptic operation technique were strictly followed to prepare the suspension of organism. The colonies of each strain of Candida albicans organism were diluted with physiological saline respectively. The turbidities of the suspensions of Candida albicans organism were adjusted to 0.5MCF (about 10^8 CFU/ml) by a turbidimeter. 1 milliliter of the 0.5MCF organism suspension was added into 9 milliliters of enrichment broth. Each suspension was diluted for ten times to contain 10^7 CFU/ml of organisms.

[00218] the requirements for aseptic technique were strictly followed to take 100 microliters of the prepared organism suspension respectively and to add the suspension into the test tubes from a to h as well as the positive control tube. No organism suspension was added into the negative control tube i.

[00219] The above tubes were cultured in a biochemical incubator at 37 °C for 24 hours. Then the growth of Candida in each tube was observed and recorded.

[00220] 3. Experimental result:

[00221] As shown in Table 22B:

Table 22B. Inhibitory effect of the combination of sodium benzoate and organic acid preservatives on the growth of Candida albicans

Bacterial strain	1	2	3	4	5	6	7	8	9
d. 0.025% sodium benzoate	+	+	+	+	+	+	+	+	+
e. 0.050% 2-phenylethanol	-	+	+	+	+	+	-	+	+
f. 0.125% sodium propionate	+	+	-	+	+	+	-	+	-
d. 0.025% sodium benzoate and 0.125% sodium propionate	-	+	-	-	-	+	-	+	-
e. 0.025% sodium benzoate and 0.050% 2-phenylethanol	-	+	-	+	-	-	-	+	+
f. 0.125% sodium propionate and 0.050% phenethyl alcohol	-	+	-	+	-	+	-	+	-
g. 0.025% sodium benzoate, 0.125% sodium propionate, and 0.050% phenethyl alcohol	-	-	-	-	-	-	-	-	-
h. positive Control	+	+	+	+	+	+	+	+	+
i. negative control	-	-	-	-	-	-	-	-	-

[00222] Remark: “+” means the growth of organisms; “-” means no growth of organisms.

[00223] 4. Conclusion:

[00224] (1) All the nine *Candida albicans* strains grew in the broth containing only
5 0.025 percent (weight/volume) of sodium benzoate; seven of the nine *Candida*
albicans strains grew in the broth containing only 0.050 percent (weight/volume) of
2-phenylethanol; six of the nine *Candida albicans* strains grew in the broth containing
only 0.125 percent (weight/volume) of sodium propionate. It was suggested that the
growth of the nine *Candida albicans* strains could not be inhibited completely by each
10 one of the three antimicrobial agents.

[00225] (2) Three of the nine *Candida* strains grew in the broth containing “0.025
percent (weight/volume) of sodium benzoate and 0.125 percent (weight/volume) of
sodium propionate”; four of the nine *Candida* strains grew in the broth containing
“0.025 percent (weight/volume) of sodium benzoate and 0.050 percent
15 (weight/volume) of 2-phenylethanol”; four of the nine *Candida* strains grew in the
broth containing “0.125 percent (weight/volume) of sodium propionate and 0.050
percent (weight/volume) of 2-phenylethanol”. It was suggested that the combinations
of any two of the three antimicrobial agents were synergistic in inhibiting against
Candida albicans.

20 **[00226]** (3) The antimicrobial agent combination group: all of the nine strains did
not grow in the broth containing “0.050 percent (weight/volume) of 2-phenylethanol,
0.025 percent (weight/volume) of sodium benzoate, and 0.125 percent
(weight/volume) of sodium propionate”. It was suggested that the combination of the
three antimicrobial agents had the strongest synergistic action in inhibiting the growth
25 of *Candida albicans*.

[00227] Experimental example 12

[00228] 1. Experimental objective: to observe the modulating effect of the gel
containing 9.0 percent (weight/volume) of maltose, 0.05 percent (weight/volume) of
2-phenylethanol, and 0.075 percent (weight/volume) of sodium benzoate on vaginal
30 flora of rhesus macaque.

[00229] 2. Experimental method:

[00230] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 9.0 percent (weight/volume) of maltose, 0.05 percent (weight/volume) of 2-phenylethanol, 0.075 percent (weight/volume) of sodium benzoate, and 2.2 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.0.

[00231] B. Animals: one adult female rhesus macaque. The vaginal secretion pH >5.4. Vaginal smear examination showed dominant Gram-negative rods (G^{-b}) and with a number of Gram-negative cocci (G^{-c}) and Gram-positive cocci (G^{+c}). Large Gram-positive rods (G^{+b}) were rare.

[00232] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. Vaginal secretion was sampled on the 6th day. The pH value of vaginal secretion was tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00233] 3. Experimental result

[00234] As shown in table 23A:

Table 23A. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
23736	5.4	$G^{-b}+++$, $G^{+c}+$, $G^{-c}+$	3.8	$G^{+b}+++$

[00235] 4. Conclusion

[00236] The administration of the gel of the present disclosure, which comprised 9.0 percent (weight/volume) of maltose, 0.05 percent (weight/volume) of 2-phenylethanol, and 0.075 percent (weight/volume) of sodium benzoate, could restore vaginal flora of rhesus macaque to normal by reducing Gram-negative rods and increasing Gram-positive rods. The vaginal secretion pH could also be reduced.

[00237] Experimental example 13

[00238] 1. Experimental objective: to observe the modulating effect of the gel containing 5.5 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.05 percent (weight/volume) of 2-phenylethanol, and 0.15 percent (weight/volume) of sodium benzoate on the BV-type flora of rhesus macaque.

5 **[00239]** 2. Experimental method:

[00240] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 5.5 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.05 percent (weight/volume) of 2-phenylethanol, 0.15 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.3.

[00241] B. Animals: two adult female rhesus macaques. The pH values of vaginal secretion of the two rhesus macaques were both 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

15 **[00242]** C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00243] 3. Experimental result

20 **[00244]** As shown in Table 23B:

Table 23B. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
3966	5.4	G ^{-b} +++	<3.8	G ^{+b} +++
1204156	>5.4	G ^{-b} +++	5.4	G ^{-b} +++ , WBC+

[00245] 4. Conclusion

[00246] The administration of the gel of the present disclosure, which comprised 5.5 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose,

0.05 percent (weight/volume) of 2-phenylethanol, and 0.15 percent (weight/volume) of sodium benzoate, restored vaginal BV-type flora of one rhesus macaque to normal. The vaginal secretion pH values of this rhesus macaque could also be reduced.

[00247] Experimental example 14

5 **[00248]** 1. Experimental objective: to observe the modulating effect of the gel containing 6.5 percent (weight/volume) of sucrose, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.05percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

[00249] 2. Experimental method:

10 **[00250]** A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 6.5 percent (weight/volume) of sucrose, 0.10 percent (weight/volume) of 2-phenylethanol, 0.05 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.8.

15 **[00251]** B. Animals: two adult female rhesus macaques. The pH values of vaginal secretion of the two rhesus macaques were both 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00252] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day.

20 The pH values of vaginal secretion were tested. The vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00253] As shown in Table 24:

Table 24. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
3966	5.4	G ^{-b} +++	3.8	G ^{+b} +++
13046	5.4	G ^{-b} +++ , G ^{+c} +++ , few	<3.8	G ^{+b} +++ mostly

		G ^{+b}		existed in epithelial cells
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[00254] 4. Conclusion

[00255] The administration of the gel of the present disclosure, which comprised 6.5 percent (weight/volume) of sucrose, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.05 percent (weight/volume) of sodium benzoate, could restore vaginal flora of rhesus macaques to normal. The vaginal secretion pH values of rhesus macaques could also be reduced.

[00256] Experimental example 15

[00257] 1. Experimental objective: to observe the modulating effect of the gel containing 1.0 percent (weight/volume) of glucose, 0.30 percent (weight/volume) of 2-phenylethanol, and 0.085 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

[00258] 2. Experimental method:

[00259] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 1.0 percent (weight/volume) of glucose, 0.30 percent (weight/volume) of 2-phenylethanol, 0.085 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.8.

[00260] B. Animals: one adult female rhesus macaque. The pH value of the vaginal secretion was 5.4. The vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00261] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH value of vaginal secretion was tested, and the vaginal smear was microscopically examined after Gram-staining. The results were compared with those before treatment.

[00262] 3. Experimental result

[00263] As shown in table 25:

Table 25. Effects of antimicrobial gel combination on vaginal flora of rhesus

macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13284	5.4	G ^{-b} +++ , few G ^{+b}	3.8 to 4.1	G ^{+b} +++ , G ^{+c} +, WBC+

[00264] 4. Conclusion

[00265] The administration of the gel of the present disclosure, which comprised 1.0 percent (weight/volume) of glucose, 0.30 percent (weight/volume) of 2-phenylethanol, and 0.085 percent (weight/volume) of sodium benzoate, could restore vaginal flora of rhesus macaques to normal. The vaginal secretion pH values of rhesus macaques could also be reduced.

[00266] Experimental example 16

[00267] 1. Experimental objective: to observe the modulating effect of the gel containing 5.0 percent (weight/volume) of fructose, 0.40percent (weight/volume) of 2-phenylethanol, and 0.09 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

[00268] 2. Experimental method:

[00269] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 5.0 percent (weight/volume) of fructose, 0.4 percent (weight/volume) of 2-phenylethanol, 0.09 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 4.1

[00270] B. Animals: two adult female rhesus macaques. The pH values of the vaginal secretion were 5.4. The vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare

[00271] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH values of vaginal secretion were tested. The vaginal smears were

microscopically examined after Gram-staining. The results were compared with those before treatment.

[00272] 3. Experimental result

[00273] As shown in table 26:

5 Table 26 Effects of antimicrobial gel combination on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13278	5.4	G ^{-b+++} , G ^{+c++}	<3.8	G ^{+b++}
6058	5.4	G ^{-b+++} , G ^{+c++} , G ^{+b+} , WBC 0~+	4.4	G ^{+b++} , WBC++

[00274] 4. Conclusion

[00275] The administration of the gel of the present disclosure, which comprised 5.0 percent (weight/volume) of fructose, 0.4 percent (weight/volume) of 2-phenylethanol, and 0.09 percent (weight/volume) of sodium benzoate, could restore vaginal flora of rhesus macaques to normal. The vaginal secretion pH values of rhesus macaques could also be reduced.

[00276] Experimental example 17

[00277] 1. Experimental objective: to observe the modulating effect of the gel containing 4.0 percent (weight/volume) of mannose, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.35 percent (weight/volume) of sodium propionate on the vaginal flora of rhesus macaque.

[00278] 2. Experimental method:

[00279] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 4.0 percent (weight/volume) of mannose, 0.10 percent (weight/volume) of 2-phenylethanol, 0.35 percent (weight/volume) of sodium propionate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.5.

[00280] B. Animals: one adult female rhesus macaque. The pH value of the vaginal secretion was 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00281] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH value of vaginal secretion was tested, and the vaginal smear was microscopically examined after Gram-staining. The results were compared with those before treatment.

[00282] 3. Experimental result

10 **[00283]** As shown in table 27:

Table 27 Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13524	5.4	G ^{-b} +++ , G ^{+c} ++ , G ^{+b} which are large and occasionally observed	4.8	G ^{+b} , G ^{+c} , smaller G ^{+c} , WBC++ mostly existed in epithelial cells

[00284] 4. Conclusion

[00285] The administration of the gel of the present disclosure, which comprised 4.0 percent (weight/volume) of mannose, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.35 percent (weight/volume) of sodium propionate, could partially restore vaginal flora of rhesus macaque toward normal. The vaginal secretion pH value of the rhesus macaque could also be reduced. But the pH value did not yet drop to 4.1 or below.

[00286] Experimental example 18

20 **[00287]** 1. Experimental objective: to observe the modulating effect of the gel containing 6.5 percent (weight/volume) of isomaltooligosaccharide, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.475 percent (weight/volume) of sodium

propionate on the vaginal flora of rhesus macaques.

[00288] 2. Experimental method:

[00289] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 6.5 percent (weight/volume) of isomaltooligosaccharide, 0.10 percent (weight/volume) of 2-phenylethanol, 0.475 percent (weight/volume) of sodium propionate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.3.

[00290] B. Animals: two adult female rhesus macaques. The pH values of the vaginal secretion were 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00291] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00292] 3. Experimental result

[00293] As shown in table 28:

Table 28. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
6048	5.4	G ^{-b+++} , G ^{+c+} , typical BV flora	3.8~4.1	G ^{-b++} , G ^{+b++} , G ^{+c++}
13132	5.4	G ^{-b+++} , G ^{+c+~++} , G ^{+b+} . WBC -, typical BV flora	<3.8	G ^{+b++}

[00294] 4. Conclusion

[00295] The administration of the gel of the present disclosure, which comprised 6.5 percent (weight/volume) of isomaltooligosaccharide, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.475 percent (weight/volume) of sodium propionate, could

restore the vaginal flora of rhesus macaques to normal. The vaginal secretion pH values of rhesus macaques could also be reduced.

[00296] Experimental example 19

[00297] 1. Experimental objective: to observe the modulating effect of the gel containing 2.0 percent (weight/volume) of maltose, 3.5 percent (weight/volume) of fructose, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.575 percent (weight/volume) of sodium propionate on the vaginal flora of rhesus macaque,

[00298] 2. Experimental method:

[00299] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 2.0 percent (weight/volume) of maltose, 3.5 percent (weight/volume) of fructose, 0.10 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.3.

[00300] B. Animal: one adult female rhesus macaque. The pH value of the vaginal secretion was 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00301] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH value of vaginal secretion was tested, and the vaginal smear was microscopically examined after Gram-staining. The results were compared with those before treatment.

[00302] 3. Experimental result

[00303] As shown in table 29:

Table 29. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13362	5.4	G ^{-b} +++, G ^{+c} +, G ^{+b} occasionally	3.8~4.1	G ^{+b} +++, WBC+, mostly existed in

		observed		epithelial cells
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[00304] 4. Conclusion

[00305] The administration of the gel of the present disclosure, which comprised 2.0 percent (weight/volume) of maltose, 3.5 percent (weight/volume) of fructose, 0.10 percent (weight/volume) of 2-phenylethanol, and 0.575 percent (weight/volume) of sodium propionate, could restore vaginal flora of rhesus macaques to normal. The vaginal secretion pH value of rhesus macaque could also be reduced.

[00306] Experimental example 20

[00307] 1. Experimental objective: to observe the modulating effect of the gel containing 4.0 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.10 percent (weight/volume) of phenoxyethanol, and 0.35 percent (weight/volume) of sodium acetate on the vaginal flora of rhesus macaque.

[00308] 2. Experimental method:

[00309] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 4.0 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.10 percent (weight/volume) of phenoxyethanol, 0.35 percent (weight/volume) of sodium acetate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.3.

[00310] B. Animals: one adult female rhesus macaque. The pH value of the vaginal secretion was 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00311] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. Vaginal secretion was sampled on the 6th day. The pH value of vaginal secretion was tested, and the vaginal smear was microscopically examined after Gram-staining. The results were compared with those before treatment.

[00312] 3. Experimental result

[00313] As shown in table 30:

Table 30. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal	Before administration	1 Day after the end of
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No.	administration			
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
	5.4	G ^b +++ , G ⁺ c++ , G ⁺ b occasionally observed	4.6	G ⁺ b++、G ⁺ c , smaller G ⁺ c , WBC+ , mostly existed in epithelial cells

[00314] 4. Conclusion

[00315] The administration of the gel of the present disclosure, which comprised 4.0 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.10 percent (weight/volume) of phenoxyethanol, and 0.35 percent (weight/volume) of sodium acetate, could restore vaginal flora of rhesus macaques to normal. The vaginal secretion pH value of rhesus macaque could also be reduced. But the pH value did not yet drop to 4.1 or below.

[00316] Experimental example 21

[00317] 1. Experimental objective: to observe the modulating effect of the gel containing 3.5 percent (weight/volume) of sucrose, 1.0 percent (weight/volume) of maltose, 0.06 percent (weight/volume) of 2-phenylethanol, 0.525 percent (weight/volume) of sodium propionate, and 0.15 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

[00318] 2. Experimental method:

[00319] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 3.5 percent (weight/volume) of sucrose, 1.0 percent (weight/volume) of maltose, 0.06 percent (weight/volume) of 2-phenylethanol, 0.525 percent (weight/volume) of sodium propionate, 0.15 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.3.

[00320] B. Animals: two adult female rhesus macaques. The pH values of the vaginal secretion were all 5.4. The vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00321] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled with a swab on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00322] 3. Experimental result

[00323] As shown in table 31:

Table 31. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
3902	5.4	$G^{-b}+++$, G^{+c} , typical BV flora	4.1	$G^{-b}++$, $G^{+b}+++$
13060-2	5.4	$G^{-b}+++$, G^{+b} occasionally observed	5.4	$G^{-b}+$, WBC++

[00324] 4. Conclusion

[00325] After vaginal administration of the gel of the present disclosure, which comprised 3.5 percent (weight/volume) of sucrose, 1.0 percent (weight/volume) of maltose, 0.06 percent (weight/volume) of 2-phenylethanol, 0.525 percent (weight/volume) of sodium propionate, and 0.15 percent (weight/volume) of sodium benzoate, the pH value of vaginal secretion of one rhesus macaque decreased from 5.4 to 4.1, while G^{-b} significantly decreased and G^{+b} significantly increased. The vaginal flora basically restored to normal. Although the pH value of vaginal secretion of the other one rhesus macaque did not change, the amount of G^{-b} bacteria in vaginal reduced. It was suggested that the composition was effective in modulating vaginal flora and could partially restore the vaginal flora of rhesus macaque as well as the vaginal pH values to normal.

[00326] Experimental example 22

[00327] 1. Experimental objective: to observe the modulating effect of the gel

containing 3.0 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of fructose, 0.065 percent (weight/volume) of 2-phenylethanol, 0.55 percent (weight/volume) of sodium propionate, and 0.12 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaques

5 **[00328]** 2. Experimental method:

[00329] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 3.0 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of fructose, 0.065 percent (weight/volume) of 2-phenylethanol, 0.55 percent (weight/volume) of sodium propionate, 0.12 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.3.

[00330] B. Animals: eight adult female rhesus macaques. The pH values of the vaginal secretion were all 5.4. The vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

15 **[00331]** C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. Vaginal secretion was sampled on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

20 **[00332]** 3. Experimental result

[00333] As shown in table 32:

Table 32. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13296	>5.4	G ^{-b+++} , typical BV flora	5.4	G ^{+b+++}
13684	5.4	G ^{-b+++} , G ^{+c+} , WBC+	<3.8	G ^{-b+} , G ^{+b+++}
3774	5.4	G ^{-b+++} , G ^{+c+} , typical BV flora	<3.8	G ^{-b+} , G ^{+b+++}

13442	5.4	G ^{-b} +++ , G ^{+c} , typical BV flora	<3.8	G ^{+b} +++ , G ^{+c} +
11290	5.4	G ^{-b} +++ , typical BV flora	4.1	G ^{-b} +, G ^{+b} +++ , WBC+
13588	5.4	G ^{-b} +++ , typical BV flora	<3.8	G ^{+b} +++
13468	5.4	G ^{-b} +++ , typical BV flora	4.1	G ^{-b} +, G ^{+b} +++ , WBC+
4086	5.4	G ^{-b} +++ , typical BV flora	5.4	G ^{-b} +++ , G ^{+c} +++

[00334] 4. Conclusion

[00335] The gel of the present disclosure, which comprised 3.0 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of fructose, 0.065 percent (weight/volume) of 2-phenylethanol, 0.55 percent (weight/volume) of sodium propionate, and 0.12 percent (weight/volume) of sodium benzoate, were applied to eight rhesus macaques. The pH values of vaginal secretion of four rhesus macaques were all decreased from 5.4 to below 3.8, while the vaginal flora restored to normal. The pH values of vaginal secretion of two rhesus macaques were decreased from 5.4 to 4.1, while the vaginal flora were dominated by large Gram-positive rods. The pH values of vaginal secretion of two rhesus macaques did not change, wherein the vaginal flora of one restored normal and the other one remained BV-typ. It was shown that the gel was effective in modulating the vaginal flora of rhesus macaques, restoring the vaginal flora, and decreasing the pH values of vaginal secretion.

[00336] Experimental example 23

[00337] 1. Experimental objective: to observe the modulating effect of the gel containing 0.10 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 0.09 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

[00338] 2. Experimental method:

[00339] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 0.10 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, 0.09 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan

gum. The pH value of the gel was adjusted to 3.3.

[00340] B. Animals: four adult female rhesus macaques. The pH values of the vaginal secretion were all 5.4. The vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

5 **[00341]** C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

10 **[00342]** 3. Experimental result

[00343] As shown in table 33:

Table 33. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13174	5.4	G ^{-b} +++ , typical BV flora	5.4	G ^{+b} +, WBC+
13126	5.4	G ^{-b} +++ , WBC+	4.1	G ^{+b} +++ , WBC+
13508	5.4	G ^{-b} +++ , G ^{+c} +, G ^{+b} occasionally observed	5.4	G ^{+b} occasionally observed, G ^{-b} +, WBC+
6924	5.4	G ^{-b} +++ , WBC+	5.4	G ^{+b} +, WBC+

[00344] 4. Conclusion

[00345] After vaginal administration of the gel of the present disclosure, which
 15 comprised 0.10 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 0.09 percent (weight/volume) of sodium benzoate, the pH value of vaginal secretion of one rhesus macaque decreased from 5.4 to 4.1, while the vaginal flora became dominated by Gram-positive bacilli. The pH values of vaginal secretion of the other three rhesus macaques did not decrease. It was
 20 suggested that the gel could modulate the vaginal flora of rhesus macaque. But the effect in restoring the vaginal flora and decreasing the vaginal pH value were not as

good as those of the saccharide-containing preparation of the present disclosure.

[00346] Experimental example 24

[00347] 1. Experimental objective: to observe the modulating effect of the gel containing 0.60 percent (weight/volume) of 2-phenylethanol and 0.70 percent (weight/volume) of sodium propionate on the vaginal flora of rhesus macaque.

[00348] 2. Experimental method:

[00349] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 0.60 percent (weight/volume) of 2-phenylethanol, 0.70 percent (weight/volume) of sodium propionate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 4.1.

[00350] B. Animals: two adult female rhesus macaques. The pH values of the vaginal secretion were both 5.4. The vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00351] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smear were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00352] 3. Experimental result

[00353] As shown in table 34:

Table 34. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13262	5.4	G ^{-b} +++ , typical BV flora	5.4	Few bacteria, WBC+
13187	5.4	G ^{-b} +++ , WBC++	5.4	Few bacteria, G ^{+b} occasionally observed, WBC+

[00354] 4. Conclusion

[00355] After administering the gel of the present disclosure, which comprised 0.60 percent (weight/volume) of 2-phenylethanol and 0.70 percent (weight/volume) of sodium propionate, the number of vaginal bacteria in two rhesus macaques were significantly reduced, while large Gram-positive bacilli (G^{+b}) were occasionally observed in one rhesus macaques. However, the pH values of vaginal secretion of both rhesus macaques were not reduced. It was suggested that the gel of the present disclosure containing “0.60 percent (weight/volume) of 2-phenylethanol and 0.70 percent (weight/volume) of sodium propionate” could inhibit vaginal bacteria, especially Gram-negative bacilli (G^{-b}). Although large Gram-positive bacilli (G^{+b}) were occasionally observed in vaginal secretion of one rhesus macaque, the flora did not restore to normal, and the pH values of vaginal secretion did not decrease to 4.1 or below.

[00356] Experimental example 25

[00357] 1. Experimental objective: to observe the modulating effect of the gel containing 2.0 percent (weight/volume) of fructose, 4.0 percent (weight/volume) of isomaltose, 0.06 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 0.12 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

[00358] 2. Experimental method:

[00359] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 2.0 percent (weight/volume) of fructose, 4.0 percent (weight/volume) of isomaltose, 0.06 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, 0.12 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum The pH value of the gel was adjusted to 3.3.

[00360] B. Animals: three adult female rhesus macaques. All the pH values of the vaginal secretion were 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00361] C. Animal treatment: 0.5ml of the gel was intravaginally administered,

once a day for consecutive 5 days. The vaginal secretion was sampled with swabs on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

5 **[00362]** 3. Experimental result

[00363] As shown in table 35:

Table 30. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
12266	5.4	G ^{-b} +++ , typical BV flora	<3.8	G ^{+b} +, WBC+
3902	5.4	G ^{-b} +++ , WBC+	<3.8	G ^{+b} +++ , WBC+
3790	5.4	G ^{-b} +++ , G ^{+c} +, G ^{+b} occasionally observed	4.1	G ^{+b} occasionally observed, G ^{-b} +, WBC+

[00364] 4. Conclusion

[00365] The administration of the gel of the present disclosure, which comprised 2.0 percent (weight/volume) of fructose, 4.0 percent (weight/volume) of isomaltose, 0.06 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 0.12 percent (weight/volume) of sodium benzoate, could restore the vaginal flora of rhesus macaques to normal. The vaginal secretion pH values of rhesus macaques could also be reduced.

15 **[00366]** Experimental example 26

[00367] 1. Experimental objective: to observe the modulating effect of the gel containing 5.5 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.10 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 0.065 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

[00368] 2. Experimental method:

[00369] A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 5.5 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.10 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, 0.065 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 4.3.

[00370] B. Animals: two adult female rhesus macaques. The pH values of the vaginal secretion were 5.4. Vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

[00371] C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. The vaginal secretion was sampled with swabs on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00372] 3. Experimental result

[00373] As shown in table 36:

Table 36. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Animal No.	Before administration		1 Day after the end of administration	
	The vaginal secretion pH	The morphology and quantity of bacteria	The vaginal secretion pH	The morphology and quantity of bacteria
13444	5.4	G ^{-b} ++++, G ^{+c} +, typical BV flora	<3.8	G ^{+b} ++, WBC+~++
13126	5.4	G ^{-b} ++++, G ^{+c} ++, G ^{+b} + occasionally observed, typical BV flora, WBC+	4.1	G ^{+b} ++

[00374] 4. Conclusion

[00375] The administration of the gel of the present disclosure, which comprised 5.5 percent (weight/volume) of maltose, 1.0 percent (weight/volume) of isomaltulose, 0.10 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of

sodium propionate, and 0.065 percent (weight/volume) of sodium benzoate, could restore the vaginal flora of rhesus macaques to normal. The vaginal secretion pH values of rhesus macaques could also be reduced.

[00376] Experimental example 27

5 **[00377]** 1. Experimental objective: to observe the modulating effect of the gel containing 2.0 percent (weight/volume) of maltose, 0.20 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 0.065 percent (weight/volume) of sodium benzoate on the vaginal flora of rhesus macaque.

10 **[00378]** A. Preparation of gel: according to the preparation method of the present disclosure, the gel was prepared by using 2.0 percent (weight/volume) of maltose, 0.20 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, 0.065 percent (weight/volume) of sodium benzoate, and 2.15 percent (weight/volume) of Xanthan gum. The pH value of the gel was adjusted to 3.3.

15 **[00379]** B. Animals: five adult female rhesus macaques. The pH values of the vaginal secretion were all 5.4. The vaginal smear examination showed dominant Gram-negative rods. Large Gram-positive rods were rare.

20 **[00380]** C. Animal treatment: 0.5ml of the gel was intravaginally administered, once a day for consecutive 5 days. Vaginal secretion was sampled with swabs on the 6th day. The pH values of vaginal secretion were tested, and the vaginal smears were microscopically examined after Gram-staining. The results were compared with those before treatment.

[00381] 3. Experimental result

[00382] As shown in table 37:

25 Table 37. Effects of antimicrobial gel on vaginal flora of rhesus macaque

Anim al No.	Before administration		1 Day after the end of administration	
	The vaginal secreti on pH	The morphology and quantity of bacteria	The vaginal secretio n pH	The morphology and quantity of bacteria

6374	5.4	G ^{-b} ++++, G ^{+c} +, typical BV flora	4.1	G ^{+b} +++
1329 6	5.4	G ^{-b} ++++, G ^{+c} ++, G ^{+b} + occasionally observed, typical BV flora, WBC+	4.1	G ^{+b} +++
1351 8	5.4	G ^{-b} ++++, G ^{+c} +, typical BV flora	4.1	G ^{+b} +++
1128 4	5.4	G ^{-b} ++++, G ^{+c} +, typical BV flora	4.4	G ^{+b} +++ , G ^{+c} +

[00383] 4. Conclusion

[00384] The administration of the gel of the present disclosure, which comprised 2.0 percent (weight/volume) of maltose, 0.20 percent (weight/volume) of 2-phenylethanol, 0.575 percent (weight/volume) of sodium propionate, and 0.065 percent (weight/volume) of sodium benzoate, could restore the vaginal flora of rhesus macaques to normal. The vaginal secretion pH values of rhesus macaques could also be reduced.

CLAIMS

What is claimed is:

1. A use of an antimicrobial agent combination in preparing a composition for vagina, wherein the composition comprises the following antimicrobial agents: (1) 2-phenylethanol and/or phenoxyethanol, total content of which is calculated as 0.05 to 0.60 percent (weight/volume) of 2-phenylethanol; (2) propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate, total content of which is calculated as 0.25 to 0.70 percent (weight/volume) of sodium propionate; and/or benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate, total content of which is calculated as 0.05 to 0.15 percent (weight/volume) of sodium benzoate.

2. The use according to claim 1, wherein the total content of 2-phenylethanol and/or phenoxyethanol calculated as that of 2-phenylethanol is from 0.10 to 0.40 percent (weight/volume); the total content of propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate calculated as that of sodium propionate is from 0.525 to 0.575 percent (weight/volume); the total content of benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate calculated as that of sodium benzoate is from 0.065 to 0.090 percent (weight/volume).

3. The composition according to claim 1, wherein the composition comprises (1) 2-phenylethanol and/or phenoxyethanol; (2) propanoic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate; (3) benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of benzoic acid.

4. The composition according to claim 1, wherein the antimicrobial agents are 2-phenylethanol, propionic acid and/or sodium propionate, and benzoic acid and/or sodium benzoate.

5. The use according to claim 1, wherein the composition for vagina further comprises one or more antimicrobial agents selected from a group consisting of dehydroacetic acid, sodium dehydroacetate, sorbic acid, potassium sorbate, sodium sorbate, diacetic acid, sodium diacetate, caprylic acid, sodium caprylate, capric acid, sodium caprate, undecylenic acid, sodium undecylenate, lauric acid, sodium laurate, natamycin, lactoferrin, lactoferrin peptide, lysozyme, antibacterial protein, antibacterial peptide, lichenic acid, bergenin, tropolone, chlorogenic acid, palmatine, benzyl alcohol, propylene phenoxyethanol, 1,2-pentanediol, 1,2-hexanediol, 1,6-hexanediol, 1,2-octanediol, 1,2-decanediol, 2-methyl-1,3-propanediol, ethylhexylglycerin, and benzoyl peroxide.

6. The use according to claim 1, wherein the composition for vagina further comprises one or more saccharides selected from a group consisting of glucose, fructose, mannose, sucrose, isomaltulose, 1-kestose, nistose trihydrate, 1F-fructofuranosylnystose, maltose, isomaltose, isomaltotriose, isomaltotetraose, isomaltopentaose, trehalose, cellobiose, melibiose, gentiobiose, gentiooligosaccharide, raffinose, panose, maltooligosaccharide, isomaltulooligosaccharide, fructooligosaccharide, glucomannan, dextrin, starch, and glycogen, with a total content of saccharides ranging from 1.0 percent to 9.0 percent (weight/volume).

7. The use according to claim 1, wherein the composition for vagina further comprises one or more amino acids selected from a group consisting of glutamic acid, glutamine, aspartic acid, asparagine, isoleucine, phenylalanine, valine, leucine, proline, and threonine, with total content of the amino acids ranging from 0.1 percent to 10.0 percent (weight/volume).

8. The use according to claim 1, wherein the composition further comprises one or more estrogen and/or phytoestrogen agents selected from a group consisting of

diethylstilbestrol, estradiol, estriol, daidzein, aglycone of daidzein, genistin, genistein, glycitein, aglycone of glycitein, biochanin, coumestrol, and formononetin, with total content of estrogen and/or phytoestrogen agents ranging from 0.001 percent to 1.0 percent (weight/volume).

9. The use according to claim 1, wherein the composition further comprises one or more substances selected from a group consisting of 0.01 to 5.0 percent (weight/volume) of aloe extract, 0.01 to 5.0 percent (weight/volume) of lavender extract, 0.001 to 1.0 percent (weight/volume) of vitamin E, 0.001 to 1.0 percent (weight/volume) of vitamin A, 0.001 to 1.0 percent (weight/volume) of vitamin D, and 0.001 to 1.0 percent (weight/volume) of vitamin C.

10. The use according to claim 1, wherein the dosage form of the composition is a solution, a water-soluble gel, or an emulsive ointment, in which the pH value of the composition is from 3.0 to 4.3.

11. A composition for vagina, wherein the composition comprises: (1) 2-phenylethanol and/or phenoxyethanol, total content of which is calculated as 0.05 to 0.60 percent (weight/volume) of 2-phenylethanol; (2) propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate, total content of which is calculated as 0.25 to 0.70 percent (weight/volume) of sodium propionate; and/or benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate, total content of which is calculated as 0.05 to 0.15 percent (weight/volume) of sodium benzoate;

wherein, the dosage form of the composition is a solution, a water-soluble gel, or an emulsive ointment, in which the pH value of the composition is from 3.0 to 4.3.

12. The composition according to claim 11, wherein the total content of 2-phenylethanol and/or phenoxyethanol calculated as that of 2-phenylethanol is from 0.10 to 0.40 percent (weight/volume); The total content of propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or

acetic acid and/or sodium acetate calculated as that of sodium propionate is from 0.525 to 0.575 percent (weight/volume); the total content of benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate calculated as that of sodium benzoate is from 0.065 to 0.090 percent (weight/volume).

13. The composition according to claim 11, wherein the composition comprises (1) 2-phenylethanol and/or phenoxyethanol; (2) propanoic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate; (3) benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of benzoic acid.

14. The composition according to claim 11, wherein the antimicrobial agents are 2-phenylethanol, propionic acid and/or sodium propionate, and benzoic acid and/or sodium benzoate.

15. The composition according to claim 11, wherein the composition further comprises one or more antimicrobial agents selected from a group consisting of dehydroacetic acid, sodium dehydroacetate, sorbic acid, potassium sorbate, sodium sorbate, diacetic acid, sodium diacetate, caprylic acid, sodium caprylate, capric acid, sodium caprate, undecylenic acid, sodium undecylenate, lauric acid, sodium laurate, natamycin, lactoferrin, lactoferrin peptide, lysozyme, antibacterial protein, antibacterial peptide, lichenic acid, bergenin, tropolone, chlorogenic acid, palmatine, benzyl alcohol, propylene phenoxyethanol, 1,2 - pentanediol, 1,2-hexanediol, 1,6-hexanediol, 1,2-octanediol, 1,2-decanediol, 2-methyl-1, 3-propanediol, ethylhexylglycerin, and benzoyl peroxide.

16. The composition according to claim 11, wherein the composition further comprises one or more estrogens and/or phytoestrogens agents selected from a group consisting of diethylstilbestrol, estradiol, estriol, daidzein, aglycone of daidzein, genistin, glycitein, aglycone of glycitein, biochanin, coumestrol, and formononetin, with total content of estrogens and/or phytoestrogens agents ranging from 0.001 to 1.0 percent (weight/volume).

17. The composition according to claim 11, wherein the composition further comprises one or more saccharides selected from a group consisting of glucose, fructose, mannose, sucrose, isomaltulose, 1-kestose, nistose trihydrate, 1F-fructofuranosylnystose, maltose, isomaltose, isomaltotriose, isomaltotetraose, isomaltopentaose, trehalose, cellobiose, melibiose, gentiobiose, gentiooligosaccharide, raffinose, panose, maltooligosaccharide, isomaltulooligosaccharide, fructooligosaccharide, glucomannan, dextrin, starch, and glycogen, with total content of saccharides ranging from 1.0 to 9.0 percent (weight/volume).

18. The composition according to claim 17, wherein the saccharides are selected from the group consisting of glucose, fructose, mannose, sucrose, maltose, isomaltulose, isomaltose, trehalose, and maltooligosaccharide, with the total content of 2.0 percent to 6.5 percent (weight/volume).

19. The composition according to claim 11, wherein the composition further comprises one or more substances selected from a group consisting of 0.01 to 5.0 percent (weight/volume) of aloe extract, 0.01 to 5.0 percent (weight/volume) of lavender extract, 0.001 to 1.0 percent (weight/volume) of vitamin E, 0.001 to 1.0 percent (weight/volume) of vitamin A, 0.001 to 1.0 percent (weight/volume) of vitamin D, and 0.001 to 1.0 percent of (weight/volume) vitamin C.

20. The composition according to claim 11, wherein the composition further comprises one or more amino acids and/or physiologically acceptable salts, which is selected from a group consisting of glutamic acid, glutamine, aspartic acid, asparagine, isoleucine, phenylalanine, valine, leucine, proline, and threonine, with total content of amino acids ranging from 0.1 to 10.0 percent (weight/volume).

21. The composition according to claim 20, wherein the content of the amino acid is 1.0 to 5.0 percent (weight/volume), and the amino acid is glutamic acid and/or aspartic acid.

22. A preparation method of a composition for vagina, wherein the method comprises the operation of adding the following agents into water, and/or water-soluble gel matrix, and/or emulsive ointment matrix: (1) 2-phenylethanol and/or phenoxyethanol, total content of which is calculated as 0.05 to 0.60 percent (weight/volume) of 2-phenylethanol; (2) propionic acid and/or sodium propionate and/or calcium propionate and/or levulinic acid and/or sodium levulinate and/or acetic acid and/or sodium acetate, total content of which is calculated as 0.25 to 0.70 percent (weight/volume) of sodium propionate; and/or benzoic acid and/or sodium benzoate and/or para-methoxybenzoic acid and/or sodium para-methoxybenzoate and/or esters of p-hydroxybenzoate, total content of which is calculated as 0.05 to 0.15 percent (weight/volume) of sodium benzoate.