

HYGIENE WASH

FIELD OF INVENTION

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The present invention generally relates to a method to formulate substances for antimicrobial cleansing of human body.

10 BACKGROUND OF THE INVENTION

Various parts of a human body are home to many species of microorganisms, mainly consisting of bacteria and fungi. Although the human body is protected by skin at the outermost layer, the skin is resided by not only beneficial microorganisms, but also
15 pathogenic microorganisms. Infestation by pathogenic microorganisms could cause skin problems, such as acne (caused by *Cutibacterium acnes* or others), rash (typically caused by yeasts), cellulitis (caused by *Streptococcus* or *Staphylococcus*), leprosy (caused by *Mycobacterium leprae*), candidiasis (caused by a type of yeast, *Candida*), ringworm (caused by *Tinea* spp.) and so on. Therefore, care should be
20 taken to maintain personal hygiene, especially at the intimate parts, where special care is usually of utmost importance.

Current formulations for washing or bathing of the human body commonly contain antimicrobial substances, which purportedly kill (bactericidal or fungicidal) or
25 inactivate (bacteriostatic or fungistatic) almost all of the microorganisms present on the skin with 90–99% efficiency upon contact. However, these antimicrobial products do not have the ability to discriminate between microorganisms that are beneficial for the human body and non-beneficial microorganisms. As such, overuse of such antimicrobial products may in turn cause discomfort and even skin problem due to
30 the loss of beneficial microorganisms that act a natural barrier to defend against attack by pathogenic microorganisms. Beneficial skin microorganisms, such as lactic acid bacteria, in fact constitute human's first line of protection in the immune system.

It would be advantageous for the present market of personal care products to have antimicrobial formulations that can selectively inactivate or eliminate pathogenic microorganisms, but not lactic acid bacteria that are beneficial to human skin.

5 Wood vinegars generally contain over 200 organic compounds, which include acetic acid, methanol, ester, acetals, ketone, formic acid and various chemical components that have strong antioxidant properties besides having antimicrobial properties suitable for use in personal care products. However, wood vinegars from different species of woody plants contain different compositions of such compounds and
10 hence, require different formulation strategies before being applied to achieve their selective antimicrobial properties. In addition, guaiacol, which contributes to undesirable pungent smell, was also detected in high amounts in wood vinegars from various plant species. It is therefore an added advantage to utilize an optimized concentration of wood vinegar from a plant species without the presence of guaiacol.

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SUMMARY OF THE PRESENT INVENTION

The present invention features an antimicrobial agent, wood vinegar (also known as
20 pyroligneous acid).

This invention more specifically relates to a method for preparing a personal care product, comprising steps of obtaining a wood vinegar and adding the wood vinegar into the personal care product so that the wood vinegar makes up 18–22% of the
25 total weight or volume of the personal care product.

The wood vinegar is obtained by pyrolysis of *Rhizophora apiculata*, wherein the wood vinegar inactivates or kills microorganisms that cause skin and urinary tract infections but retains a substantial amount of beneficial microorganisms, which helps defend
30 against attack by pathogenic microorganisms.

Preferably, the wood vinegar is obtained without having guaiacol.

Preferably, the beneficial microorganisms retained are lactic acid bacteria.

Preferably, the wood vinegar makes up 20% of the total weight or volume of the personal care product.

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Further in the proposed method, the wood vinegar is left to age for at least three months before being purified and added into a personal care product. Then, the wood vinegar is purified by filtration or distillation to remove impurities.

10 The parts of *Rhizophora apiculata* used for pyrolysis include barks, stems, branches, roots, leaves or any combination thereof.

The purified wood vinegar contains, but not limited to, syringol, benzoic acid, maltol, catechol and vanillin.

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In some embodiments, the personal care product includes, for example, feminine intimate wash, shower gel, soap, facial and cosmetic products, perfume, body lotion, hair shampoo and conditioner, hair grooming products, ointment, antiseptics, and other skin and oral care products.

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Preferably, an antidegradation stabilizer is added into the personal care product.

Preferably, a surfactant or detergent to reduce surface tensions of liquid–liquid or liquid–solid interfaces is added into the personal care product.

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Preferably, a gelling agent and/or emulsifier is added into the personal care product.

In another embodiment, this invention describes a personal care product comprising a purified wood vinegar that is added into the personal care product so that the wood
30 vinegar makes up 18–22% of the total weight or volume of the personal care product. The wood vinegar is obtained by pyrolysis of *Rhizophora apiculata*, wherein the wood vinegar inactivates or kills microorganisms that cause skin and urinary tract infections but retains a substantial amount of beneficial microorganisms.

The present invention consists of features and a combination of parts hereinafter fully described and illustrated in the accompanying drawings, it being understood that various changes in the details may be made without departing from the scope of the invention or sacrificing any of the advantages of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

To further clarify various aspects of some embodiments of the present invention, a more particular description of the invention will be rendered by references to specific embodiments thereof, which are illustrated in the appended drawings. It is appreciated that these drawings depict only typical embodiments of the invention and are therefore not to be considered limiting of its scope. The invention will be described and explained with additional specificity and detail through the accompanying drawings in which:

Figure 1 illustrates the process flow from the pyrolysis of woods and leaves of *Rhizophora apiculata* to the condensation of smoke to wood vinegar, which is then purified and added into personal care products.

Figure 2 is a dot plot histogram showing antimicrobial activities (minimal inhibition concentration and minimal germicidal concentration) of the tested extract against 28 microbial strains.

Figure 3 is a bar chart showing the reduction efficacy of 20% concentration of wood vinegar extract against *E. coli* ATCC25922, *Staphylococcus aureus* LCUM0005, *Candida albicans* ATCC90028 and *Lactobacillus pentosus* LCUM1001 measured in percentage of reduction (%) (All data points with negative percentage reading were recorded as zero percent of reduction).

DETAILED DESCRIPTION OF THE INVENTION

The general principles of the present invention relate to a method to produce and incorporate wood vinegar, an antimicrobial extract, into the formulation of personal care products.

Referring to Figure 1, a wood vinegar (203, 301) is derived from the pyrolysis (100) of woods and leaves (102) of *Rhizophora apiculata* (101), a mangrove plant (also known as *bakau minyak*). Pyrolysis (100), that is the heating at high temperatures above the ceiling temperatures of polymers (temperatures at which polymers tend to revert to their monomers), may take place in the form of dry distillation, hydrous pyrolysis or vacuum pyrolysis. Dry distillation takes place in the absence of air and water. Hydrous pyrolysis takes place in the presence of superheated water or steam, whereas vacuum pyrolysis takes place in a closed, vacuum environment.

The pyrolysis (100) process generates smoke (103) and burnt solids called char (104). The smoke (103) is subjected to condensation (200) in a condenser, through which cooling water is passed in from one end and goes out from the other end of the condenser to cool down the smoke (103). Excessive, unwanted gas (201) is released, and a condensate (202) is collected at the bottom of the condenser. The condensate (202) is further separated into freshly extracted wood vinegar (203), bio-oil and bitumen. Freshly extracted wood vinegar (203) is allowed to age for three months or more prior to purification (300) by filtration or distillation to remove impurities in the wood vinegar.

The purified wood vinegar (301) is added into personal care products (302) at a final concentration of 18–22%, preferably 20%, of the total weight or volume of the personal care product (302). This is because a product containing 20% of purified wood vinegar (301) from *Rhizophora apiculata* (101) for three minutes was found to efficiently and selectively act against (inactivate or kill) commonly found skin bacteria, particularly enteric bacteria and *Staphylococcus* that are potentially harmful to human and produce bad body odour, but not against lactic acid bacteria,

such as *Lactobacillus*, which is involved in the protection of human gut, urinary tract and intimate body parts.

5 The use of wood vinegar (203, 301) from *Rhizophora apiculata* (101) is proposed to replace common antimicrobial agents, such as triclosan, triclocarban, benzalkonium chloride, benzethonium chloride and chloroxylenol.

The major compounds in the wood vinegar (203, 301) are described in Example 1.

10 Each of the various compounds in the wood vinegar (203, 301) has different microbial inactivation mechanism and efficiency. Therefore, the ideal concentration of the wood vinegar (203, 301) to be applied to selectively inactivate non-beneficial and harmful skin microorganisms, while retaining beneficial microorganisms, was optimized.

15 The antimicrobial and selective effects of the present invention is further described with reference to specific examples (Examples 2–4) showing the efficacy tests conducted on 27 strains of pathogenic microorganisms (various species) and one strain of beneficial bacteria.

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Example 1

The major active antimicrobial compound in the wood vinegar (203, 301) from the pyrolysis (100) of *Rhizophora apiculata* (101) is syringol (IUPAC chemical name: 2,6-dimethoxyphenol), which constitutes 28.8% by weight of the wood vinegar.

25 Antioxidants, such as benzoic acid (constitutes 4.8468%), maltol (IUPAC chemical name: 3-hydroxy-2-methylpyran-4-one, constitutes 4.1448%), catechol (IUPAC chemical name: benzene-1,2-diol, constitutes 2.7173%) and vanillin (IUPAC chemical name: 4-hydroxy-3-methoxybenzaldehyde, constitutes 1.3902%) are also present in the wood vinegar (203, 301), contributing to its antimicrobial activity.

30 of these compounds have also been reported to be generally present in wood vinegars from other species. However, guaiacol, which contributes to undesirable pungent smell, was also detected in high amounts in wood vinegars from other plant species. Guaiacol was not detected in the wood vinegar from *Rhizophora apiculata*.

In fact, syringol and its derivatives, which accounted for about 30% of the chemical components detected by gas chromatography-mass spectrometry (GCMS) analysis, were found to be the major components in the wood vinegar from *Rhizophora apiculata*. Therefore, the aroma of the wood vinegar from *Rhizophora apiculata* has strong, woody and smoky smells contributed by syringol.

The library/ID of major compounds and their corresponding Chemical Abstract Service (CAS) registry numbers and concentrations (R1) detected in the wood vinegar from *Rhizophora apiculata* (101) by GCMS are shown in Table 1.

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Table 1

No	Library/ID	CAS	R1
1	Phenol, 2,6-dimethoxy- (Syringol)	000091-10-1	28.8083 - 28.9754%
2	Benzoic acid	000065-85-0	4.8468- 7.0655%
3	Diethyl Phthalate	000084-66-2	1.3504- 4.5513%
4	1,2-Benzenediol, 3-methoxy-	000934-00-9	3.7460- 3.9451%
5	Maltol	000118-71-8	3.4793-4.1448%
6	Hexanedioic acid, bis(2-ethylhexyl) ester	000103-23-1	0.2733- 2.3285%
7	Catechol	000120-80-9	2.1471- 2.7173%
8	Vanillin	000121-33-5	1.3902-1.8106%
9	3-Buten-2-ol, 2-methyl-	000115-18-4	0.3616-1.0709%
10	2-Oxo-n-valeric acid	001821-02-9	0.0000- 15.3409%
11	2,6-Octadiene-4,5-diol	004486-59-3	0.0000- 6.8894%
12	Benzoic acid, 4-hydroxy-3-methoxy-	000121-34-6	0.0000- 6.8720%
13	1-Hepten-3-ol, 3-methyl	024424-68-8	0.0000- 19.2399%
14	Tetrahydrofuran, 2-propyl-	003208-22-8	0.0000- 9.5503%
15	2H-Pyran-2-one, 3-acetyl-4-hydroxy-6-methyl-	000771-03-9	0.0000- 5.0413%
16	Phenol, 2,6-dimethyl-4-nitro-	002423-71-4	0.0000-3.1326%
17	Butanoic acid, 4-hydroxy-	000591-81-1	0.0000-2.8276%
18	1,1'-Biphenyl, 3,3',4,4',5,5'-hexamethoxy-	056772-00-0	0.0000-1.4318%
19	3-Methylcarbazole	004630-20-0	0.0000-1.3388%
20	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	000080-71-7	0.0000-1.2921%
21	2(3H)-Furanone, dihydro-5-methyl-	000108-29-2	0.0000-1.1584%
22	9H-Carbazole-9-methanol	002409-36-1	0.0000-1.0947%

Example 2

The antimicrobial activity of the wood vinegar is tested on a wide range of bacterial and fungal species and strains, which include *Escherichia coli*, *Enterobacter* spp.,

Enterococcus faecalis, *Enterococcus faecium*, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Bacillus cereus*, *Candida glabrata*, *Candida tropicalis*, *Candida albicans*, *Candida kefyr*, *Candida parapsilosis* and *Lactobacillus pentosus* (as described in Table 2).

Table 2

Microorganisms	Strains	Description
<i>Escherichia coli</i> (non-pathogenic)	<i>E. coli</i> ATCC25922	Cause urinary tract infection
	<i>E. coli</i> LCUM0100	
<i>Escherichia coli</i> (pathogenic)	ETEC LCUM0010	Cause diarrhea disease in human
	DAEC LCUM0011	
	EAEC LCUM0012	
	EIEC LCUM0013	
	EHEC LCUM0014	
	EPEC LCUM0015	
<i>Enterobacter</i> spp.	<i>Enterococcus</i> spp. LCUM0105	Common commensal organisms in the intestines of humans
<i>Enterococcus faecalis</i>	<i>E. faecalis</i> ATCC33186	Can cause endocarditis and septicemia, urinary tract infections, meningitis, and other infections in humans
<i>Enterococcus faecium</i>	<i>E. faecium</i> ATCC700221	Common gut bacteria; but may be pathogenic, causing diseases such as neonatal meningitis or endocarditis.
<i>Acinetobacter</i> spp.	<i>Acinetobacter</i> spp. LCUM099	Can cause skin and wound infections, bacteremia, and meningitis in immuno-compromised patient
<i>Pseudomonas aeruginosa</i>	<i>P. aeruginosa</i> LCUM0111	An opportunistic, nosocomial pathogen of immune-compromised individuals; typically infects the airway, urinary tract, burns, and wounds, and also causes other blood infections
	<i>P. aeruginosa</i> LCUM0112	
<i>Proteus mirabilis</i>	<i>P. mirabilis</i> ATCC7002	Can cause urinary tract infection with distinct fishy odor; can also cause wound infections, septicemia, and pneumonia, mostly in hospitalized patients

<i>Staphylococcus aureus</i>	<i>S. aureus</i> LCUM0005	A common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning
<i>Staphylococcus epidermidis</i>	<i>S. epidermidis</i> ATCC14990	Common skin bacteria; can cause disease in immuno-compromised patient; universally found inside affected acne vulgaris pores
	<i>S. epidermidis</i> ATCC35984	
<i>Staphylococcus saprophyticus</i>	<i>S. saprophyticus</i> ATCC15305	Normal flora of the female genital tract and perineum; common cause of community-acquired urinary tract infections particularly in female; sexual activity increases the risk of <i>S. saprophyticus</i> urinary tract infections
<i>Bacillus cereus</i>	<i>B. cereus</i> LCUM0001	Common cause of food poisoning
	<i>B. cereus</i> LCUM0002	
<i>Candida glabrata</i>	<i>C. glabrata</i> RJ4	Causes urinary tract infection
<i>Candida tropicalis</i>	<i>C. tropicalis</i> RJ8	Causes urinary tract infection
<i>Candida albicans</i>	<i>C. albicans</i> ATCC90028	Cause urinary tract infection
	<i>C. albicans</i> ATCC90029	
<i>Candida kefyr</i>	<i>C. kefyr</i> ATCC204093	Causes urinary tract infection
<i>Candida parapsilosis</i>	<i>C. parapsilosis</i> ATCC90018	Causes urinary tract infection
<i>Lactobacillus pentosus</i>	<i>L. pentosus</i> LCUM1001	Beneficial bacteria associated to healthy vaginal flora; the healthy vaginal flora is mostly composed of lactobacilli

Example 3

- Antimicrobial activities of the wood vinegar extract measured in terms of minimal inhibition concentration and minimal germicidal concentration was tested against
- 5 28 microbial strains – *Escherichia coli* (*E. coli*) ATCC25922, *E. coli* LCUM0100, Enterotoxigenic *E. coli* (ETEC) LCUM0010, Diffusely Adherent *E. coli* (DAEC)

LCUM0011, Enteroinvasive *E. coli* (EIEC) LCUM0013, Enterohemorrhagic *E. coli* (EHEC) LCUM0014, Enteropathogenic *E. coli* (EPEC) LCUM0015, *Enterobacter* spp. LCUM0105, *Enterococcus faecalis* ATCC33186, *Enterococcus faecium* ATCC700221, *Acinetobacter* spp. LCUM099, *Pseudomonas aeruginosa* LCUM0111, *Pseudomonas aeruginosa* LCUM0112, *Proteus mirabilis* ATCC7002, *Staphylococcus aureus* LCUM0005, *Staphylococcus epidermidis* ATCC14990, *Staphylococcus epidermidis* ATCC35984, *Staphylococcus saprophyticus* ATCC15305, *Bacillus cereus* LCUM0001, *Bacillus cereus* LCUM0002, *Candida glabrata* RJ4, *Candida tropicalis* RJ8, *Candida albicans* ATCC90028, *Candida albicans* ATCC90029, *Candida kefyr* ATCC204093, *Candida parapsilosis* ATCC90018 and *Lactobacillus pentosus* LCUM1001.

Referring to Figure 1, germicidal effect generally occurred at extract concentrations that are higher than the concentrations required to achieve inhibition effect. Beyond 20% concentration, all of the tested strains were killed except the beneficial bacterial strain, *Lactobacillus pentosus* LCUM1001.

Example 4

Reduction efficacy with 20% concentration of the wood vinegar extract against *Escherichia coli* ATCC25922, *Staphylococcus aureus* LCUM0005, *Candida albicans* ATCC90028 and *Lactobacillus pentosus* LCUM1001 was measured in percentage of reduction (%) after 1 minute, 3 minutes and 5 minutes of exposure to the wood vinegar (as illustrated in Figure 2). Exposure of 3 minutes to the wood vinegar gave the highest average percentage of reduction of harmful microorganisms (*Escherichia coli* ATCC25922, *Staphylococcus aureus* LCUM0005 and *Candida albicans* ATCC90028) and considerably low percentage of reduction of beneficial bacteria (*Lactobacillus pentosus*). The optimized wood vinegar concentration selectively inactivated 99.99% of Gram-negative enteric bacteria, 99.40% of Gram-positive *Staphylococcus* and 73.47% of *Candida*, all of which cause skin and urinary tract infections. However, the product was inefficient against *Lactobacillus* because only 26.03% of *Lactobacillus* was inactivated upon 3 minutes of contact with the product. Other than the species being studied here, beneficial lactobacilli that are potentially conserved also comprises *L. acidophilus*, *L. brevis*,

L. casei, *L. fermentum*, *L. paraplantarum*, *L. plantarum* and many more, as well as other genus of common probiotic bacteria, such as *Bifidobacterium bifidum*.

5 Examples of personal care products include feminine intimate wash, shower gel, soap, facial and cosmetic products, perfume, body lotion, hair shampoo and conditioner, hair grooming products, ointment, antiseptics, and other skin and oral care products.

10 In addition to wood vinegar, the method for preparing such personal care product may include mixing with other compounds to improve the characteristics of the personal care product. Among many compounds are those acting as surfactants, such as:

Anionic surfactants, which are categorized as sulphate, sulfonate, phosphate, and carboxylates. Prominent alkyl sulphates include ammonium lauryl sulphate, 15 sodium lauryl sulphate, sodium dodecyl sulphate, and the related alkyl-ether sulphates like sodium laureth sulphate and sodium myreth sulphate. Carboxylates comprise the alkyl carboxylates (soaps), for example, sodium stearate, whereas more specialized species of carboxylates include sodium lauroyl sarcosinate and carboxylate-based fluorosurfactants).

20 Cationic surfactants, which are categorized as pH-dependent primary, secondary, or tertiary amines like octenidine dihydrochloride. Cationic surfactants also include permanently charged quaternary ammonium salts like cetrimonium bromide (CTAB), cetylpyridinium chloride (CPC), benzalkonium chloride (BAC), benzethonium chloride (BZT), dimethyldioctadecylammonium chloride or 25 dioctadecyldimethylammonium bromide (DODAB).

Zwitterionic surfactants, which include sultaines like CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) and cocamidopropyl hydroxysultaine; and betaines like cocamidopropyl betaine (having a carboxylate with ammonium).

30 Non-ionic surfactants, which are categorized as ethoxylates, fatty acid esters, alkyl polyglucosides, amine oxides, lauryldimethylamine oxide, sulfoxides, dimethyl sulfoxide and phosphine oxides.

Antidegradation stabilizer (for example, antioxidants like phosphite esters and alkylated phenols, as well as sequestrants or chelating agents like disodium ethylenediamine tetraacetic acid, ammonium polyaspartate and tetrasodium etidronate) could be added into the formulation of the personal care product to
5 prevent degradation of the product due to inactivation by metal ions, pH changes, exposure to UV or light and oxidation. Stabilizers also help to maintain clarity, protect fragrance compounds, and prevent rancidity.

Additionally, thickener or gelling agent (for example, polyethylene glycol, polyacrylic
10 acid, vegetable gums and waxes), and emulsifier (common examples include emulsifying wax, cetearyl alcohol, polysorbate 20, and ceteareth-20), some of which also act as stabilizer, could be further added into the formulation of the personal care product to promote homogenous mixing and thickening of substances in the product in order to achieve stability and ease of application.

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The present invention may be embodied in other specific forms without departing from its essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore indicated by the appended claims rather than by the foregoing description.
20 All changes, which come within the meaning and range of equivalency of the claims, are to be embraced within their scope.