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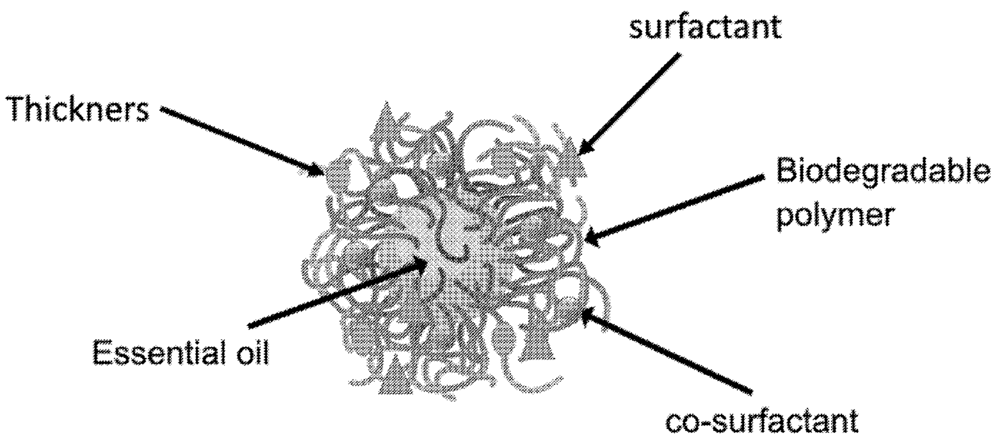

FIG. 1

(57) Abstract: A nanoemulsion composite mixture for antimicrobial activities is provided having biodegradable polymers, co-surfactants, surfactants and thickeners together entrapping an oil. The mixture is dissolved in glacial acetic acid and dichloromethane. Water is used as the vehicle to hold the mixture, and in the mixture: the biodegradable polymers are within 5-8%, the glacial acetic acid is within 0.5-1.5%, the oil is within 0.5-1.5%, the surfactant is within 0.5-1.5%, the thickener is within 15-25%, the dichloromethane is within 2.5-10%, and the co- surfactants is within 0.5-1.5%. These nanoemulsion composite mixture exhibits superior antimicrobial abilities, can kill 99.99% of different bacteria without causing any harmful side effect to humans. The nanoemulsion composite mixture can be incorporated in products like hand gel sanitizers, soap, detergents, animal feed, and more.

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[Continued on next page]

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NANOEMULSION COMPOSITE MIXTURE OF ESSENTIAL OILS, BIODEGRADABLE POLYMERS AND CO-SURFACTANTS FOR ANTIMICROBIAL ACTIVITIES

FIELD OF THE INVENTION

This invention relates to nano-emulsion of oil mixtures with biodegradable polymers and co-surfactants.

BACKGROUND OF THE INVENTION

Microbial infection is prevailing world-wide and is a high reason for mortality and morbidity leading to economic burden arising from both the decreased healthiness and the high cost spent on health care. Traditional disinfectants contain harsh chemical and alcohols, and have major problems related to dermal toxicities which may on the long run cause severe health issue such as asthma, lung cancer, skin cancer, eye infection and many others. The present invention advances the art with new nano-emulsion composites addressing at least some of these problems.

SUMMARY OF THE INVENTION

The present invention provides a nanoemulsion composite mixture for antimicrobial activities. The nanoemulsion composite mixture can be defined in three forms: comprising, consisting essentially of, or consisting of: biodegradable polymers, co-surfactants, surfactants and thickeners together entrapping an oil. The nanoemulsion composite mixture is dissolved in glacial acetic acid and dichloromethane, where water is used as the vehicle to hold the nanoemulsion composite mixture, and wherein in the nanoemulsion composite mixture: the biodegradable polymers are within 5-8%, the glacial acetic acid is within 0.5-1.5%, the oil is within 0.5-1.5%, the surfactant is within 0.5-1.5%, the thickener is within 15-25%, the dichloromethane is within 2.5-10%, and the co-surfactants is within 0.5-1.5%.

In one embodiment, the thickener is glycerin.

In another embodiment, the surfactant is Tween 80.

In yet another embodiment, the biodegradable polymers are Chitosan, 1,3 Propyleneglycolalginate, Gelatin, Pectin, 3-hydroxypentanoic acid, Glucose, Poly (acrylates), Albumin, Gluten, Poly (alkyl cyanoacrylate), Alginate, Glycerol and various, Poly (e-caprolactone), Alginic acid, Hydroxyethyl starch, poly(lactic-co-glycolic acid), Carrageenan, Hydroxypropylstarch, Polyanhydrides, Casein Cellulose, Lactic acid, Polylactic acid, Cellulose, Lactic acid, butyl ester, Polyphosphazenes, Cellulose acetate, Lignocellulose, polyvinyl alcohol, Cellulose acetate butyrate, Sucrose, Propyleneglycolalginate, Cellulose derivative, zein, starch, Chitin, Dextrin, Edible Starch, Hydrolysed Starch or Dextrin.

In yet another embodiment, the oil is Green tea essential oil, Ajwain Essential Oil, Almond Oil, Ambrette Seed Essential Oil, Angelica root Oil, Basil Essential oil, Bay oil, Bergamot oil, Black Pepper Oil , Black Seed essential oil, Cade oil, Cajeput oil, Calamus Essential Oil, Caraway seed, Carrot Seed Oil , Cassia Bark Oil, Castor Oil, Chamomile Essential Oil, Cinnamon essential oil, Citronella Essential Oil, Coconut Oil, Coriander Seed Oil, Cumin oil, Curry Leaf Oil, Cypress oil, Davana Essential Oil, Dill Seed Essential Oil, E. Citriodora Essential Oil, Sesame oil, Fennel Essential Oil, Fractionated Coconut Oil, Frankincense Essential Oil, Garlic Oil, Geranium Essential Oil, Ginger Essential Oil, Globulus Essential Oil, Grapefruit, Green tea essential oil, Holy Basil Essential Oil, Indian Rosemary Essential Oil, Jojoba Oil, Juniper Berry Oil, Karanj Oil, Lavender Oil, Lemongrass Essential Oil, Lemongrass Essential Oil, Lemongrass Essential Oil, Lime Oil, Mandarine, Marigold Essential Oil, Mentha Arvensis Essential Oil, Mentha Citrata Essential Oil, Mentha Peperata Essential Oil, Mentha Spicata Essential Oil, Moringa Seed oil, Nagarmotha Essential oil, Neem Oil, Nutmeg Essential Oil, Palmarosa Essential Oil, Patchouli Essential Oil, Peanut Oil, Sage Essential Oil, Soybean Oil, Spearmint, Sweet Marjoram oil, Tagetes Minuta Essential Oil, Tamannu Essential Oil, Tea tree essential oil, Tea Tree Oil, Vetiver Essential Oil, Orange Oil, Thyme red Essential Oil, Wormwood, or Ylang Ylang Essential Oil.

In still another embodiment, the co-surfactants are Benzalkonium chloride, Behentrimonium chloride, Carbethopendecinium bromide, Didecyldimethylammonium chloride, Benzalkonium chloride, Cetalkonium chloride, Dimethyldioctadecylammonium bromide, Benzethonium chloride, Cetrimonium bromide, Dimethyldioctadecylammonium chloride, Benzododecinium bromide, Cetrimonium chloride, Dioleoyl-3-trimethylammonium propane, Bronidox, Cetylpyridinium chloride, Domiphen bromide, Lauryl methyl gluceth-10 hydroxypropyl dimonium chloride, Pahutoxin, Tetramethylammonium hydroxide, Octenidine dihydrochloride, Stearalkonium chloride, Thonzonium bromide, Olaflur, N-Oleyl-1,3-propanediamine, or Octenidine dihydrochloride.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows according to an exemplary embodiment of the invention a nano-emulsion system is composed of five different type of main components: Four components: biodegradable polymer such as chitosan, co-surfactant such as benzalkonium chloride, surfactants and thickeners. These four type of components enhance the entrapment of the fifth component type such as green tea essential oil.

FIGs. 2-4 show according to exemplary embodiments of the invention antimicrobial activities for the 6 formulations against 6 different microbial strains. Each block of bar graphs is in the order of formula 1 through 6 from left to right in each respective block of graph bars. For color interpretation of these graphs the reader is referred to the priority document of this application.

FIG. 5 shows according to an exemplary embodiment of the invention pour plate colony counting antimicrobial assay against S. aureus and E. coli.

FIG. 6 shows according to an exemplary embodiment of the invention improved essential oils thermal stability.

FIG. 7 shows according to an exemplary embodiment of the invention improved antioxidant activity.

DETAILED DESCRIPTION

The objective of the present invention was to develop a natural and eco-friendly mixture of oils (e.g. fixed oils, mineral oils, oleo-resin oils, and essential oils) and biodegradable polymers and mixing all of them (with different components, not limited to, such as: co-surfactants and/or viscosity modifiers and/or stabilizers and/or pH adjusters and/or humectants and/or moisturizing and/or preservatives and/or chelating agents and/or buffering agents and/or skin conditioning and/or solvents and/or antioxidants) by using conventional and mixers and/or high speed homogenizers under certain speed and duration, to have a final product as a natural disinfectant/antiseptic/antioxidant with broad antimicrobial activities and skin regeneration and cleansing activities.

The mixing step of all ingredients determines the final product, for example, either composite mixture of all ingredients with mixers and/or nanoscale transformation of the composite mixture by high-speed homogenizers. The latter makes the mixture exhibiting superior improvement in the antibacterial/disinfectant/antioxidant properties compared to their composite bulk solutions and can be prepared as nanoparticles and nano-emulsions, nano-suspensions and solubilized preparations.

The nanoemulsion composite mixture can be prepared as liquid solution and gel and powder additive forms to be used as natural disinfectant/antiseptic/antioxidant, and can also be incorporated in all cosmetics products, pesticides and animal feed, food beverages to enhance their antimicrobial and antioxidant and preservation properties while maintaining their natural flavor and function. **Table 1** shows a composition of key components of the nano-emulsion composition.

Table 1. A list of key components of the nano-emulsion composition.

Each 1L contains:				
S.N.	Ingredient name	Quantity	Function	Specification
1-	Chitosan	65 g	Biodegradable polymer with two functions: Co-surfactant and antiseptic	USP 40
2-	Glacial acetic acid	10 g	Solvent	
3-	Green tea essential oil	10 g	Solvent	
4-	Tween 80	11.2 g	Surfactant	
5-	Glycerin	200 g	Thickener	
6-	Dichloromethane	50 g	Solvent	
7-	Benzalkonium chloride	10 g	Co-surfactant and antiseptic	
8-	Distilled water	1000 gm	Vehicle	USP40

These different components added to the nano-emulsion formula each playing different roles. The following components are key components, yet some components could be varied as will be discussed *infra*.

- Benzalkonium chloride is a quaternary ammonium antiseptic and disinfectant with actions and uses similar to those of other cationic surfactants. It can also be used as an antimicrobial preservative for pharmaceutical products.
- Glacial acetic acid is the anhydrous (undiluted or free of water) form of acetic acid. Acetic acid is considered an organic compound and has the chemical formula CH_3COOH. A diluted solution of acetic acid is known as vinegar or ethanoic acid or ethylic acid. This acid is classified as a weak acid, and it is used as a solvent to dissolve chitosan molecules.

- Green tea essential oil is an anti-free radical, an antioxidant and a skin regenerating (anti-wrinkles, anti-ageing) as well as their ability of killing microorganisms due to their phenolic contents.
- Tween 80 (polysorbate 80, polyoxyethylene sorbitan monooleate) is a non-ionic surfactant that is widely used as an emulsifier both in the laboratory and in industry. It is often included in laboratory experiments without any comments or evaluations of possible side effects.
- The presence of glycerol in an oil-in-water emulsion will increase the solubility of the non-polar oil molecules in the surrounding aqueous phase since a glycerol solution is less polar than pure water.
- Dichloromethane is a clear colorless, volatile, sweet-smelling lipophilic liquid. It is commonly used as a solvent of green tea essential oil.
- Chitosan is a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide. Chitosan has fascinating antibacterial activity, good biodegradation, outstanding biocompatibility, non-toxicity and excellent physical and chemical properties. As a result, chitosan has been widely used in the field of antibacterial. Chitosan and its derivatives show antibacterial activity against fungi, gram-positive bacteria and gram-negative bacteria. In recent years, there have been some reviews about the antibacterial activity of chitosan and its derivatives.

The mixing step of all ingredients determines the final shape of the product, for example, either composite mixture of all ingredients with mixers and/or nanoscale transformation of the composite mixture by high-speed homogenizers. Briefly, chitosan solution (1% w/v) is prepared after dissolving chitosan powder in glacial acetic acid (1% w/v). Then, Tween 80 is stirred with chitosan solution forming the aqueous phase of the emulsion. In dichloromethane, green tea essential oil is dissolved forming the oily phase of the emulsion. On the semi-industrial scale rotor-stator homogenizer, in an exemplary embodiment, the oily phase is homogenized with the aqueous phase at 3000 rpm for 20 min to get an oil in water emulsion. Then benzalkonium chloride, glycerin and distilled water are added step wise forming the antiseptic nanoemulsion composite mixture.

The latter makes the nanoemulsion composite mixture exhibiting superior improvement in the antibacterial, disinfectant, antioxidant properties compared to their composite bulk solutions.

The final forms of the mixed products (nanoemulsion composite mixture) can be prepared as liquid solution and gel and powder additive forms that to be used as natural disinfectant/antiseptic/antioxidant, and can also be incorporated in all cosmetics products, and added to all food beverages to enhance their antimicrobial and antioxidant and presentation properties while maintaining their natural flavor and function and can also be added as additives to pesticides and animal feed. The nanoemulsion composite mixture showed:

(i) Enhanced essential oils water solubility

(ii) Improved essential oils thermal stability

(iii) Boosted the antioxidant activity of essential oil

(iv) Improved in the microbial activities of essential oils against both Gram-positive, Gram-negative bacteria, fungi, and viruses.

Enhanced essential oils water solubility

Nano-emulsions constitute one interesting vehicle for enhancing solubility, stability and delivering natural oils, by encapsulating them into nanosized micelles with sizes ranging from 20 – 200 nm. They gather some unique characteristics as small size, increased surface area and stability which can increase efficiency and biological effects of pharmaceutical dosage forms.

Improved essential oils thermal stability

Thermogravimetric analysis (TGA) analysis is performed to study the thermal stability of green tea essential oil, after encapsulation. The temperature at which a material is subjected to the highest rate of weight loss is known as the temperature of maximum degradation rate (Td) TGA studies showed a significant improvement of the thermal stability of nanoencapsulated green tea essential oil by about 1.74 folds (or in another embodiment more than 1.5 folds) over the bulk green tea essential oil.

Boosted the antioxidant activity of essential oil

DPPH radical scavenging ability is a method that used to estimate the antioxidant properties of the was identified for the formula. Gallic acid was used as positive control. Surprisingly the antioxidant activity of green tea essential oil is increased significantly by about 2-fold (or in another embodiment more than 2-folds) after nanoencapsulation process with IC50 (concentration required to scavenge DPPH radicals by 50%) of 0.34 mg/mL (or in another embodiment more than 0.3 mg/mL).

Hypoallergic feature

The most updated animal test results showed that the nanoemulsion composite mixture samples didn't show any sensitivity reactions on the skin on three different rabbits that are used in this trials testing. Accordingly, the nanoemulsion composite mixture samples showed conformance with requirements according to the tests mentioned in the Egyptian standards.

Improved the antimicrobial activities of essential oils against both Gram-positive, Gram-negative bacteria and fungi

Inhibition zone method was followed in order to test the antimicrobial efficiency of the selected nanoemulsion composite mixture samples against 6 chosen strains of gram-positive bacterial strains, 6 strains of gram-negative bacterial strains 6 strains of fungi as shown in the following list;

1-Gram-positive bacterial:

a) Staphylococcus aureus

b) Bacillus subtilis

c) Micrococcus spp.

d) Streptococcus mutants

e) Methicillin-resistant Staphylococcus aureus (MRSA)

f) Enterococcus faecalis

2-Gram-negative bacteria:

a) Enterobacter colacae

b) Salmonella Typhium

c) Eisherecia coli

d) Klebsella pneumonia

e) Proteus vulgaris

f) Pseudomonas aeruginosa

3-Fungi:

a) Aspergillus fumigatus

b) Aspergillus niger

c) Candida albicans

d) Geotricum candidum

e) Penicillium italicum

f) Fusarium moniliform

The nanoemulsion composite mixture was tested to find out the percentage of dilution that can stop the growth of microbes. This method is known as (MIC= minimum inhibitory concentration). At the time, the results showed that the sample can stop the growth of microbes even after 13 times of dilution with some types of microbes such as staph. mutants. Sample showed antimicrobial activities with an ability close to commercial preparations containing strong chemicals or alcohols especially against Geo. Candidium, staph. mutants, B. subtilis, Entero. faecllialis, and E. colace. Moreover, sample that proved effectiveness were also tested to see if the samples had the ability to kill microbes (bactericidal effect = cidal) or could only stop their growth (bacteriostatic effect = static). This method is known as (MIC= minimum toxic concentration). As shown in (**Table 2**), The results showed that the nanoemulsion composite mixture samples can kill different types of microbes, and in other cases, they were only able to stop the growth of some microbes.

Table 2. The antimicrobial activity of the nanoemulsion composite mixture against gram-positive, gram-negative bacteria and fungi.

Microbial strains		**Nanoemulsion composite mixture**
Gram-positive bacteria	Staphylococcus aureus	Bactericidal
	Bacillus subtilis	Bacteriostatic
	Micrococcus spp.	Bacteriostatic
	Streptococcus mutants	Bacteriostatic
	MRSA	Bacteriostatic
	Enterococcus faecalis	Bacteriostatic
Gram-negative bacteria	Enterobacter colacae	Bacteriostatic
	Salmonella Typhium	Bactericidal

	Klebsella pneumonia	Bactericidal
	Proteus vulgaris	Bactericidal
	Pseudomonas aeruginosa	Bactericidal
Fungi	Aspergillus fumigatus	Bactericidal
	Aspergillus niger	Bactericidal
	Candida albicans	Bactericidal
	Geotricum candidum	Bactericidal
	Penicillium italicum	Bactericidal
	Fusarium moniliform	Bactericidal

Improved the antimicrobial activities of essential oils against COVID-19

The antiviral activity for the all the nanoemulsion composite mixture samples was performed at Center of Scientific Excellence for Influenza Viruses and Consultant Unit for Viruses Research and Bioassays at National Research Centre (NRC). The nanoemulsion composite mixture samples shows high cytotoxic value for COVID-19. The nanoemulsion composite mixture samples show antiviral activity that reaches almost 100% with addition of harsh chemicals. Specifically, after the addition of quaternary ammonium compounds. The IC50 value is 0.0167 uL (or in another embodiment less than 0.05 uL).

Alternatively, the invention can be described as a composite Mixture of Essential Oils with biodegradable polymers and co-surfactants for broad antimicrobial activities and enhanced thermal stability and antioxidant activities. Mixing procedure determines the final compositional structure of nanoemulsion composite mixture. For the later, the following are the key aspects in the preparation:

1- Mixing all components, as for example shown in **Table 1**, into nano-emulsion formulation is based on high-speed Homogenization process with mechanical stirring range 300-30000 rpm.

2- Controlling the preparation parameters such as (i) homogenization speed (1000 - 3000 rpm) and homogenization time (10 min - 30 min).

3- Controlling the solution parameters such as the selection of the desired essential oil(s), polymer and co-surfactant as well as their concentration as described in Table 7.

4- Identifying the antioxidant activities of the nanoemulsion composite mixture.

5- Investigating the antibacterial activities and determination of both minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of the prepared nanoemulsion composite mixture against different gram-positive and gram-negative bacteria, viruses and fungi.

6- Investigating the thermal stability of the nanoemulsion composite mixture .

7- Estimating the IC50 of the nanoemulsion composite mixture against covid-19 (microbial) strains

8- Investigating the cytotoxicity of the nanoemulsion composite mixture in-vitro and on the skin of different animals (to study skin irritation as well).

9- Estimating the antimicrobial contact time needed for the In-vitro release of the active components (i.e. essential oils) from the nanoemulsion composite mixture at different pH values.

10- Studying the chemical stability of the essential oils after nanoencapsulation

11- Validation of the nanoemulsion composite mixture by testing at different industrial sites

The innovation is not limited to green tea essential oil, and it can include the following essential oils that are listed in **Table** 3.

Table 3: List of other Essential oils

List of Essential Oils		
Ajwain Essential Oil	Almond Oil	Ambrette Seed Essential Oils
Angelica root Oil	Basil Essential oil	Bay oil
Bergamot oil	Black Pepper Oil	Black Seed essential oil
Cade oil	Cajeput oil	Calamus Essential Oil
Caraway seed	Carrot Seed Oil	Cassia Bark Oil
Castor Oil	Chamomile Essential Oil	Cinnamon essential oil
Citronella Essential Oil	Coconut Oil	Coriander Seed Oil
Cumin oil	Curry Leaf Oil	Cypress oil
Davana Essential Oil	Dill Seed Essential Oil	E. Citriodora Essential Oil
Sesame oil	Fennel Essential Oil	Fractionated Coconut Oil
Frankincense Essential Oil	Garlic Oil	Geranium Essential Oil
Ginger Essential Oil	Globulus Essential	Grapefruit
Green tea essential oil	Holy Basil Essential Oil	Indian Rosemary Essential Oil
Jojoba Oil	Juniper Berry Oil	Karanj Oil
Lavender Oil	Lemongrass Essential Oil	Lemongrass Essential Oil
Lemongrass Essential Oil	Lime Oil	Mandarine
Marigold Essential Oil	Mentha Arvensis Essential Oil	Mentha Citrata Essential Oil
Mentha Peperata Essential Oil	Mentha Spicata Essential Oil	Moringa Seed oil
Nagarmotha Essential oil	Neem Oil	Nutmeg Essential Oil
Palmarosa Essential Oil	Patchouli Essential Oil	Peanut Oil
Sage Essential Oil	Soybean Oil	Spearmint
Sweet Marjoram oil	Tagetes Minuta Essential Oil	Tamannu Essential Oil
tea tree essential oil	Tea Tree Oil	Vetiver Essential Oil

Orange Oil	Thyme red Essential Oil	Wormwood
Ylang Ylang Essential Oil		

The innovation is not limited to chitosan, and it can include the following biodegradable polymers that are listed in **Table** 4.

Table 4. List of Other Biodegradable polymers

List of biodegradable polymers		
1,3 Propyleneglycolalginate	Gelatin	Pectin
3-hydroxypentanoic acid	Glucose	Poly (acrylates)
Albumin	Gluten	Poly (alkyl cyanoacrylate)
Alginate	Glycerol and various	Poly (e-caprolactone)
Alginic acid	Hydroxyethyl starch	poly(lactic-co-glycolic acid)
Carrageenan	Hydroxypropylstarch	Polyanhydrides
Casein Cellulose	Lactic acid	Polylactic acid
Cellulose	Lactic acid, butyl ester	Polyphosphazenes
Cellulose acetate	Lignocellulose	polyvinyl alcohol
Cellulose acetate butyrate	Sucrose	Propyleneglycolalginate
Cellulose dervatives,	zein	starch
Chitin	Dextrin	Starch, edible
Dextrin	Starch, hydrolysed	

The innovation is not limited to benzalkonium chloride, and it can include the following co-surfactants that are listed in **Table 5**.

Table 5. List of Other Co-surfactants

List of co-surfactants		
Behentrimonium chloride	Carbethopendecinium bromide	Didecyldimethylammonium chloride
Benzalkonium chloride	Cetalkonium chloride	Dimethyldioctadecylammonium bromide
Benzethonium chloride	Cetrimonium bromide	Dimethyldioctadecylammonium chloride
Benzododecinium bromide	Cetrimonium chloride	Dioleoyl-3-trimethylammonium propane
Bronidox	Cetylpyridinium chloride	Domiphen bromide
Lauryl methyl gluceth-10 hydroxypropyl dimonium chloride	Pahutoxin	Tetramethylammonium hydroxide
Octenidine dihydrochloride	Stearalkonium chloride	Thonzonium bromide
Olaflur	N-Oleyl-1,3-propanediamine	Octenidine dihydrochloride

The nanoemulsion composite mixture with exemplary formula components is preferably used with percentages and amount ranges as shown in **Table 6**. It is noted that these ranges and percentages apply to other examples discussed herein for the biodegradable polymers, oils and co-surfactants.

Table 6. Percentage and amount ranges of the formula components

Each 1L contains					
Ingredient name	**Quantity range**		**Percentage range (%)**		**Function**
	Minimum	**Maximum**	**Minimum**	**Maximum**	
Chitosan USP 40	50 g	80 g	5	8	Biodegradable polymer with two functions: Co-surfactant and antiseptic
Glacial acetic acid	5 g	15 g	0.5	1.5	Solvent
Green tea	5 g	15 g	0.5	1.5	Solvent
Tween 80	5 g	15 g	0.5	1.5	Surfactant
Glycerin	150 g	250 g	15	25	Thickener
Dichloromethane	25 g	100 g	2.5	10	Solvent
Benzalkonium chloride	5 g	15 g	0.5	1.5	Co-surfactant and antiseptic
Distilled water USP 40 to	1 L	1 L	100	100	Vehicle

Antimicrobial activities of the different formulations against 18 different microbial strains were studied as shown in **Table** 7. The following formulae were defined:

- Formula 1 (F1): Green tea essential oil
- Formula 2 (F2): Chitosan solution
- Formula 3(F3): benzalkonium solution
- Formula 4 (F4): control (antimicrobial compound)
- Formula 5 (F5): Green tea essential oil + Chitosan solution
- Formula 6 (F6): The final formula of the nanoemulsion composite mixture

Table 7. Antimicrobial activity of the 6 formulations expressed as % inhibition

		F1	F2	F3	F4	F5	F6
Gram-positive bacteria	*Staphylococcus aureus*	12	50	50	60	38	50
	Bacillus subtilis	10	41	72	72	30	61
	Micrococcus spp.	20	45	64	57	37	38
	Streptococcus mutants	20	60	45	47	10	63
	MRSA	25	85	90	27	54	80
	Enterococcus faecalis	40	75	60	50	23	80
Gram-negative bacteria	*Enterobacter colacae*	30	60	94	85	25	60
	Salmonella Typhium	27	52	41	47	41	83
	Eisherecia coli	19	58	63	73	58	58
	Klebsella pneumonia	26	62	80	38	53	63
	Proteus vulgaris	20	63	95	62	10	100
	Pseudomonas aeruginosa	40	51	66	60	40	51
Fungi	*Aspergillus fumigatus*	29	33	52	35	25	70
	Aspergillus niger	30	38	70	30	56	70
	Candida albicans	25	35	50	50	93	45
	Geotricum candiaum	44	100	89	46	30	44
	Penicillium italicum	32	80	96	29	58	90
	Fusarium moniliform	20	64	90	29	46	81

Inhibition zone method was followed in order to test the antimicrobial efficiency of the 6 selected nanoemulsion composite mixture samples against 6 chosen strains of gram-positive bacterial strains, 6 strains of gram-negative bacterial strains 6 strains of fungi.

It is found that some of the selected samples have high antibacterial efficiency against most of the chosen bacterial strains when compared to the control such as MRSA and Enterobacter faecalis. Interestingly, it was found that some samples show good antibacterial activity even against highly resistant bacterial strains such as MRSA.

It is found that some of the selected samples have high antibacterial efficiency against most of the chosen bacterial strains when compared to the control. Interestingly, it was found that the two samples possess high antibacterial activity that is equal or greater than that of the control when tested with specific strains such as Salmonella Typhium, Proteus vulgaris, and Pseudomonas aeruginosa. Moreover, It is found that some of the selected samples have high antifungal efficiency against most of the chosen bacterial strains when compared to the control. Interestingly, it was observed that those two samples showed good antimicrobial results against gram-positive bacteria, gram-negative bacterial and fungi. Therefore, they could be considered as promising antimicrobial sanitizers against wide range of microorganisms. It should be mentioned that the benzalkonium chloride (BAC) solution showed the highest antimicrobial activity against most of the microbial strains however, many studies have shown the harmful effects of BAC on the human body, such as skin irritation and allergic contact dermatitis. Highly toxic effects of BAC have also been found in animal studies. BAC orally administered to rats causes significant lethality, and the lethal dose 50 (LD50) has been reported to be 234-525 mg/kg.

Stability testing of the optimum formula

The stability for the nanoemulsion composite mixture formula was studied using the visual, DLS analysis as well as SEM analysis (**FIG. 5**). The results showed a good stability over one-month storage. According to the pH impact on the different nano-systems, the acidic media negatively affected the stability of the formula and induce their degradation. Thermogravimetric analysis (TGA) showed a significant improvement of the thermal stability of encapsulated essential oil 1.74 folds over the pure non-capsulated bulk essential oil. The thermal degradation of the essential oil occurred at temperature above 350 °C.

Table 8. Main differences between nano-emulsions and nanoparticles

	Nanoparticles	**Nanoemulsions**
Definition	Nanoparticles are small or ultrafine particles in a nanoscale range (normally between 1 – 100 nm) that could be in Solid, liquid, or gas states	Emulsion with droplet (referred to as micelles) size in the nanoscale range (normally between 20 – 200 nm) that in a liquid state
Formation	The preparation of nanoparticles is achieved either by: (i) Bottom-up approaches such as chemical synthesis, precipitation, or self-assembly (ii)Top-down approaches such as milling, grinding, or lithography	The preparation of nanoemulsion is achieved by emulsification techniques such as high-pressure homogenization, sonication, microfluidization, and phase inversion.
Composition	Comprises various materials, including metals, polymers, lipids, silica, or quantum dots	Comprises of oil nanosized droplets in dispersed in water or water nanosized droplets dispersed in oil

Stability	nanoparticles are susceptible to degradation, sedimentation, and agglomeration	nanoemulsion exhibit enhanced stability due to small droplet size and presence of co-surfactants that stabilizes the emulsion and prevent coalescence of droplets
Drug Encapsulation	nanoparticles can encapsulate drugs within the nanoparticle matrix	Nanoemulsion can encapsulate drugs within the oil or water droplets
Drug Release	The drug release from nanoparticles can be controlled via modifying the particle surface, matrix, or through stimulus-responsive systems	The drug release can be controlled from nanoemulsion via modifying the emulsion composition, droplet characteristics, or stimuli-responsive systems
Toxicity	The toxicity of nanoparticles depends on their composition and surface properties	The toxicity of nanoemulsions is primarily determined by the components used, such as surfactants or co-surfactants

Advantages

Using Nano-Encapsulation Technology to produce Eco-friendly disinfectants without any harsh chemicals or harmful materials by using natural materials which play a crucial role in enhancing the penetration and disruption of microbial membranes that cause inhibition effect on cell functional properties and eventually causing leakage of the internal contents of the microbial cell leading to death of the microbe. These novel nanoemulsion composite mixture exhibit superior antimicrobial abilities, can kill 99.99% of different bacteria without causing any harmful side effect to humans. These nanoemulsion composite mixtures can be incorporated in many products (hand gel sanitizers, soap, detergents, animal feed, and many more).

The unique power of Nano-Encapsulation Technology that it offers the ability to work near the atomic level to create structures with fundamentally new and superior beneficial physical properties and behaviors compared to any other material. The different nanoemulsion composite mixture produced are non-toxic, and controllable. They can be used in different forms (solution, suspension, emulsion, gel and powder). More importantly, we do not use any harsh chemicals or alcohol in our products, thus we offer the first of its kind Eco-friendly nanocapsulation technology-based disinfectant. More interestingly, the formulations can be added to any existing products (beverages, detergents, textile, cosmetics, pesticides, animal feed, etc).

The innovative nanoemulsion composite mixture could be added as a liquid additive with a percentage that ranges between 0.05-30% on the beverages, detergents, textile, cosmetics, pesticides, animal feed products.

1- Broad spectrum antimicrobial effect
2- Close antimicrobial effect compared to commercially available chemical-based disinfectants.
3- FDA approved and EPA registered ingredients.
4- Non-toxic, Biodegradable and eco-friendly
5- hypoallergenic
6- Powerful antioxidant properties
7- Long lasting protection.

8- Different forms (liquid, spray, soaps, powder)

9- High thermal stability of the natural components

10- High water stability

11- Works on both nonporous and porous surfaces

12- used both indoors and outdoors.

CLAIMS

What is claimed is:

1. A nanoemulsion composite mixture for antimicrobial activities, comprising: biodegradable polymers, co-surfactants, surfactants and thickeners together entrapping an oil,

   wherein the nanoemulsion composite mixture is dissolved in glacial acetic acid and dichloromethane,

   wherein water is used as the vehicle to hold the nanoemulsion composite mixture, and

   wherein in the nanoemulsion composite mixture:

   the biodegradable polymers are within 5-8%,

   the glacial acetic acid is within 0.5-1.5%,

   the oil is within 0.5-1.5%,

   the surfactant is within 0.5-1.5%,

   the thickener is within 15-25%,

   the dichloromethane is within 2.5-10%, and

   the co-surfactants is within 0.5-1.5%.

2. The nanoemulsion composite mixture as set forth in claim 1, wherein the thickener is glycerin.

3. The nanoemulsion composite mixture as set forth in claim 1, wherein the surfactant is Tween 80.

4. The nanoemulsion composite mixture as set forth in claim 1, wherein the

biodegradable polymers are Chitosan, 1,3 Propyleneglycolalginate, Gelatin, Pectin, 3-hydroxypentanoic acid, Glucose, Poly (acrylates), Albumin, Gluten, Poly (alkyl cyanoacrylate), Alginate, Glycerol and various, Poly (e-caprolactone), Alginic acid, Hydroxyethyl starch, poly(lactic-co-glycolic acid), Carrageenan, Hydroxypropylstarch, Polyanhydrides, Casein Cellulose, Lactic acid, Polylactic acid, Cellulose, Lactic acid, butyl ester, Polyphosphazenes, Cellulose acetate, Lignocellulose, polyvinyl alcohol, Cellulose acetate butyrate, Sucrose, Propyleneglycolalginate, Cellulose derivative, zein, starch, Chitin, Dextrin, Edible Starch, Hydrolysed Starch or Dextrin.

5. The nanoemulsion composite mixture as set forth in claim 1, wherein the oil is Green tea essential oil, Ajwain Essential Oil, Almond Oil, Ambrette Seed Essential Oil, Angelica root Oil, Basil Essential oil, Bay oil, Bergamot oil, Black Pepper Oil , Black Seed essential oil, Cade oil, Cajeput oil, Calamus Essential Oil, Caraway seed, Carrot Seed Oil , Cassia Bark Oil, Castor Oil, Chamomile Essential Oil, Cinnamon essential oil, Citronella Essential Oil, Coconut Oil, Coriander Seed Oil, Cumin oil, Curry Leaf Oil, Cypress oil, Davana Essential Oil, Dill Seed Essential Oil, E. Citriodora Essential Oil, Sesame oil, Fennel Essential Oil, Fractionated Coconut Oil, Frankincense Essential Oil, Garlic Oil, Geranium Essential Oil, Ginger Essential Oil, Globulus Essential Oil, Grapefruit, Green tea essential oil, Holy Basil Essential Oil, Indian Rosemary Essential Oil, Jojoba Oil, Juniper Berry Oil, Karanj Oil, Lavender Oil, Lemongrass Essential Oil, Lemongrass Essential Oil, Lemongrass Essential Oil, Lime Oil, Mandarine, Marigold Essential Oil, Mentha Arvensis Essential Oil, Mentha

Citrata Essential Oil, Mentha Peperata Essential Oil, Mentha Spicata Essential Oil, Moringa Seed oil, Nagarmotha Essential oil, Neem Oil, Nutmeg Essential Oil, Palmarosa Essential Oil, Patchouli Essential Oil, Peanut Oil, Sage Essential Oil, Soybean Oil, Spearmint, Sweet Marjoram oil, Tagetes Minuta Essential Oil, Tamannu Essential Oil, Tea tree essential oil, Tea Tree Oil, Vetiver Essential Oil, Orange Oil, Thyme red Essential Oil, Wormwood, or Ylang Ylang Essential Oil.

6. The nanoemulsion composite mixture as set forth in claim 1, wherein the co-surfactants are Benzalkonium chloride, Behentrimonium chloride, Carbethopendecinium bromide, Didecyldimethylammonium chloride, Benzalkonium chloride, Cetalkonium chloride, Dimethyldioctadecylammonium bromide, Benzethonium chloride, Cetrimonium bromide, Dimethyldioctadecylammonium chloride, Benzododecinium bromide, Cetrimonium chloride, Dioleoyl-3-trimethylammonium propane, Bronidox, Cetylpyridinium chloride, Domiphen bromide, Lauryl methyl gluceth-10 hydroxypropyl dimonium chloride, Pahutoxin, Tetramethylammonium hydroxide, Octenidine dihydrochloride, Stearalkonium chloride, Thonzonium bromide, Olaflur, N-Oleyl-1,3-propanediamine, or Octenidine dihydrochloride.

7. A nanoemulsion composite mixture for antimicrobial activities, consisting essentially of: biodegradable polymers, co-surfactants, surfactants and thickeners together entrapping an oil,

wherein the nanoemulsion composite mixture is dissolved in glacial acetic acid and dichloromethane,

wherein water is used as the vehicle to hold the nanoemulsion composite mixture, and wherein in the nanoemulsion composite mixture:

the biodegradable polymers are within 5-8%,

the glacial acetic acid is within 0.5-1.5%,

the oil is within 0.5-1.5%,

the surfactant is within 0.5-1.5%,

the thickener is within 15-25%,

the dichloromethane is within 2.5-10%, and

the co-surfactants is within 0.5-1.5%.

8. The nanoemulsion composite mixture as set forth in claim 7, wherein the thickener is glycerin.

9. The nanoemulsion composite mixture as set forth in claim 7, wherein the surfactant is Tween 80.

10. The nanoemulsion composite mixture as set forth in claim 7, wherein the biodegradable polymers are Chitosan, 1,3 Propyleneglycolalginate, Gelatin, Pectin, 3-hydroxypentanoic acid, Glucose, Poly (acrylates), Albumin, Gluten, Poly (alkyl cyanoacrylate), Alginate, Glycerol and various, Poly (e-caprolactone), Alginic acid, Hydroxyethyl starch, poly(lactic-co-glycolic acid), Carrageenan, Hydroxypropylstarch, Polyanhydrides, Casein Cellulose, Lactic acid, Polylactic acid,

Cellulose, Lactic acid, butyl ester, Polyphosphazenes, Cellulose acetate, Lignocellulose, polyvinyl alcohol, Cellulose acetate butyrate, Sucrose, Propyleneglycolalginate, Cellulose derivative, zein, starch, Chitin, Dextrin, Edible Starch, Hydrolysed Starch or Dextrin.

11. The nanoemulsion composite mixture as set forth in claim 7, wherein the oil is Green tea essential oil, Ajwain Essential Oil, Almond Oil, Ambrette Seed Essential Oil, Angelica root Oil, Basil Essential oil, Bay oil, Bergamot oil, Black Pepper Oil , Black Seed essential oil, Cade oil, Cajeput oil, Calamus Essential Oil, Caraway seed, Carrot Seed Oil , Cassia Bark Oil, Castor Oil, Chamomile Essential Oil, Cinnamon essential oil, Citronella Essential Oil, Coconut Oil, Coriander Seed Oil, Cumin oil, Curry Leaf Oil, Cypress oil, Davana Essential Oil, Dill Seed Essential Oil, E. Citriodora Essential Oil, Sesame oil, Fennel Essential Oil, Fractionated Coconut Oil, Frankincense Essential Oil, Garlic Oil, Geranium Essential Oil, Ginger Essential Oil, Globulus Essential Oil, Grapefruit, Green tea essential oil, Holy Basil Essential Oil, Indian Rosemary Essential Oil, Jojoba Oil, Juniper Berry Oil, Karanj Oil, Lavender Oil, Lemongrass Essential Oil, Lemongrass Essential Oil, Lemongrass Essential Oil, Lime Oil, Mandarine, Marigold Essential Oil, Mentha Arvensis Essential Oil, Mentha Citrata Essential Oil, Mentha Peperata Essential Oil, Mentha Spicata Essential Oil, Moringa Seed oil, Nagarmotha Essential oil, Neem Oil, Nutmeg Essential Oil, Palmarosa Essential Oil, Patchouli Essential Oil, Peanut Oil, Sage Essential Oil, Soybean Oil, Spearmint, Sweet Marjoram oil, Tagetes Minuta Essential Oil, Tamannu Essential Oil, Tea tree essential oil, Tea Tree Oil, Vetiver Essential Oil, Orange Oil,

Thyme red Essential Oil, Wormwood, or Ylang Ylang Essential Oil.

12. The nanoemulsion composite mixture as set forth in claim 7, wherein the co-surfactants are Benzalkonium chloride, Behentrimonium chloride, Carbethopendecinium bromide, Didecyldimethylammonium chloride, Benzalkonium chloride, Cetalkonium chloride, Dimethyldioctadecylammonium bromide, Benzethonium chloride, Cetrimonium bromide, Dimethyldioctadecylammonium chloride, Benzododecinium bromide, Cetrimonium chloride, Dioleoyl-3-trimethylammonium propane, Bronidox, Cetylpyridinium chloride, Domiphen bromide, Lauryl methyl gluceth-10 hydroxypropyl dimonium chloride, Pahutoxin, Tetramethylammonium hydroxide, Octenidine dihydrochloride, Stearalkonium chloride, Thonzonium bromide, Olaflur, N-Oleyl-1,3-propanediamine, or Octenidine dihydrochloride.

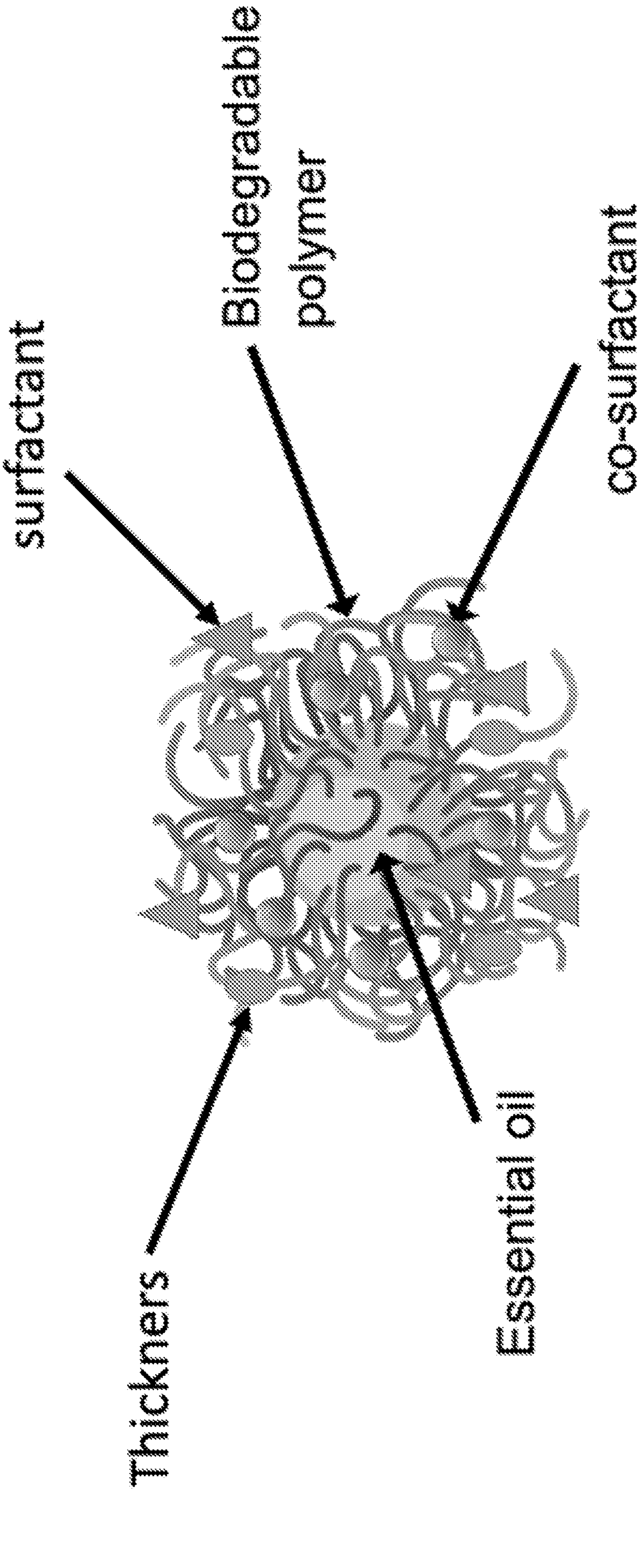

surfactant
Biodegradable polymer
co-surfactant
Thickners
Essential oil

FIG. 1

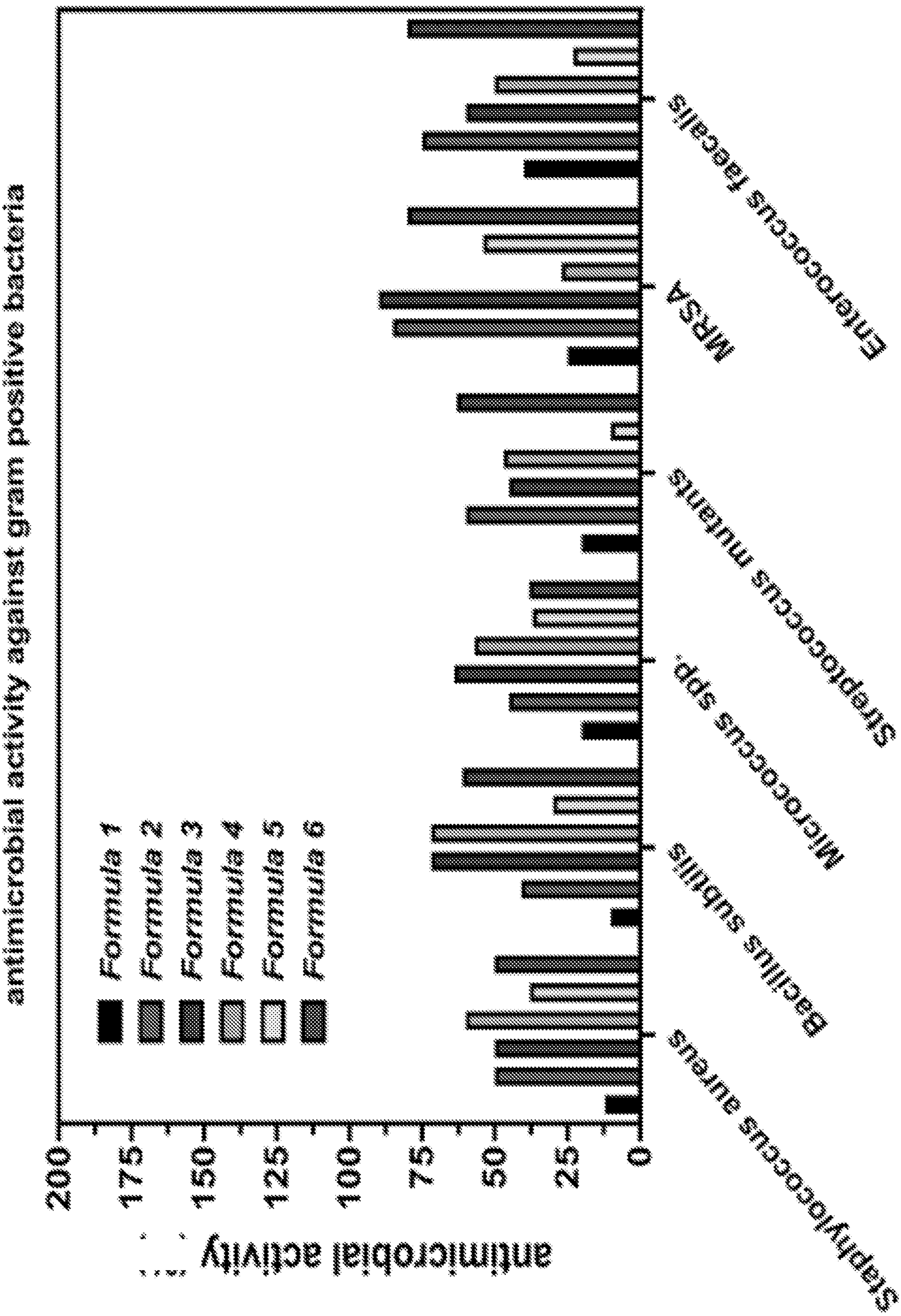

antimicrobial activity against gram positive bacteria
antimicrobial activity
200
175
150
125
100
75
50
25
0
Formula 1
Formula 2
Formula 3
Formula 4
Formula 5
Formula 6
Staphylococcus aureus
Bacillus subtilis
Micrococcus spp.
Streptococcus mutants
MRSA
Enterococcus faecalis

FIG. 2

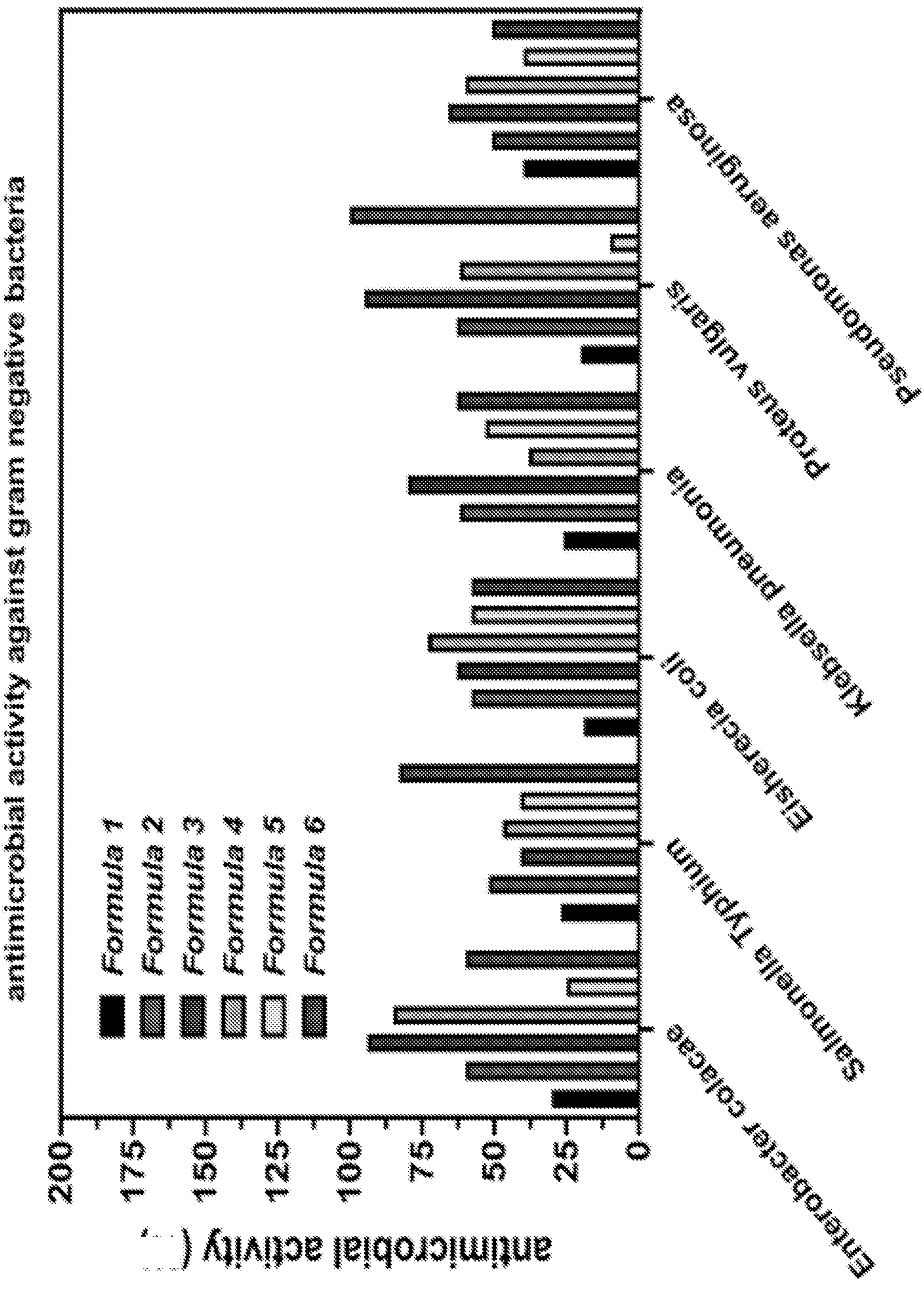
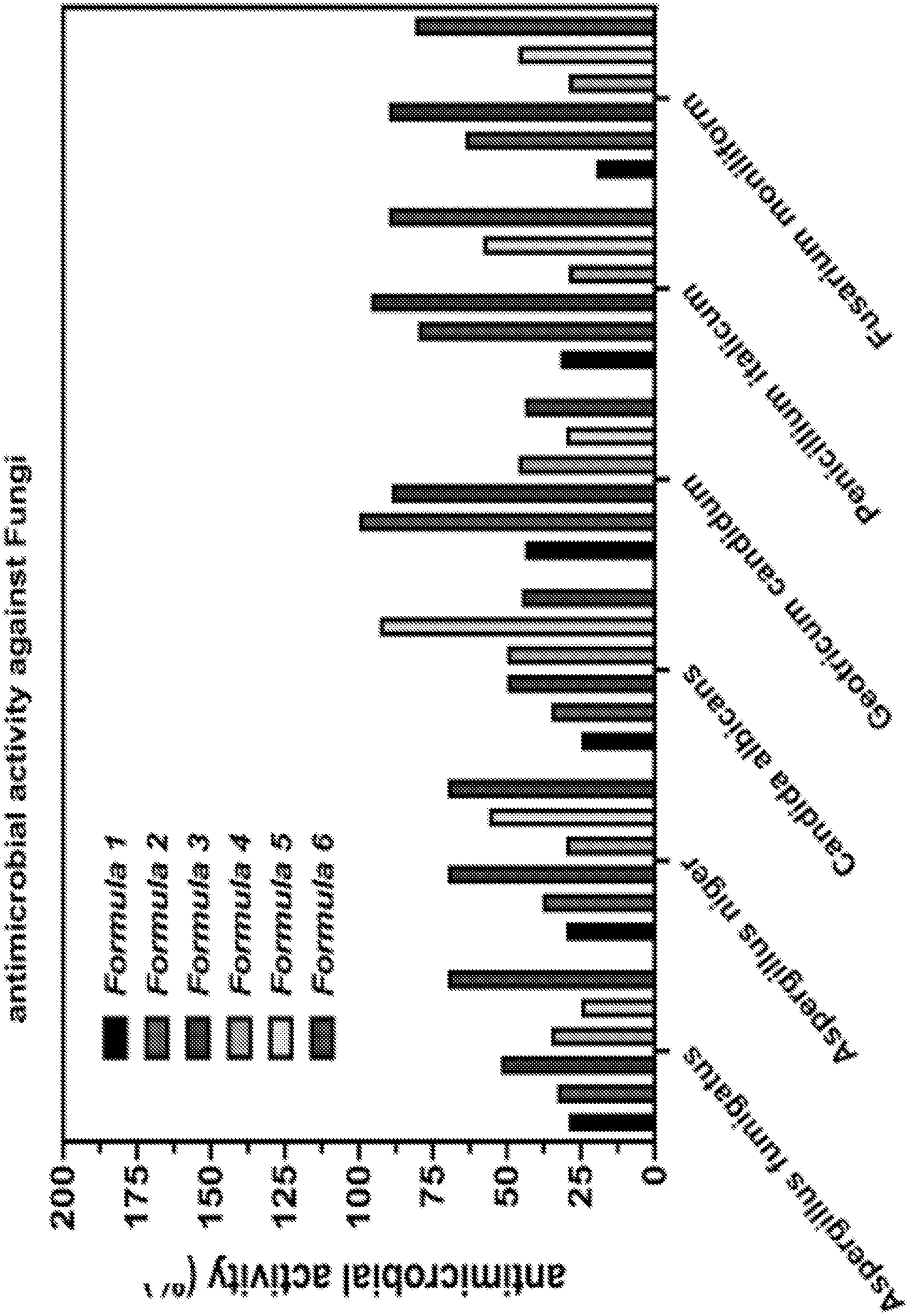

antimicrobial activity against gram negative bacteria
antimicrobial activity (
200
175
150
125
100
75
50
25
0
Formula 1
Formula 2
Formula 3
Formula 4
Formula 5
Formula 6
Enterobacter colacae
Salmonella Typhium
Eisherecia coli
Klebsella pneumonia
Proteus vulgaris
Pseudomonas aeruginosa

FIG. 3



antimicrobial activity against Fungi
antimicrobial activity (%)
200
175
150
125
100
75
50
25
0
Formula 1
Formula 2
Formula 3
Formula 4
Formula 5
Formula 6
Aspergillus fumigatus
Aspergillus niger
Candida albicans
Geotricum candidum
Penicillium italicum
Fusarium moniliform

FIG. 4

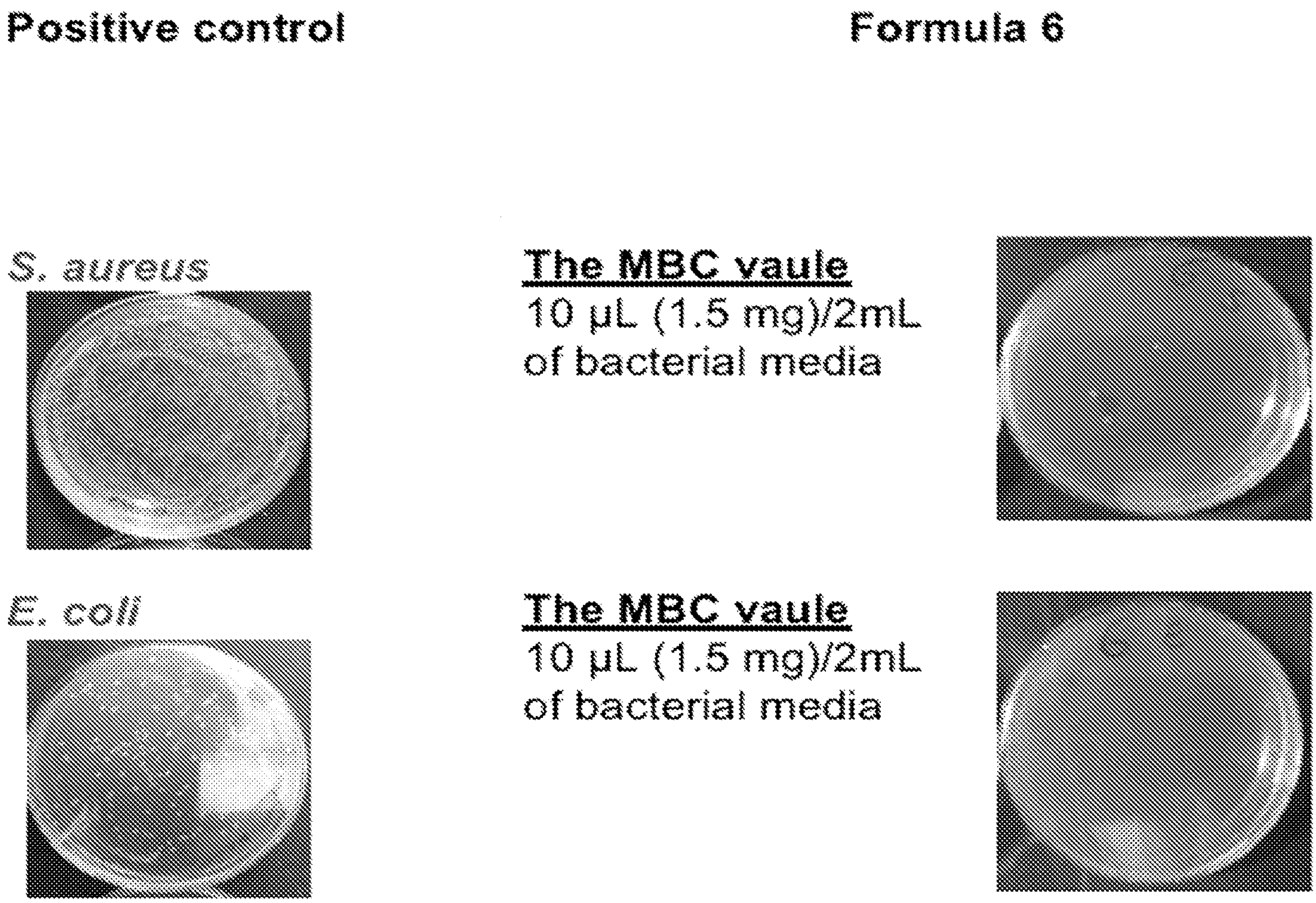

Positive control
Formula 6
S. aureus
The MBC vaule
10 µL (1.5 mg)/2mL
of bacterial media
E. coli
The MBC vaule
10 µL (1.5 mg)/2mL
of bacterial media

FIG. 5

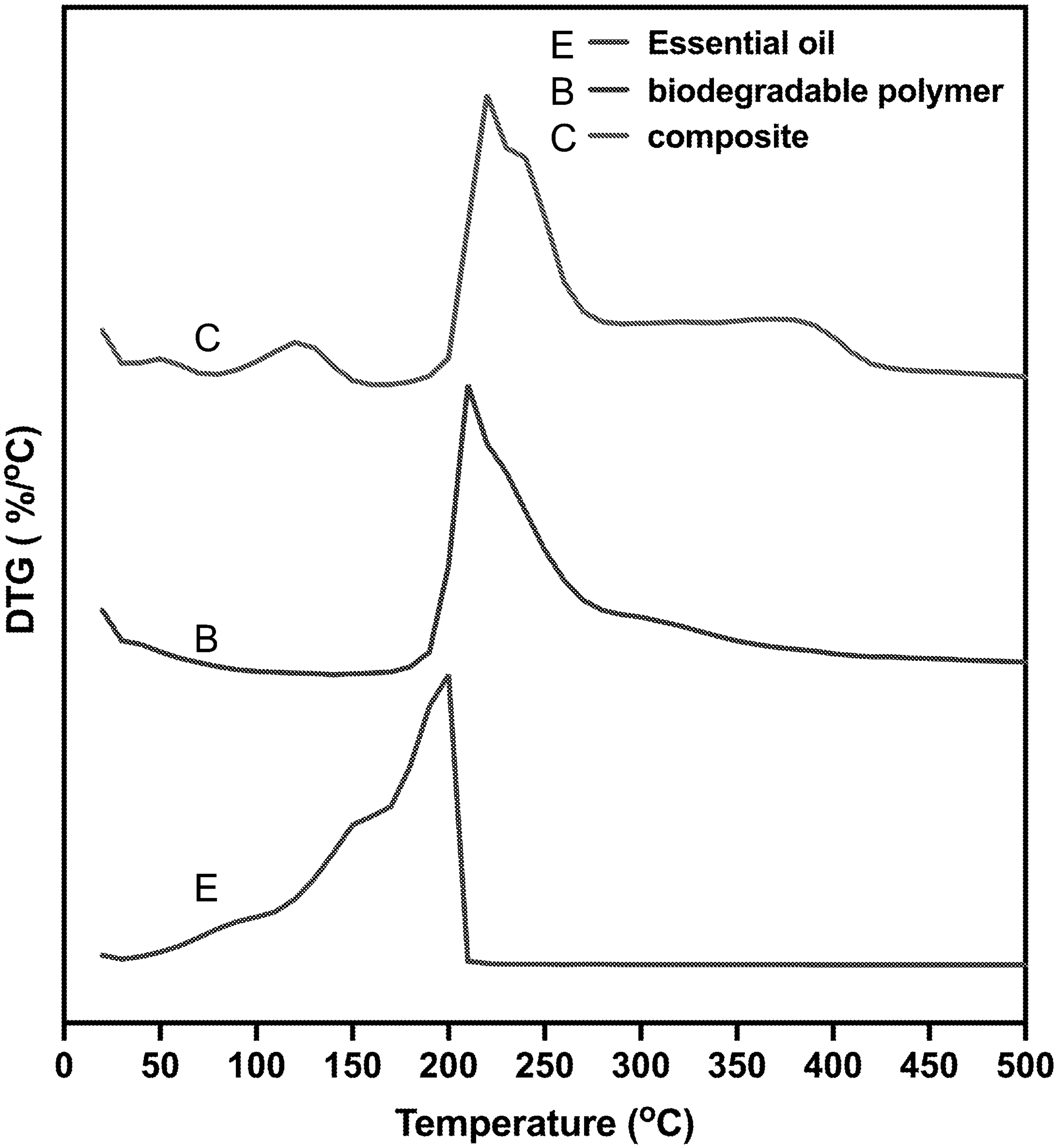

E Essential oil
B biodegradable polymer
C composite
C
B
E
DTG (%/°C)
0
50
100
150
200
250
300
350
400
450
500
Temperature (°C)

FIG. 6

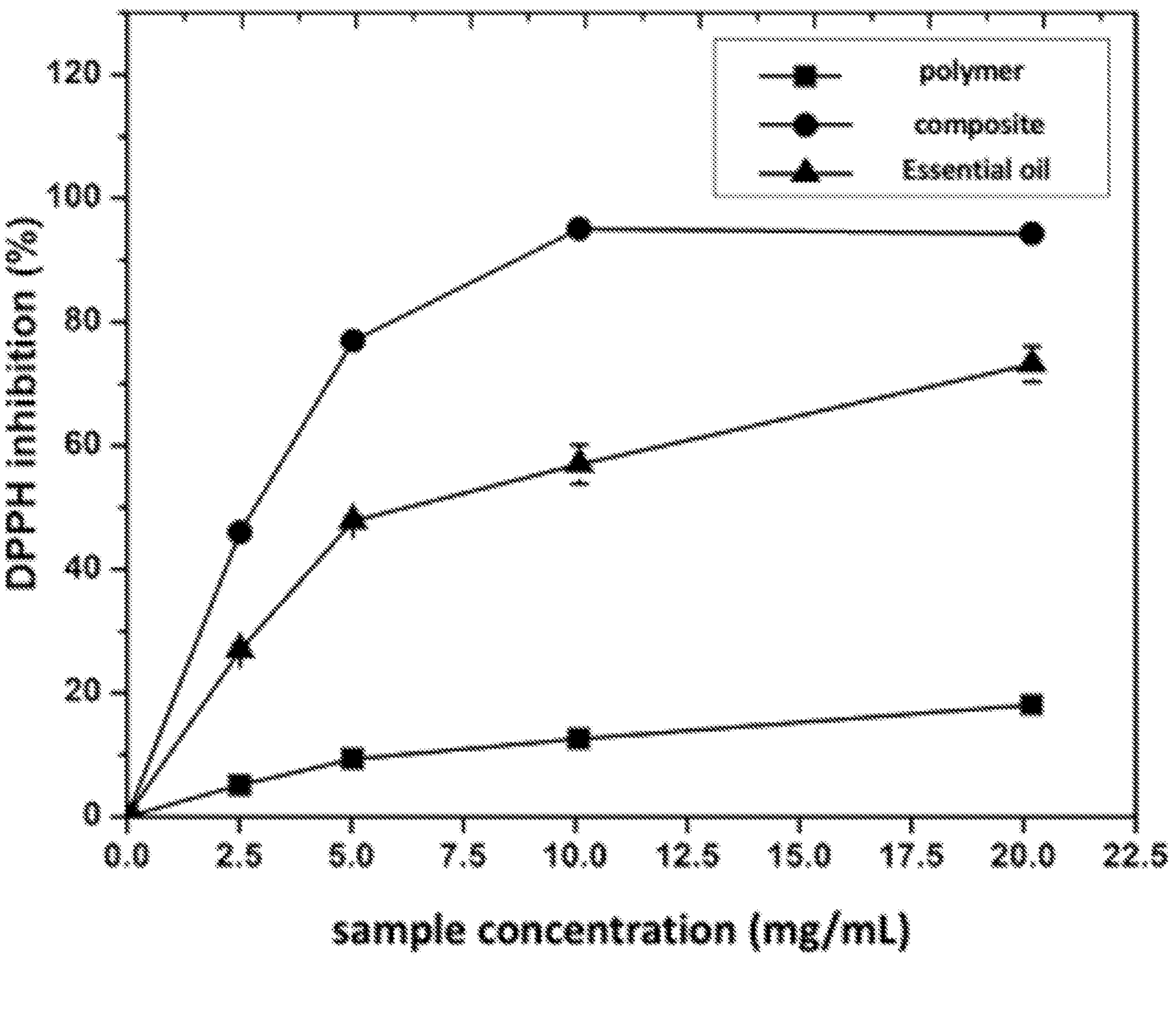

polymer
composite
Essential oil
DPPH inhibition (%)
0
20
40
60
80
100
120
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
sample concentration (mg/mL)

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/26070

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 9/10; A01P 1/00; A61K 9/14; A61K 9/16 (2023.01)

ADD. A61K 9/06; A61K 9/20; A61K 9/51 (2023.01)

CPC - INV. A61K 9/1075; A01P 1/00; A61K 9/145; A61K 9/1652; A61K 9/2059

ADD. A61K 9/06; A61K 9/5161; A61K 2039/55566

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2021/0236629 A1 (JUBILANT PHARMA HOLDINGS INC.) 05 August 2021; paragraphs [0027], [0029], [0066], [0117], [0130], [0131], [0134], [0135], [0144]; Table 2	1-5, 7-11 --- 6, 12
Y	US 2009/0264653 A1 (BARTOLINI et al.) 22 October 2009; paragraph [3574]	6, 12
A	WO 2012/075534 A1 (NS TECHNOLOGIES PTY LTD) 14 June 2012; entire document.	1-12
A	WO 2021/026391 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 11 February 2021; entire document	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 August 2023 (16.08.2023)	SEP 21 2023

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300