

NANOPARTICULATE TITANIUM DIOXIDE NANOMATERIAL MODIFIED WITH
FUNCTIONAL GROUPS AND WITH CITRIC EXTRACTS ADSORBED ON THE
SURFACE, FOR THE REMOVAL OF A WIDE RANGE OF MICROORGANISMS

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FIELD OF THE INVENTION

The present invention relates to the synthesis and preparation of a mixture of herbal and fruit extracts, and its absorption on titanium dioxide nanomaterial modified with functional groups, with a general formulation of $E/M_aO_{2(c)}(OH)_v(PO_4)_w(SO_4)_xCl_y(NH_2)_z$, where E is a complex solution obtained from grapefruit, lemon, tangerine, and other citrus extracts, and M is titanium. The matrix particle size, acidity, matrix mean pore size and particle size are controlled during synthesis. The nanomaterial of the present invention is used to inactivate viruses, remove bacteria, mycobacteria, fungi and spores. The invention is intended to the use of these nanoparticles for disinfection and sanitizing of biological surfaces and is not limited to a specific application field.

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BACKGROUND ART

From the beginning of times, human beings have tried to alter matter, and recently, scientists have acquired the ability to manipulate matter, the scientist ability to manipulate materials at atomic and molecular

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scale through the use of nanotechnology has evolved from science fiction to science reality in common life. Today, nanotechnology is being developed in order to prevent, diagnose and treat infectious diseases, with some products about to enter the clinical trial phase. Advances in this field are exponential (1-6). Interdisciplinary nanoscience and researches including chemists, physicists, biologists and engineers are concerned on the necessity of developing ecological and sustainable methods for the synthesis of nanomaterials. There is a trend of excitement to integrate green chemistry approaches in the design of environmentally benign materials and processes. Quick advances are taking place in the synthesis of biocompatible mixed oxides or metallic nanomaterials and single bimetallic oxides, as well as the surface modification thereof intended for bioactivity and nanomedicine applications. Biosynthesis of nanoparticles as an emerging ending point from nanotechnology and biotechnology interaction has been increasingly drawing attention due to the increasing necessity to develop environmentally friendly technologies regarding material synthesis. Biomolecules as reagents have been found to have a significant advantage over equivalent molecules as protective agents (7-13).

Materials properties can change notably when their particle size is reduced to a nanometer scale. In

materials science "particle" is a general term for describing small solid objects having sizes ranging from the atomic scale (10^{-10} m) to microscopic scale (10^{-3} m). However, particle size is often found between 10^{-9} to 10^{-5} m. Large particles ($> 10^{-6}$ m) are commonly called grains (i.e. zeolites, carbon, Raney metals) and small particles (< 15 nm) of mixed (metal) oxides, i.e. $\text{TiO}_2\text{-SiO}_2$, TiO_2 or SiO_2 are often added (14-20). All the materials consist of grains (particles) formed by agglomeration of nanoparticles.

In conventional materials, grains have a sized comprised between 100 micrometers and millimeters (mm), while nanomaterial particles are within the range of a billionth of a meter (10^{-9}). The mean diameter of human hair is approximately one nanometer. The radius of an atom is 1 to 3 Angstrom ($\text{\AA}$), and one nanometer is equal to 10 $\text{\AA}$. Nanomaterials are solid, rigid, resistant and ductile at high temperatures, they are resistant to degradation, erosion and corrosion, as well as chemically very active. The physical and chemical properties of each nanomaterial or nanoparticle material are determined by the type of compounds and the interactions by which nanoparticles are functionalized; thus electron density and hydroxyl concentration in the network has an important role in the rupture of pathogenic DNA.

One of the areas where the importance of nanoparticles has increased is the disinfection field, where a particle distribution having well defined shape and size will be obtained in order to improve disinfection activity. In particular, it is necessary to obtain highly dispersed particles where most of the atoms are located on the surface. The structure includes a solid area, pore size, as well as shape and volume of pores. These parameters are also important, since they are responsible for increasing the microorganism disinfection rate. The adsorption of functional groups on the surface makes them selective to pathogenic microorganisms and the adsorption of citrus extracts confers them disinfectant power.

Although the activity can be directly related to the total contact area between the material and the organism, determination of the surface is considered an important requirement in the characterization of said material. Also, it is necessary to specify the nature of pore structure since it is responsible for controlling the transport of reagents and products.

Titanium dioxide can be found naturally in three crystalline phases: anatase, rutile, and brookite (Fig. 2). Anatase and brookite can transform into rutile at high temperatures. Anatase can irreversibly transform into rutile by heating. Several factors contribute in phase

shifting, such as particle size, crystal morphology, but in particular, the influence of ions on network poisoning. Literature cites one of the three phases, anatase, as having high chemical stability and corrosion resistance, being inert to biological agents and having high specific surface area. However, the commercial titanium oxide is a mixture (Degussa P25) containing 60 to 80 % anatase. The only problem in obtaining anatase is that rutile is thermodynamically more stable. Anatase and rutile structures are tetrahedral, while brookite is orthorhombic, each titanium atom is bonded to 6 almost equally distant oxygen atoms, and each oxygen atom is bonded to three titanium atoms.

The need for disinfectants and antiseptics having specific action to inactivate virus and kill bacteria, mycobacteria, mycoplasma, fungi, protozoa and spores with proven high efficiency against these and other microorganisms has increased.

This has a relationship with the increase in new infections (e.g. HIV, influenza and avian flu) and the re-emergence of previously controlled infections due to drug resistance, environmental changes and lifestyle alterations. Besides, the use of novel medical devices, which cannot be sterilized through conventional techniques, such as heat treatment, can spread some infectious

diseases. Nanotechnology will have a deep impact on nosocomial infections and the diseases caused thereby, for improved diagnosis, prevention and detection, directed therapies, and antibacterial, antiviral, antimycotical, 5 antimycobacterial and sporicidal materials. According to literature, antimycobacterial activity is closely linked to sporicidal activity mainly around the *Bacillus Subtilis*.

Diagnosis technologies combine a recognition system and a detection system, comprising a small 10 cantilever that moves over the antigen-binding site with nanowires detecting current of cell binding immunity.

For prevention, nanotechnology-based microbicides are tested against HIV and other viruses, and are now in early clinical trials. Laboratory studies on new vaccines 15 against hepatitis B, tuberculosis, HIV, influenza and antibacterial surface coatings or materials, including those for the medical sector, look promising. These coatings can reduce the problem related to bacteria or virus adherence to hospital surfaces and have a beneficial 20 impact on intrahospital transmission of multiresistant bacteria, virus, spores, fungi, etc., which is a serious problem not yet solved. Titanium dioxide has a specific interaction with many biological molecules, microbes, algae, cells and living tissues. Specific interactions mean 25 that they are different from common reactions between non-

5 viable materials and biomolecules or living tissues. Interactions are mostly beneficial from the point of view of biotechnology applications. Titanium dioxide is known to form a direct bond with living tissues that can be used in biomaterials applications. Other application fields of the titanium dioxide include biosensors, tissue engineering, genus therapy, controlled delivery of therapeutic agents, and environmental protection (21-30).

10 Microbial safety is still a significant concern in priority health topics, regulatory organizations, and industries around the world. Traditionally, many strategies have been used to control microorganisms. Although synthetic antimicrobials are approved in many countries, recent trends have been towards the use of natural products, which requires exploration of safe, effective and acceptable antimicrobials from alternative sources.

20 In recent years nanoparticle assembly for disinfection of viral particles, virus-cell interactions, and viral pathogenesis, have taken into account these approaches for the development and design of new strategies. The rotavirus is a genus of double-stranded RNA virus in the family Reoviride (double-stranded (ds)). RNA viruses are a diverse group of viruses with a wide range of hosts (humans, animals, plants, fungi and bacteria), genome segment, organization and number (one to twelve), and

virion (T number, capsid layers or turrets).

Influenza, commonly known as flu, is an infectious disease caused by ARN viruses. The type A influenza virus particle or virion is about 80-120 nm in diameter and generally approximately spherical, although filamentous shapes can occur. Unusually for a virus, the influenza type A virus genome is not a single piece of nucleic acid, but a segmented eight-pieces of antisense RNA (13.5 kilobases total), encoding 11 proteins (HA, NA, NP, M1, M2, NS1, NEP, PA, PB1, PB1-F2, PB2). The best features of these viral proteins are hemagglutinin and neuraminidase, two large glycoproteins found on the outside of viral particles. Functionalized nanoparticulate biocatalysts of the present patent break ARN bonds and protein structure of this type of virus.

Extracts

Plants contain numerous components and constitute valuable sources of novel biologically active molecules having antimicrobial properties. Said components are extracted from certain plants either as standardized extracts or as a source of pure compounds, providing unlimited opportunities for control of microbial proliferation due to its chemical diversity. Many plant extracts have antimicrobial activity against various

bacteria, yeasts and molds, but variations in quality and quantity of bioactive components are significant drawbacks. The development of effective isolation procedures that produce standardized extracts, as well as the safety and toxicology assessment of said antimicrobials require further research (45-47).

Antimicrobial properties of essential oils (EOs) have been acknowledged for centuries and with the increasing demand for changes in consumer trends, legislation and isolation of antibiotic-resistant pathogenic agents, alternatives to chemical products based on bactericides must be found. Citrus oils are not only useful in foods, but they are also generally recognized as safe (GRAS), and have been found to be inhibitory, in both oil and vapor form, against a range of both Gram-positive and Gram-negative bacteria. This group of oils can provide natural antimicrobials, which are required to meet many requirements (45-47).

State-of-the-art patents

U.S. Patent No. 6,117,814. This patent describes a titanium oxide containing support, which also incorporates both silica and alumina as binder in the structure thereof. The purpose of said binder is to impart improved mechanical properties to the support. The

support's size range is of about 20 to 120 microns. Said support comprises about 50 % of binder manufactured by a sol-gel process.

U.S. Patent No. 6,087,405. This invention
5 describes a support for Fischer-Tropsch reaction of synthesis gas. Said support structure contains metals from Group VII. The rutile-anatase ratio within said structure is a distinctive feature of this patent.

WO/2003/064324. The invention relates to a
10 titanium oxide-based polymer composition. The inventive composition comprises a $\text{TiO}_x(\text{OH})_y$ and $(\text{H}_2\text{O})_z$ ($x + y + z = 3$) titanium oxide-based polymer in the form of a gel or sol. Said polymer, which has a one-dimensional (1D) structure, is made from concentric fibers having a
15 periodicity, which is deduced from the spacing between said fibers, of between 3.5 and 4. Each fiber comprises TiO_6 octahedrons and each TiO_6 octahedron shares two opposite edges with two adjacent octahedrons ($2 \times 2.92 \text{ \AA}$) in order to form infinite chains that develop along the fiber's
20 axis. According to the invention, two adjacent chains form double lines as a result of the shared edges ($2 \times 3.27 \text{ \AA}$). The inventive polymer is suitable for use as a photosensitive element in a photovoltaic cell, such as a window sunscreen.

25 WO/2006/079757. Method of preparing stable oxide

ceramic precursor sol-gel solutions based on lead, titanium, zirconium and lanthanide(s) and method of preparing said ceramic. The invention relates to a method of preparing a stable oxide ceramic precursor sol-gel solution based on lead, titanium, zirconium and lanthanide(s). The invention comprises the following successive steps consisting in: a) preparing a sol-gel solution by bringing a molecular lead precursor, a molecular titanium precursor, a molecular zirconium precursor and a molecular lanthanide precursor into contact with a medium comprising a diol solvent and optionally an aliphatic mono-alcohol; b) leaving the solution thus obtained to stand for a sufficient period of time in order to obtain a solution having an essentially-constant viscosity; and c) diluting the solution obtained in the preceding step with a diol solvent identical to that used in step a, or a solvent that is miscible with said solvent, at a predetermined rate. The invention can be used for preparing an oxide ceramic material comprising lead, a lanthanide metal, titanium and zirconium.

WO/2007/141590. Sol-gel nanostructured titanium oxide reservoirs for use in the controlled release of drugs in the central nervous system and method of synthesis. The invention is related to a sol-gel nanostructured titanium dioxide reservoir and its synthesis which is biocompatible

with brain tissue. The pore size distribution, crystallite size and the extent of the crystalline phase distribution of anatase, rutile and brookite can be fully controlled. This device may be used to contain neurological drugs. It
5 may be inserted directly into brain tissue for the purpose of the controlled time release of drugs over a period of from 6 months to three years. The patent uses the sol-gel method and is limited to the manufacture of particles contrary to the method of the present patent where a pre-
10 manufactured material is used and its surface is modified by means of impregnation.

WO93/21969. Novel coating materials for biomedical applications, particularly for the use on biomedical implants, said coating material comprising gel-
15 derived titanium oxide, wherein said material is capable of inducing calcium phosphate formation onto its surface under in vitro conditions, e.g. in a simulated body fluid and/or under in vivo conditions, processes for the preparation of the coating materials, as well as their use in biomedical
20 implant technology.

US 8,404,743 B2. Compounds comprising a zinc oxide complex chemically bonded to one or more molecules having an acidic hydrogen such as, for example, an organic acid. The invention further provides for compositions
25 comprising said compounds and method for making the same.

US 2012/0244086 A1. Compositions for dental use, comprising zingiber officinale extract by using zinc-derived materials, with antibacterial properties.

US 2012/0237455 A1. Compositions for oral use,
5 comprising zizyphus joazeiro extract by using zinc-derived materials, with antibacterial properties.

EP 1,981,513 A2. A topical skin care composition comprising kakadu plum extract or acai berry extract, or a combination of both. The composition can have a high oxygen
10 radical absorbance capacity (ORAC) value. The composition can improve skin's visual appearance, physiological functions, clinical properties, and/or biophysical properties. Nanoparticles are not used therein, but the invention shows antibacterial properties.

15 US 2012/0225147 A1. A topical composition for visually improving skin appearance comprising an effective amount of Malpighia puniceifolia (acerola), Myrciaria dubia (camu camu), and Ribes nigrum (black currant) extracts, and a dermatologically acceptable vehicle comprising water,
20 glycerin, dimethicone or cyclomethicone, stearic acid, carbomer, and sodium hydroxide. It exhibits antibacterial properties.

EP 2,099,429 A2. Micellar nanoparticles comprising polyethylene glycol (PEG), phospholipids,
25 cholesterol, glycolipid fatty acids, bile acids, and

saponins encapsulating the botulinum toxin in order to reduce its toxic effects. They exhibit antibacterial properties.

US 8,372,382 B2. Non-ionic oil-in water emulsion
5 comprising less than 50 % by weight of water, a combination
of non-ionic emulsifiers and non-ionic emulsion
stabilizers, a combination of humectant skin conditioning
agents, moisturizers and a combination of UV absorbing
agents. The emulsion can be stable and have an SPF of at
10 least 30. Nanoparticles are not used therein, but the
invention shows antibacterial properties.

EP 2,470,159 A1. Compositions and methods for
treating skin comprising a chemically compatible
combination of skin active ingredients comprising palmitoyl
15 tetrapeptide-7, methylsilanol mannuronate, and
Lactobacillus ferment, a chemically compatible combination
of skin active ingredients comprising plant extracts from
Punica granatum, Castanea sativa, Gossypium hirsutum, and
Euterpe oleracea, and a dermatologically acceptable
20 vehicle. The compositions can be substantive in that they
can remain on a person's skin during sleep. Nanoparticles
are not used therein, but the invention exhibits
antibacterial properties.

US 5,792,793 A. A complex formed by the
25 coordination between a thiol group-containing compound and

a silver ion; an antibacterial, antifungal, and antiviral agent containing the same as the active agent; and an antibacterial, antifungal, and antiviral agent compatible with various vehicles and carriers, sustains its activity for long, and has reduced peroral rout toxicity, skin irritation and mucosa irritation.

EP 2,448,416 A1. (Text from WO/2011/002929A1)
Preservative antimicrobial compositions comprising low concentrations of botanical extracts, in synergistic combinations with alkanediols in a solvent system, optionally with fruit acids. Furthermore, the present invention relates to a preservative or antimicrobial compositions which comprise a silver compound, an essential oil or individual constituent, one or more zinc salts, and one or more alkanediol. The compositions of the invention may be used in personal care products including wound care products or in veterinary use. Preferably, the compositions of the invention have little or no human-detectable fragrance.

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SUMMARY

The main object of the present disclosure is the use of nanotechnology for the development of a titanium dioxide nanomaterial with herbal or fruit extracts adsorbed on the surface thereof for use in inactivating any type of

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virus, and killing of bacteria, fungi, mycoplasma, mycobacteria, protozoa and spores.

In one aspect of the present disclosure, there is provided a composite nanomaterial for the preparation of disinfectant, antiseptic, healing or cleaning composition, said composite nanomaterial comprising:

a nanoparticulate titanium dioxide (TiO_2) support having a surface and pores, a mean diameter particle size between 1 and 100 nm and a surface area of at least 50 m^2/g , the nanoparticulate titanium dioxide being in a crystalline structure;

functional groups chemically adsorbed on the surface of the nanoparticulate titanium dioxide support, the functional groups comprising an hydroxyl as organic functional groups and inorganic radicals; and

herbal and fruit extracts physically adsorbed on the surface and pores of the titanium dioxide (TiO_2) support, the herbal and fruit extracts comprising particles of size between 0.3 to 10 nm,

wherein the fruit and herbal extracts comprise a mixture of at least three fruit and/or herbal extracts and the mixture comprises citric extracts.

In another aspect of the present disclosure, there is provided a method for preparing a composite nanomaterial for the preparation of disinfectants or

antiseptics as defined herein, the method comprising:

- a) preparing herbal and fruit extracts;
- b) providing a support comprising nanoparticulate titanium dioxide (TiO_2), comprising a surface and pores, a
5 mean particle diameter between 1 and 100 nm and a surface area of at least $50 \text{ m}^2/\text{g}$, the nanoparticulate titanium dioxide being in crystalline structure;
- c) providing precursor solutions which provide organic functional groups and inorganic radicals as
10 functional groups;
- d) performing a chemical modification of the nanoparticulate titanium dioxide by adsorption of the functional groups by the addition of the precursor solutions to the nanoparticulate titanium dioxide, by
15 agitation at a constant temperature;
- e) providing herbal and fruit ethanolic extracts, comprising particles size between 0.3 to 10 nm;
- f) performing physical modification of the nanoparticulate titanium dioxide by adsorption of fruit and
20 herbal ethanolic extracts by adding a mixture of at least three herbal and fruit ethanolic extracts that comprises citric extracts to the nanoparticulate titanium dioxide, after the adsorption of the functional groups, by agitation at constant temperature to the nanoparticulate.

In yet another aspect of the present disclosure, there is provided a disinfectant, antiseptic, healing or cleaning composition comprising:

up to 40% of the nanomaterial as defined herein;
5 and various proportions of selected raw materials.

The optimization of said nanomaterial allowing for the control of the following parameters: support acidity, BET area, pore size distribution, particle size, functionalization extent, dispersion of adsorbed extracts
10 onto the support is significant in order to gain a high activity to break CC and CN bonds of protein, RNA and DNA in pathogenic microorganisms. It is of significance that said matrix-supported extracts be fully dispersed all over the support so as to obtain a high cracking efficiency on
15 protein and nucleotide CC and CN bonds.

The nanomaterial support is a nanoparticulate inorganic metal oxide functionalized through an impregnation process. Said material have been functionalized and evenly dispersed with extracts having a
20 small particle size (0.3-10 nm).

The present disclosure addresses current issues of disinfectants, most of which are pollutants, irritants, toxic, non-biodegradable or even carcinogenic. Besides being selective, no harm is caused to

human beings, which is a further advantage versus non-toxic disinfectants.

BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1. Extraction system of herbal and fruit

active agents and organic oils.

The system is a traditional vapor entrainment system with a first vapor generating flask. With a second flask where raw materials, which will undergo extraction, are placed, and a third flask for liquid collection.

Figure 2. Crystalline phases of titanium dioxide.

Crystalline phases of titanium dioxide: anatase, rutile and brookite. Anatase phase is meta-stable and has oxygens on the outside of the crystal.

Figure 3. Infrared spectrum of the titanium dioxide nanomaterial.

Figure 4. X-ray diffraction spectrum confirming presence of the anatase phase of titanium dioxide.

Figure 5. Electron microscopy confirming particle size.

Scan electron microscopy, where nanoparticle clusters are seen with sizes between 1 and 100 nm. Transmission microscopy showing the existence of particles $\leq 1-2$ nm.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the conjugate of a titanium dioxide nanomaterial with herbal and/or fruit extracts adsorbed onto the surface thereof, synthesized by means of industrial impregnation process, using

nanoparticulate (1-100 nm) inorganic oxides as support. Said oxide nanoparticles are functionalized with organic functional groups, inorganic radicals and plant extracts adsorbed thereon, which provide the property of an anti-
 5 microbial agent. With such configuration, said material has a high disinfectant and antiseptic power for killing bacteria, fungi, mycobacteria, spores, mycobacteria, protozoa and viruses.

This nanomaterial is obtained using an
 10 impregnation process, which modify the surface thereof and disperse citrus extract particles of 0.3-10 nm in size over said support surface.

Important factors in the design of the support material

15 Crystalline structure	Crystalline metal oxide
Chemical composition	$E/M_aO_{2(c)}(OH)_v(PO_4)_w(SO_4)_xCl_y(NH_2)_z$ where E is an extract complex solution; M is metal
Grain, particle or crystal size	Distribution from 1 to 100 nm
Surface	50 m ² /g

EXTRACTION OF HERBAL AND/OR FRUIT ACTIVE AGENTS

20 The production of extracts involves two steps. First, the ethanolic step where seeds, leaves, peels and shells of selected fruits, said fruits being grape, tangerine, orange, grapefruit, lemon, guava, among other plants, are placed in contact with at least 70 % ethyl
 25 alcohol solution under constant stirring between 100 and

400 RPM, at a temperature between 30 and 50 °C, for a period of time between 24 and 48 hours. The alcoholic portion is removed from the extract by filtration.

The herbal residue collected from the ethanolic step in a system such as that shown in figure 1 where distilled water is placed in the first flask and heated to between 100 and 130 °C. The vapor generated is transferred through a tube to another flask wherein herbal residue is placed and maintained hot between 40 and 60 °C. Vapor is collected on the outlet of the second flask in a condenser with circulating cold water at a temperature between 10 and 4 °C. The resultant liquid is mixed with that obtained in the first step.

The mixture is allowed to stand at room temperature for 12 hours. The resultant liquid must be of variable color, depending on the plants and fruits used, non-viscous, with an intense bitter flavor and pH from 2 to 5.

20 Functionalization of the support

The process for functionalizing the support was improved in order to include extracts.

Significant aspects of said nanomaterial are the chemical characteristics thereof. First of all, the aspect that characterizes the invention as nanotechnology, which

is one of its major attributes and an advantage for removing microorganisms, is that it has a particle size between 1 and 100 nm and a crystalline structure, which is as well important since the material's oxygen atoms must be on the outside of the crystal allowing for the access or addition process of functional groups as well as extract adsorption.

As a starting process it is required to prepare solutions providing hydroxyl, phosphate, sulfate, chloride, amino, methyl, and phosphate functional groups at a concentration of 1.4 %. For said solutions the following solutes are used:

Table 1. Functional groups precursors providing selectivity properties to the nanomaterial.

hydroxyl	phosphate	sulfate	chloride	amino	methyl	folate
Water	Sodium phosphate	Sodium sulfate	Sodium chloride	ethyl amine	Methanol	Folic acid
Ethanol	Phosphoric acid	Sulfuric acid	Hydrochloric acid	Gamma-amino-butyric acid	Formic acid	Pteroyl glutamic acid

The industrially pre-manufactured titanium dioxide nanomaterial purchased from the chosen provider, preferably Degussa P25, which meets the surface area characteristics of 50 m²/g and particle size between 1 and 100 nm, is placed in a flask with water in a oxide-water ratio of 1:200. Agitation is set to constant speed between about 100 and 400 RPM, and from that point temperature is

maintained between 30 and 100 °C. Agitation and temperature must be maintained within these ranges for the whole process.

Previously prepared solutions are completely added drop wise one at a time, in the order as recited, maintaining agitation of the overall suspension between about 100 and 400 RPM. Once the addition of one solution is completed the next solution should be added after a waiting time of 5 to 30 minutes. The waiting time is in order to allow the complete adsorption of each functional group to the material surface before the addition of the next solution.

Then it is allowed to dry at temperatures between 30 and 100 °C in order to remove any remaining liquid.

Adsorption of extracts

In order to realize the incorporation of extracts, the oxide to be used should have a surface area greater than or equal to 50 m²/g.

Extracts added to the support can be from different parts of a plant such as flowers, buds, seeds, leaves, bark, grass, wood, fruit and roots; as well as from different plants such as citruses, grapes, pomegranate, barks like cinnamon and seeds like pepper, leaves like oregano, and many other plant extracts, which have proven

to possess antimicrobial properties.

An aqueous solution of 70 % extracts is placed in a flask under constant stirring at a speed of between about 100 and 400 RPM, then it continues at a temperature between 30 and 50 °C, and the previously treated titanium dioxide nanomaterial is added slowly so as to incorporate the functional groups from the previous process, and stirring is maintained at a speed of between about 100 and 400 RPM for 24 hours.

70% of said solution is comprised of an equitative mixture of extracts from at least three herbal or fruit sources, for example tangerine, grapefruit, orange and lemon.

Characterization tests

In these exemplary tests titanium oxide was used as a support.

The infrared transmittance spectrum shows a central band at 3667 cm^{-1} . This band is attributed to OH stretching vibration. Generally, this band is observed at 3700 cm^{-1} with pure titanium dioxide, and it is due to the presence of terminal hydroxyl groups, which because of OH stretching vibrations, leads to both Lewis and Brönsted acidic sites. The corresponding OH bending vibrations are centered at 1633 cm^{-1} . Infrared bands associated with

stretching vibrations of amine groups were observed at 3230 cm^{-1} . These observations are consistent with the fact that the complex could have lost only one atom and probably some decomposition of said complex have occurred as a result of any TiO_2 . Within the spectrum low energy region a centered wide band is observed at 1095 cm^{-1} with a shoulder at 1228 cm^{-1} . These bands are due to stretching vibrations ($-\text{O}-\text{Si}-\text{O}-$). The nanomaterial has several characteristics as observed from the infrared spectrum. In particular, a centered HNH deformation band at 1548 cm^{-1} and an asymmetric stretching band at 3230 cm^{-1} are evident. The UV-Vis spectra and thermal analysis show that when citrus extracts are adsorbed on the nanomaterial surface the decomposition and evaporation temperatures thereof increase, which means that extracts are protected from ambient factors, thus lengthening its useful life as well as extending its usage or storage range. The infrared profile is shown in figure 3, and the X-ray diffraction spectrum confirming presence of the anatase phase is shown in figure 4.

Said material has disinfectant and antiseptic properties so it can be used in cleaning, disinfection, antiseptic and healing products. To these ends, it can be included in diverse formulations, which can comprise:

	Ingredient	Minimum value (%)	Maximum value (%)
	Nanoparticulate material	0.0001	40
	Soft water	10	98
	Sodium lauryl sulfate	10	70
	Sodium lauryl ether sulfate	10	70
	Ammonium lauryl sulfate	10	70
	Ammonium lauryl ether sulfate	10	70
5	Monoethanolamine lauryl sulfate	10	70
	Sodium lauryl ether sulfosuccinate	10	70
	Triethanolamine lauryl sulfate	10	70
	Decyl polyglycoside	10	70
	Alkyl polyglycoside	10	70
	Cocoamidopropyl betaine	10	70
	Coconut fatty acid diethanolamine	10	50
	Polyethylene glycol	10	10
	Propylene glycol	10	40
	Ethylenediaminetetraacetic acid (EDTA)	10	10
10	Citric acid	10	20
	Sodium chloride	10	30
	Glycerin	10	50
	Mineral oil	10	50
	Coconut palm oil with ethylene oxide	10	50
	Stearic acid	10	70
	Acrylate/acrylamide copolymer	10	40
	Polysorbate 85	10	40
	Sodium acrylate copolymer	10	20
	Sodium acrylomethyl taurate copolymer	10	20
15	Trideceth 6	10	20
	Polyacrylic acid	10	20
	Triethanolamine	10	20
	Xanthan gum	10	20
	Guar gum	10	20
	Fragrance	10	20
	Flavor	10	20
	Egg yellow	0	0.1
	Red #6 color	0	0.1
	Rhodamine color	0	0.1
	Lemon pop yellow	0	0.1
20	Aniline blue	0	0.1
	Emerald green	0	0.1
	Orange yellow	0	0.1
	Blue #1	0	0.1
	Green #5	0	0.1
	Yellow #5	0	0.1
	Green #8	0	0.1
	Violet Woll	0	0.1

Testing of virucidal activity

Cell cultures

MDCK cell line cultured on 25 cm² flasks with minimum essential medium (MEM) (Gibco/BRL, NY, USA) supplemented with 10 % bovine fetal serum (Invitrogen, México D.F.) and antibiotics (penicillin 100 IU/mL, streptomycin 100 mg/mL and amphotericin B 10 mg/mL) (Sigma-Aldrich, Inc., St. Louis M. USA) at 37 °C with 5 % CO₂ are used. Cells must be allowed to grow to 80 % confluence.

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Virus titration by hemagglutination assay (HA)

Inside test tubes or on V-well plates, dual dilutions of a virus-containing sample are mixed together, with a constant suspension of erythrocytes (use generally 10,000,000 cells/mL), and then incubated. In order to evaluate the result, the amount of cells added are quantified using a spectrophotometer, the last dilution showing complete hemagglutination (HA) is considered as the limit dilution and expressed as hemagglutinating units (HAU's).

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Preparation of erythrocytes

Erythrocytes from chickens of 3 to 5 days of age are used. Chickens are euthanized by blood extraction; the blood is placed in Alsever's solution. Cells are washed

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several times by centrifugation at 1800 xg for 5 min, once supernatant becomes clear it is removed and cells are adjusted to 10 % in PBS. When using the solution it is adjusted to 0.5 % in PBS.

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Titration of hemagglutinin

Each virus batch must be titrated. Dual dilutions of 1:10 to 1:2560 are made by placing a volume of 0.05 mL of each dilution in wells of 96-V-bottom-well plates. One
10 well for erythrocytes as control must be included.

Erythrocyte suspensions are added to each well with gentle mixing so as not to rupture the erythrocytes. They are incubated at room temperature for 1 to 2 hours.

HA titration is determined by reading the highest
15 dilution capable of agglutinating the erythrocytes.

The titration is reported as the reciprocal of the highest dilution capable of agglutinating the erythrocytes. And it is interpreted as hemagglutinating units: HAU/0.05 mL virus.

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Determination of virus titration to be used

The amount of viruses that is needed for testing as well as the effect of the sanitizer on cells is standardized. The solution of titanium dioxide nanomaterial
25 with herbal or fruit extracts adsorbed on the surface

thereof is used at the recommended dilution from the sanitizer container (prepared by pouring 75 mL in 5 liters of water). 40 μ l of virus are admixed to dilution at different HAU (40, 20 and 4 HAU) and incubated at different points of time: 1, 5 and 15 minutes. Mixtures from each interaction time (virus-nanomaterial) are inoculated with confluence monolayers of MDCK cells, and incubated for two hours at 37 °C under humid atmosphere and 5 % CO₂. Thereafter, the viral inoculum is removed, fresh culture medium without fetal bovine serum is added, and the monolayers are incubated for 24 hours.

Effect of the titanium dioxide nanomaterial with herbal or fruit extracts adsorbed onto the disinfectant surface thereof on a viral infection

MDCK cells are cultivated on 24-well plates until confluence. Different volumes of the sanitizing solution mixed with a constant infective dose of virus are employed. These are incubated for 10 to 15 min at room temperature and then inoculated with confluent cell monolayers on the microtitration plates for two hours.

Then, the sanitizing solution and virus mixture is removed, washed with sterile PBS (pH 7.45), and 1.5 mL of 2.0 % methylcellulose in MEM is added to each well so as to incubate again at 37 °C with 5 % CO₂ until lytic plates

are formed (maximum 10 days). The methylcellulose is removed, washed again, 200 μ l of 75 % methanol is added, then removed after 20 min and the 1 % violet crystal is added for 15 min. Then it is washed with tap water, examined, and lytic plates are counted under the microscope. The minimum dose of sanitizing solution is calculated according to the virus cytopathic ability. Virus without sanitizing solution is used as an infective control and uninfected cells are used as negative controls. Since it is a qualitative test, an approximate percentage of reduction of plates formed is calculated.

With these tests, it has been found that the subject invention of the present description inhibits up to 100 % of virus infectivity, such as influenza virus, within 0 to 5 minutes.

Testing of Bactericidal and fungicidal activity

For laboratory analysis and sample retention, randomly select a product representative subsample to be analyzed, and record the lot number.

To determine the antimicrobial activity, only one method is used, based on determining the reduction percentage of a given number of microorganisms when in contact with a germicide under specific test conditions.

Preparation of solutions0.25 M Phosphate buffer solution

In a 1000 mL volumetric flask, dissolve 34 g of potassium monobasic phosphate in 500 mL of water, adjust pH
5 between 7.1 and 7.3 with sodium hydroxide solution, bring to volume with water, mix and divide into 100 mL portions. Sterilize in autoclave at 394 °K (121 °C) for 15 min, allow to cool, and keep under refrigeration.

10 Diluted phosphate buffer solution

Place 1.25 mL of 0.25 M phosphate buffer solution into a 1 L volumetric flask and bring to volume with water, mix, and divide into 9 mL and 99 mL portions in test tubes and flasks respectively, sterilize in autoclave at 394 °K
15 (121 °C) for 15 min.

Concentrated neutralizing solution

Admix 40 g of azolecithin with 280 mL of polysorbate 80 mL and 1.25 mL of phosphate buffer solution,
20 dilute with water to obtain 1 L; adjust pH to 7.2 with the sodium hydroxide volumetric solution or hydrochloric acid volumetric solution, then distribute into 100 ml portions. Sterilize in autoclave at 394 °K (121 °C) for 20 min.

Diluted neutralizing solution

Mix 100 ml of concentrated neutralizing solution with 25 ml of 0.25 M phosphate buffer solution, add 1675 ml of water, mix together and distribute into 9 ml portions in
 5 20 mm x 150 mm threaded test tubes. Sterilize in autoclave at 394 °K (121 °C) for 20 min.

Preparation of culture media

Prepare and sterilize culture media according to
 10 the manufacturer instructions on the product label. In the case of agar culture media for the standard method with neutralizing solution and before sterilizing, add 25 mL of neutralizing solution to one liter of agar culture media for the standard method.

15

Neutralizing broth

Mix together the components shown in table 1, heat until dissolution occurs, adjust pH to 7.2, put in autoclave and sterilize at 394 °K (121 °C) for 15 min.

20

TABLE 1. Components of neutralizing broth

Tryptone	5.0 g
Yeast extract	2.5 g
Dextrose	10.0 g
Sodium thioglycolate	1.0 g
Sodium thiosulfate	6.0 g
Sodium bisulfite	2.5 g
Polysorbate 80	5.0 g
Soy lecithin	7.0 g
Bromocresol purple	0.02 g
25 Distilled water	1 L

Test microorganisms and culture media

Staphylococcus aureus (EMB agar)

Escherichia coli (EMB agar)

Pseudomona aeruginosa

5 Salmonella sp (EMB agar)

Enterobacter sp (E.M.B. Agar)

Klebsiella pneumoniae (EMB agar)

Candida albicans (EMB agar)

Aspergillus niger (EMB agar)

10

SAMPLE PREPARATION AND CONDITIONINGPreservation of test microorganisms

15 Preserve microorganism strains by weekly
reseeding in 16 mm x 125 mm test tubes with inclined
culture media (7 ml nutritive agar), incubate for 20 hr to
24 hr at a temperature of 308 °K to 310 °K (35 °C to 37 °C)
and keep under refrigeration.

Preparation of test microorganism suspension

20 Before testing, make two reseedings of each test
microorganism and incubate for 20 to 24 hr at a temperature
of 308 °K to 310 °K (35 °C to 37 °C).

From these cultures, reseed each test
microorganism in 22 mm x 175 mm test tubes each containing
25 12 mL of inclined nutritive agar and incubate at the

indicated conditions.

Remove growth from each tube with 3 mL of saline solution, transfer the supernatant to a sterile test tube and continue dilution with the same solution until
5 obtaining a suspension with readings between 3 % and 5 % transmittance at a wavelength of 580 nm.

Determine the CFU number / mL in the suspension and indicate the percent transmittance of a suspension containing 75 to 125 x 10⁸ CFU / mL. The latter is verified
10 according to the provisions of the Mexican Official Standard NOM-092-SSA1 (see 2 references) and these values are taken into account for future analyses.

Determination of initial viable count

15 To an Erlenmeyer flask containing 99 mL of sterilized diluted phosphate buffer solution, transfer 1 mL of the test microorganism suspension and make the necessary decimal dilutions in order to obtain plates containing between 25 and 250 colonies each.

20 Place in sterilized Petri dishes, 1 mL of each dilution in duplicate, add to each plate 15 mL to 18 mL of agar for standard methods, homogenize and allow to solidify, turn the Petri dishes over and incubate for 48 h at 303 °K - 308 °K (30 °C - 35 °C). Count colonies
25 contained in each dish in a colony counter.

PROCEDURE

1. Strain seeding and culturing;
2. Determination of surviving cells;
3. Sample preparation;

5 If necessary, make an appropriate dilution with water to reach product's concentration as recommended by the manufacturer on the package label.

4. Sample inoculation

 For each test microorganism, measure precisely
10 and in duplicate 99 mL of the product or its dilution, transfer to 250 mL sterilized Erlenmeyer flasks with screw caps.

 Agitate flasks, stop agitation just before inoculation, so that the residual liquid still have motion
15 to facilitate incorporation of the inoculum. Individually inoculate each flask with each test microorganism in the center of the liquid surface, avoiding contact of the pipette with the neck or walls of the flask.

 Agitate the flask with the inoculated sample
20 inside and exactly 30 seconds after inoculation, transfer 1 mL of the same to a test tube containing 9 mL of the diluted neutralizing solution or neutralizing broth, mix together and transfer in duplicate 1.0 mL aliquots to sterilized Petri dishes, and continue dilution until the
25 necessary dilutions are obtained to form plates containing

25 to 250 colonies, add 15 mL to 18 mL of agar for standard method as a neutralizer to each plate, homogenize, allow to solidify, turn over the plates and incubate for 48 hr at 308 °K to 310 °K (35 °C to 37 °C).

- 5 After the incubation period, count the CFU number in the plates.

EXPRESSION OF RESULTS

Determination of % reduction

- 10 Average the results from plates of the initial viable count and surviving cells, then calculate % reduction using the following formula:

$$\% \text{ reduction} = 100 - \frac{S \times 100}{V.C.}$$

- 15 where:

S are the surviving cells CFU / mL, and
V.C. is the initial viable count.

Report the percentage of reduction obtained on the product sample.

20

Interpretation of results

- A product labeled as germicide must have a 99.999 percentage reduction of viable count within 30 seconds of contact at the recommended concentration, when the initial
25 viable count is between 75 and 125 x 10⁸ CFU / mL.

Sporicidal activity tests

Effectiveness tests were carried out based on the AOAC 966.04 methodology, where 59 out of 60 replicas
5 against *Bacillus Subtilis* were obtained.

It is important to point out that the tests have been carried out according to NMX-BB-040-SCFI-1999 "METODO GENERAL DE ANALISIS-DETERMINACION DE LA ACTIVIDAD ANTIMICROBIANA EN PRODUCTOS GERMICIDAS" (General method for
10 analysis-determination of antimicrobial activity in germicide products") where, according to said NMX standard 100 % of *Escherichia coli*, *Salmonella paratyphi*, *Bacillus subtifis*, *Vibrio cholerae*, *Listeria monocytogenes*, *Streptococcus faecalis* and *Saccharomyces diastaticus* were
15 killed in a period of time of 0 to 5 minutes, while the effectiveness was also of 100 % according to said NMX within a time interval of 5 to 10 minutes, and 99.995 % within a time interval of 0 to 5 seconds for *Aspergillius niger* and *Staphylococcus aureus*, and 100 % within a time
20 interval of 10 to 15 minutes and 99.995 % within a time interval of 0 to 10 minutes for *Pseudomonas aeruginosas*.

CLAIMS:

1. A composite nanomaterial for the preparation of disinfectant, antiseptic, healing or cleaning composition, said composite nanomaterial comprising:

5 a nanoparticulate titanium dioxide (TiO_2) support having a surface and pores, a mean diameter particle size between 1 and 100 nm and a surface area of at least 50 m^2/g , the nanoparticulate titanium dioxide being in a crystalline structure;

10 functional groups chemically adsorbed on the surface of the nanoparticulate titanium dioxide support, the functional groups comprising an hydroxyl as organic functional groups and inorganic radicals; and

herbal and fruit extracts physically adsorbed on
15 the surface and pores of the titanium dioxide (TiO_2) support, the herbal and fruit extracts comprising particles of size between 0.3 to 10 nm,

wherein the fruit and herbal extracts comprise a mixture of at least three fruit and/or herbal extracts and
20 the mixture comprises citric extracts.

2. The nanomaterial of claim 1, wherein the organic functional groups and inorganic radicals are selected from an hydroxyl, a phosphate, a sulfate, a
25 chloride, an amino, a methyl and a folate.

3. The nanomaterial of claim 1, wherein the fruit extracts are selected from extracts of different parts of citrus plants or fruits, grape, tangerine, orange, grapefruit, lemon, guava or pomegranate.

4. The nanomaterial of claim 1, wherein the herbal extracts are selected from extracts of oregano, cinnamon or pepper.

10

5. A method for preparing a composite nanomaterial for the preparation of disinfectants or antiseptics according to any one of claims 1 to 4, the method comprising:

15 a) preparing herbal and fruit extracts;

b) providing a support comprising nanoparticulate titanium dioxide (TiO_2), comprising a surface and pores, a mean particle diameter between 1 and 100 nm and a surface area of at least $50 \text{ m}^2/\text{g}$, the nanoparticulate titanium dioxide being in crystalline structure;

20

c) providing precursor solutions which provide organic functional groups and inorganic radicals as functional groups;

d) performing a chemical modification of the nanoparticulate titanium dioxide by adsorption of the functional groups by the addition of the precursor solutions to the nanoparticulate titanium dioxide, by
5 agitation at a constant temperature;

e) providing herbal and fruit ethanolic extracts, comprising particles size between 0.3 to 10 nm;

f) performing physical modification of the nanoparticulate titanium dioxide by adsorption of fruit and
10 herbal ethanolic extracts by adding a mixture of at least three herbal and fruit ethanolic extracts that comprises citric extracts to the nanoparticulate titanium dioxide, after the adsorption of the functional groups, by agitation at constant temperature to the nanoparticulate.

15

6. The method according to claim 5, wherein the organic functional groups and inorganic radicals in the precursor solutions are selected from an hydroxyl, a phosphate, a sulfate, a chloride, an amino, a methyl and a
20 folate.

7. The method of claim 5, wherein the chemical modification of the nanoparticulate titanium dioxide is performed under a constant agitation at an average speed in
25 the range of 100 to 400 RPM.

8. The method of claim 5, wherein the chemical modification of the nanoparticulate titanium dioxide is made at constant temperature which is in the range of 30 to 100 °C.

9. The method of claim 5, wherein physical modification of the nanoparticulate titanium dioxide is made at constant temperature which is in the range of 30 to 50 °C.

10. The method of claim 5, wherein the precursor solutions are added dropwise one by one leaving a waiting time between the addition of each organic functional group or inorganic radical precursor solution.

11. The method according to claim 10, wherein said waiting time after addition of each precursor solution is from 5 to 30 minutes.

12. The method of claim 11, wherein said modification of the nanoparticulate titanium dioxide further comprises drying the nanoparticulate titanium dioxide obtained at a temperature from 30 to 100 °C before adding the fruit and herbal extracts.

13. The method of claim 12, wherein the step of performing the physical modification of the nanoparticulate titanium dioxide comprises performing physical adsorption
5 of the herbal and fruit extracts in an aqueous extracts mixture solution at 70 %.

14. The method of claim 13, wherein the step of performing the physical modification of the nanoparticulate
10 titanium dioxide for the adsorption of the herbal and fruit extracts requires an agitation period of 24 hours.

15. The method according to claim 14, wherein the fruit extracts are selected from different parts of citrus
15 plants or fruits, grape, tangerine, orange, grapefruit, lemon, guava or pomegranate extracts.

16. The method according to claim 14, wherein the herbal extracts are selected from oregano, cinnamon or
20 pepper extracts.

17. A disinfectant, antiseptic, healing or cleaning composition comprising:

up to 40% of the nanomaterial of any one of
claims 1 to 4; and

various proportions of selected raw materials.

NANOPARTICULATE TITANIUM DIOXIDE NANOMATERIAL MODIFIED WITH
FUNCTIONAL GROUPS AND WITH CITRIC EXTRACTS ADSORBED ON THE
SURFACE, FOR THE REMOVAL OF A WIDE RANGE OF MICROORGANISMS

5

ABSTRACT

The present invention relates to the conjugate of
a titanium dioxide nanomaterial and nanoparticulate (1-100
nm) herbal and/or fruit extracts, synthesized by means of
10 impregnation, using titanium dioxide as support, to which
are adsorbed organic functional groups, inorganic radicals
and plant extracts that confer thereon the property of
being an anti-microbial agent with a high disinfectant and
antiseptic power, removing bacteria, fungi, mycobacteria,
15 spores, mycobacteria, protozoa and viruses.

This invention is a solid nanomaterial in liquid
suspension. Said material is prepared by means of impregnation
to disperse the functional groups and the extract particles,
with control of the temperature to stabilize the interactions
20 within the network of the support.

The virucidal, bactericidal, fungicidal,
mycobactericidal, mycoplasmicidal, antiprotozoal and
sporocidal activity of the nanoparticulate biomaterial

depends on the particle size of the support oxide, the functionalization and the dispersion of extracts adsorbed on the surface. The functional groups may include hydroxyl, carboxyl, amine, sulfate, phosphate, and the supports may be
5 titanium dioxide, silica, zirconia, zinc oxide, alumina and other metal oxides, without being restricted to these functional groups, extracts or supports.

No suitable figure