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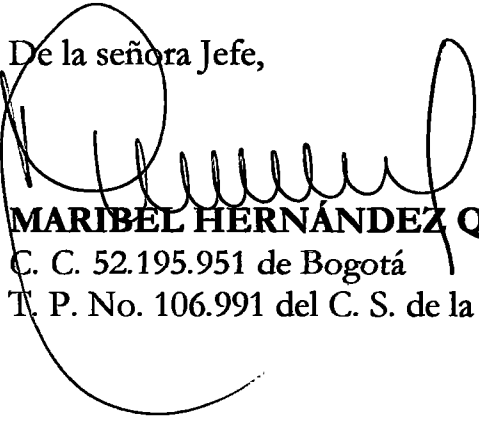
**MANUEL VIAMONTE & LAURENCE
SHANLEY.**

Solicitud internacional: PCT/US03/24598

MARIBEL HERNÁNDEZ QUINTERO, mayor de edad, domiciliada y
residenciada en la ciudad de Bogotá D. C., identificada con la cédula de
ciudadanía No. 52.195.951 de Bogotá y tarjeta profesional No. 106.991 del
Consejo Superior de la Judicatura, en mi calidad de apoderada de los señores
MANUEL VIAMONTE & LAURENCE SHANLEY, por medio de este
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solicitud de la referencia.

Se adjunta recibo de pago No. 07-105815, por valor de
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De la señora Jefe,


MARIBEL HERNÁNDEZ QUINTERO

C. C. 52.195.951 de Bogotá

T. P. No. 106.991 del C. S. de la J.



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(12) **United States Patent**
Viamonte, Jr.

(10) **Patent No.:** **US 6,296,882 B1**
 (45) **Date of Patent:** **Oct. 2, 2001**

(54) **NON-TOXIC MUCOSAL DISINFECTANT
 CONTAINING ISOPROPYL ALCOHOL,
 SESAME OIL, AND LEMON OIL**

(76) **Inventor:** **Manuel Viamonte, Jr.**, 1643 Brickell
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(*) **Notice:** Subject to any disclaimer, the term of this
 patent is extended or adjusted under 35
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(22) **Filed:** **Jun. 12, 2000**

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(52) **U.S. Cl.** **424/736**

(58) **Field of Search** **424/736**

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(57) **ABSTRACT**

A non-toxic mucosal disinfectant for topical application in the nose comprising a composition of 91% isopropyl alcohol of at least 50% by weight; sesame oil not exceeding 49.5% by weight and lemon oil of about 0.5% by weight. All of the components are mixed homogeneously with the sesame oil supplementing and neutralizing the dehydrating effect of the alcohol.

12 Claims, No Drawings

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NON-TOXIC MUCOSAL DISINFECTANT CONTAINING ISOPROPYL ALCOHOL, SESAME OIL, AND LEMON OIL

FIELD OF THE INVENTION

The present invention is generally directed toward a topical disinfectant and more specifically is directed toward a non-toxic mucosal disinfectant effective against various pathogenic organisms which cause infectious processes.

DESCRIPTION OF THE PRIOR ART

Infectious diseases remain the leading cause of death and morbidity. Even the domestication of animals has resulted in a number of illnesses (Diphtheria came from the water buffalo; mycobacteria from ungulates; measles from canine distemper, etc.).

The aerosolization of water in western society, ranging from the widespread use of showers instead of baths to the spraying of products in supermarkets, to air conditioning, has probably played a significant role in the emergence of Legionnaire's disease and mycobacterium avium infection in both normal and immunocompromised hosts. Legionella pneumophila, is an infectious agent of predatory protozoans found widely in nature.

Some microorganisms are pathogens (a microorganism capable of causing disease) but most microorganisms that are seen in the human body are innocuous. For example, more than 600 species of bacteria inhabit the large bowel of a human. Not only are the majority of human microorganisms innocuous but they play useful, if unseen roles. These microorganisms provide a necessary part of the development pathways required for the maturation of human intestinal mucosa and our innate local immune system protects us against harmful microorganisms and helps the digestion of food.

Most of human microbes are commensal. Commensal or transient microbes can be an opportunistic pathogen of humans; namely, they can cause disease if one or more defense mechanisms are breached by accident, medical intent, or an underlying metabolic or even infectious disorder.

Human beings are exposed to nosocomial as well as nosohusial infections. Many microorganisms are adapted exclusively to humans and other animals and many pathogenic microorganisms have learned to circumvent, exploit, subvert or avoid our normal cellular mechanisms to multiply at human expense. Some microbes have made the transition from harmless commensal to potentially fatal infectious agents.

Increases in the world population, rapid travel between distant regions, high concentration of individuals in small areas, the wide spread use of air conditioning and heating equipment without air exchange, the large number of people traveling in confined areas (i.e., aircraft, trains, buses, and automobiles), have resulted in the increase in the number of pathogenic organisms and the increase of mutations of organisms. Thus, there is a need for effective protective measures to decrease the number and severity of respiratory infections.

Some societies use face masks as a protection against respiratory infections. However, the use of face masks are an impractical, inefficient and largely ineffective way to prevent dissemination of infection.

There are a number of highly contagious respiratory infections which result in fatal illness, such as African Ebola

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viruses. Some people have a decreased or defective immunological response to such infections. People who reside in high density cities are at risk for a large variety of respiratory illnesses. Many of the respiratory illnesses do not become confined to the respiratory tract (upper and/or lower) and can disseminate to other organs or become systematic infections.

It is estimated that over 66 million colds occur annually in the United States. An American catches an average of two to four colds a year. As few as ten cold viruses may be enough to cause infection. When symptoms first become noticeable there are thousands of viruses in the lung. The droplets of a sneeze travel as fast as 150 feet a second and as far as 12 feet.

It is highly desirable to protect the nasal mucosa from pathogenic organisms. In those individuals with active respiratory infection, it is also desirable to decrease the likelihood of dissemination of infection by decreasing the number and/or virulence of the pathogenic organisms expelled during exhaling, sneezing, and/or coughing through the use of a topically applied nasal disinfectant and to also reduce or eliminate disease transmissions through the fingers and hand of an infected person.

BACKGROUND OF THE INVENTION

Respiratory infections are the result of the exposure to pathogenic bacteria, viruses, and fungi. The immunological response to infections consists of the neutralization and destruction of the invader by immunoglobulins circulating in the liquid part of the blood (plasma) and phagocytosis from neutrophils, monocytes, and tissue macrophages. When the infection exceeds the effectiveness of the defense mechanism, illness will result.

Good health, and particularly a strong immunological system, is protective against infections. However, at this time, we cannot avoid the effects of the inhalation of pathogens, particularly when subjects are in a closed environment (buildings, airplanes, buses, trains, etc.) or are in physical proximity to individuals who have active respiratory infections and are sneezing, coughing, and expelling microdroplets with a high concentration of pathogens.

Prophylaxis is better than treatment and it appears to be quite limited for the prevention of upper and lower respiratory tract infections. Avoidance of environments with a high concentration of airborne pathogenic material is not always possible. However, the application of a topical antiseptic solution to the nose and avoidance of introducing pathogenic material into the nose by fingers that may be contaminated, appear to be a valid, simple, inexpensive practical method of helping human beings to prevent upper respiratory infection.

When considering the application of a disinfectant to a human subject, it must be recognized that the human capacity for smell is highly developed and easily desensitized which limits the use of a number of disinfectants. Humans have roughly 1000 receptors capable of recognizing some 10,000 distinct odors and over five million smell-sensing cells having neurons with eight or more stringy cilia. Olfactory neurons undergo constant renewals with an average replacement every one to two months. Olfactory receptor cells have bipolar neurons that are located in the olfactory epithelium under the dorsal aspect of the nasal cavity, the septum and part of the superior turbinates in the nose. Turbinates in the nose create airflow patterns that allow volatile compounds to reach the olfactory cells. Olfactory receptors bind odorants and belong to the 2-G-protein-coupled receptor superfamily associated with the adenylyl cyclase and phosphoinositol signaling. Coding for odor

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1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

2. Once the problem is identified, the next step is to define the objectives and goals of the project. This helps to clarify what needs to be achieved and provides a clear direction for the team.

3. The third step is to develop a plan or strategy to address the problem. This involves breaking down the problem into smaller, manageable tasks and determining the resources needed to complete each task.

4. The fourth step is to implement the plan. This involves putting the strategy into action and monitoring progress regularly to ensure that the project is on track.

5. The final step is to evaluate the results of the project. This involves comparing the actual outcomes with the objectives and goals to determine the effectiveness of the project and identify areas for improvement.

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JOHN DEWITT, JR., 1894-1900

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quality and identification may involve the specific temporal sequences of firing that is compound specific. Axons of olfactory bipolar cells traverse through the small holes in the cribriform plate of the ethmoidal bone to the olfactory bulb where they form synapses in intricate masses called glomeruli. There is, thus, a neuroanatomical overlap which provides an anatomical basis for the capacity of odor to produce hedonic responses. Olfactory information is ultimately transmitted to the hypothalamus and this anatomical structure emphasizes the importance of olfaction in eating and nutrition. Thus, it is extremely important when treating disease that these sensor functions are not desensitized or overpowered by the chemical compound being used to treat the infection.

Rubbing alcohol (isopropyl alcohol, isopropanol) is used as a 70% mixture with water for rubdowns because it cools the skin by evaporation and causes pores to close. Isopropyl alcohol has been used for preparing needles and syringes for hypodermic injection. Isopropyl alcohol does not contain ethyl or grain alcohol. It is also used as a solvent for medicine, as a sterilant for instruments and as a skin cleanser before drawing blood or giving injections. Isopropyl alcohol has been shown to be an excellent antiseptic product. It appears to be lethal to bacterial, fungi and viruses, including the AIDS virus. It has also been effectively used as a solution and as a spray for its antiseptic properties as is shown in U.S. Pat. Nos. 5,441,723 and 5,145,663. It is a disinfectant which is nontoxic, biodegradable, material compatible and highly effective.

The use of topical disinfectants for application to body surfaces is noted in:

U.S. Pat. No. 3,445,564 shows various spray germicides used for sanitizing surfaces.

U.S. Pat. No. 4,209,509 describes Aerosol sprays including an anhydrous alcohol used with a fragrance or perfume.

U.S. Pat. No. 4,678,658 shows numerous disinfecting compounds which are effective as germicides for disinfecting surfaces.

U.S. Pat. No. 5,145,663 describes biodegradable disinfectant containing anhydrous alcohol and propylene glycol for use on skin and hard surfaces.

U.S. Pat. No. 5,441,723 shows a composition of various alcohol, propylene glycol; 1,3 Propanediol; 1,2 butanediol PEG 400; glycerol 1,4 butanediol or mixtures all of which reduce the surface glaze formed by alcohol as well as surface tension to be used on body surfaces and hard surfaces.

An examination of the prior art fails to present an effective, non-toxic, disinfectant topically applied through a spray or contact application.

The present invention is based on the discovery that animals and humans have a limited ability to neutralize high concentrations of airborne pathogens and that the present invention can effectively destroy airborne pathogenic material.

SUMMARY OF THE INVENTION

The present invention relates to a non-toxic mucosal disinfectant topically applied which effectively destroys various pathogenic organisms and which does not quickly evaporate and does not interfere with sensory identification of odors.

The non-toxic topical disinfectant comprises a novel composition of isopropyl alcohol, sesame oil and lemon oil, in specific relative proportions by weight. The non-toxic topical solution may be used safely to disinfect the nasal

mucosa through application of spray or droplets. The interactive composition comprises of 50% isopropyl alcohol, 49.5% sesame oil, and 0.5% of lemon oil by weight.

It is an object of this invention is to prophylactically apply a solution of the composition comprising isopropyl alcohol, sesame oil, and lemon oil to the distal nasal mucosa (close to the nares) to destroy pathogenic bacteria viruses and fungi which are present therein and which are inhaled. Normally, present flora is destroyed as well, but repopulates the nose, generally 6 hours after the use of the topical disinfectant.

The present invention provides a method of utilizing a disinfectant for the prevention of upper respiratory tract infection.

It is another object of the present invention to treat skin infections with the present inventive composition.

It is also an object of the invention to treat paronychia infection around the fingers or toenails with the inventive composition.

It is yet another object of the invention to provide for the relief of pain and for the treatment of infections in the external auditory canal.

These and other objects, advantages, and novel features of the present invention will become apparent when considered with the teachings contained in the detailed disclosure.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Numerous disinfectants and delivery systems have been developed aimed at destroying various pathogenic organisms. The chemical and biological effectiveness of such disinfectants are limited by their chemical and biological effects.

The invention of this non-toxic disinfectant composition, by virtue of its most active ingredient (isopropyl alcohol) is effective against various pathogenic organisms which include bacteria (salmonella, staph, pseudomonas, etc.) viruses, (including herpes, and HIV), and fungi, by topical application of this product in the nasal mucosa. The preferred embodiment of the composition comprises 91% isopropyl alcohol 50% by weight, sesame oil 49.5% by weight and lemon oil 0.5% by weight. Other testing has shown effective treatment with isopropyl alcohol being used in a range of about 50 to 75% weight; sesame oil in the range of about 49.5%+ to 24.5%+ and lemon oil in a range of about 0.1 to 0.5%.

The isopropyl alcohol provides the primary bactericidal and virucidal effect (the killing effect of pathogenic organisms) while sesame oil provides lubrication of the nasal mucosa or infection area while also acting as a disinfectant. The dehydrating effect of isopropyl alcohol is also neutralized by its dilution with the sesame oil. The lemon oil which is added to the composition provides lubrication, fragrance and vitamin C to the nasal mucosa. Both the sesame oil and lemon oil provide prolonged contact time of the alcohol to the nasal mucosa or infection area (in excess of four hours up to six hours).

It can, thus be seen that non-toxic mucosal disinfectant of the subject inventive composition includes three distinct products which actively complement each other. One is the primarily active disinfectant, isopropyl alcohol. The others, an additional disinfectant, sesame oil, mixed together with lemon oil, provide mucosal protection which delay the evaporation of the alcohol. The lemon oil adds Vitamin C to the infection site which is effective against infections.

Sesame Oil, Ben (n) e, Gingelly, Simsin, til or ufta Oil is the oil extracted from the seeds of Sesamum indicum

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(Pedaliaceae). Sesame Oil is an oily product which has 22% total fat, 10% of saturated fat, and polyunsaturated and monounsaturated fat. It has no sodium and no carbohydrate as well as no protein and is not a significant source of cholesterol, dietary fiber, vitamin A and C, calcium and iron. The best sesame oil is the 100% pure expeller pressed.

Lemon Oil is an essential oil meaning containing the essence of lemons. It takes 1000 pounds of fresh lemons to make 10 pounds of pure lemon oil. This oil is derived primarily from lemon peels and contains no scents or preservatives. The lemon oil used in the present composition should be 100% pure USP Grade.

The disinfectant isopropyl alcohol, when used by itself in a 91% solution, is an irritant to the nasal mucosa and its strong scent generally would make it unacceptable. Also, individuals should not be subjected to prolonged inhalation of vapors of any type of alcohol.

Alcohol and oil do not interact chemically and the composition must be thoroughly mixed. A mixture of sesame oil and isopropyl alcohol not only dilutes the alcohol but provides a lubricating surface to the mucosa of the distal part of the nose. When lemon oil is added to these other two ingredients, it contributes to the lubrication of the surface and introduces a scent which makes the combination of substances more agreeable, without harming the sensory receptors. Furthermore, lemon oil has Vitamin C which has well-known beneficial properties, including protection against infections. Preparation of the composition is shown in the following illustrative examples.

EXAMPLE I

The disinfectant properties of the present composition in the dosage recommended (one to three drops in each nasal cavity), occur without toxic effects or damage to the mucosal surface of the nose. The optimum proportional relationships of the ingredients by weight is isopropyl alcohol, 50%; sesame oil, 49.5% and lemon oil, 0.5%. The components are mixed at an ambient temperature with a standard mixing apparatus and topically applied as follows:

The composition of Example I was topically applied by an eye dropper to the external nasal mucosa of a human subject. The nose was then squeezed using sterile gloves to provide a uniform distribution of the solution in the nose.

Subject A, a human male, had a baseline sampling of his right nostril taken at 8:00 a.m. Five minutes after the instillation of inventive solution into his right nostril (Sample 1), another sample was obtained (Sample 2). One hour later, Sample 3 was obtained from the right nostril and at two hours (Sample 4), three hours (Sample 9), four hours (Sample 10), five hours (Sample 11), and at six hours (Sample 12).

The left nostril of Subject A was used as a control. A baseline sample was obtained from the left nostril (Sample 5). Five minutes after using distilled water in the left nostril another sample was obtained (Sample 6). One hour later another sample was obtained from the left nostril (Sample 7). Finally, at two hours, another sample was obtained from the left nostril (Sample 8).

Subject B was a human woman with acute, severe, upper respiratory infection. Topical application of the inventive solution was undertaken in the same manner of application to Subject A. Sample 13 was obtained from the right nostril as a baseline. Ten minutes after application of the inventive solution in the right nostril another sample was obtained (Sample 14). One hour later another sample was obtained from the right nostril (Sample 15). Finally, a two hour

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sample was obtained from the right nostril (Sample 16). The 28 and 48 hour result showed growth of bacteria. The culture was an Agar blood medium (red background). Colonies of bacteria were visually obvious in the four Agar plates. Each plate was divided into four quadrants:

Plate 1: Samples 1 through 4

Plate 2: Samples 5 through 8

Plate 3: Samples 9 through 12

Plate 4: Samples 13 through 16

The growth of bacteria was classified as few, moderate, or heavy.

RESULTS

Sample 1: Heavy growth (baseline)

Sample 2: Moderate growth (five minutes after instilling the inventive solution)

Sample 3: One hour later and showed moderate growth

Sample 4: Two hours later and showed minimal growth

CONTROL STUDY (After using Water)

Sample 5: Heavy growth

Sample 6: Heavy growth

Sample 7: Moderate growth (one hour after water)

Sample 8: Moderate growth (one hour after water)

Samples 9, 10, 11 and 12 from right nostril.

Sample 9: Minimal growth (three hours)

Sample 10: Moderate growth (four hours)

Sample 11: Moderate growth (five hours)

Sample 12: Minimal growth (six hours)

The Subject B with upper respiratory infection showed heavy growth in Sample 13 (baseline); heavy growth on Sample 14 (10 minutes after instilling the inventive solution. Very minimal growth was present one hour after instilling solution, rare growth (two hours after the inventive solution)- Sample 16.

CONCLUSIONS

1. The present inventive solution is effective in decreasing bacterial growth when compared to normal bacterial growth occurring in the instillation of distilled water.

2. The present inventive solution showed dramatic control on the bacterial growth on the Subject B with acute respiratory infection.

3. The duration of the effects of the inventive solution is as follows:

a. In the normal subject, four hours after instilling the inventive solution only moderate bacterial growth was observed.

b. In the Subject B with acute respiratory infection up to two hours, there was insignificant growth of bacteria. The subject with respiratory infection was studied only during the first two hours after instilling the inventive solution.

Photographs were obtained of the four plates and of the individual plates showing the growth of bacteria. There were no studies performed on fungi or viruses.

IN SITU APPLICATION OF INVENTIVE SOLUTION

In the Agar/Blood plates with Samples 13 and 14, drops of the inventive solution were applied to Sample 13 and

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1. The first part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are written in a cursive script, and the addresses are listed below them. The list includes names such as "Mr. J. H. Smith", "Mr. W. H. Jones", and "Mr. R. H. Brown".

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1. The first step in the process of the development of a new product is the identification of a market need. This is often done through market research, which can be conducted in a variety of ways, including surveys, focus groups, and interviews. The goal is to understand what customers want and need, and to identify any gaps in the current market.

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1. The first part of the document is a list of names and titles, including "The Hon. Mr. Justice" and "The Hon. Mr. Justice".

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1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

2. Once the problem is identified, the next step is to define the objectives and goals of the project. This helps to clarify what needs to be achieved and provides a clear direction for the team.

3. The third step is to develop a plan or strategy to address the problem. This involves breaking down the problem into smaller, manageable tasks and determining the resources needed to complete each task.

4. The fourth step is to implement the plan. This involves putting the strategy into action and monitoring progress to ensure that the project is on track.

5. The final step is to evaluate the results of the project. This involves assessing the outcomes against the objectives and goals and identifying any areas for improvement.

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drops of Virahol®, a commonly used disinfectant, were applied to Sample 14. Both samples showed complete destruction of bacteria within one hour.

The effectiveness of isopropyl alcohol on destroying bacteria, fungi, and viruses, make it an ideal substance to be applied in a topical form to the distal part of the nose. The oily components of this solution (sesame and lemon) produce a type of lubrication which lasts several hours (in general, more than four).

EXAMPLE II

The solution was prepared with a range of isopropyl alcohol of 50 to 75% by weight; sesame oil in a 25 to 49.5% range and lemon oil in a range of 0.1 to 0.5% by weight. A comparative 40% alcohol solution was effective up to two hours. A 30% alcohol solution was effective only for one hour. Two other concentrations, a 20% and a 10% alcohol solutions were not effective at all.

EXAMPLE III

The base solution (50% alcohol) of Example II and higher concentrations of the isopropyl alcohol (65-75%) sesame oil 24.5 to 34.5%, lemon oil about 0.5% appear to be effective against numerous pathogenic organisms when applied to the skin or hard surfaces.

EXAMPLE IV

The solution of Example I was topically applied with an applicator to a skin infection of several human subjects. The infections were cured by the inventive solution.

EXAMPLE V

The solution of Example I was topically applied with an applicator to a fingernail infection of several human subjects. The paronychia was quickly cured.

EXAMPLE VI

The solution of Example I was instilled into the external auditory canal of three subjects with ear pain secondary to infections of the external auditory canal. Within minutes the pain disappeared in all the three patients and the infection was used within the first day.

EXAMPLE VII

The solution of Example I was topically applied with an applicator to aphthous ulcers of the buccal mucosal and the infection was cured.

Several spouses who slept next to a spouse with an upper respiratory infection had the inventive solution applied to both. The unaffected spouse did not develop symptoms of a cold up to two weeks of observation.

In the foregoing description, the invention has been described with reference to a particular preferred embodiment, although it is to be understood that specific

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details as shown are merely illustrative, and the invention may be carried out in other ways without departing from the true spirit and scope of the following claims.

What I claim is:

1. A non-toxic mucosal disinfectant for topical application in the nose comprising a composition of isopropyl alcohol of at least 50% by weight; sesame oil not exceeding 49.5% by weight and lemon oil not exceeding 0.5% by weight, mixed homogeneously, said sesame oil supplementing and neutralizing the dehydrating effect of the alcohol.

2. A non-toxic topical mucosal disinfectant according to claim 1, wherein said isopropyl alcohol ranges from about 50 to 75% by weight, said sesame oil ranges from about 24.9% to 49.9% by weight.

3. A non-toxic topical mucosal disinfectant according to claim 1, wherein at least 50% by weight of said disinfectant is a 91% isopropyl alcohol and not more than 50% by weight consists of sesame oil and lemon oil.

4. A non-toxic topical mucosal disinfectant according to claim 1, wherein said composition has an effective treatment life of at least four hours and a shelf life of at least two years.

5. A disinfectant composition for treatment of an infection for topical application in the human nose comprising a composition of a solution of isopropyl alcohol of about 50% by weight; sesame oil of about 49.5% by weight and lemon oil of about 0.5% by weight, mixed homogeneously, said sesame oil supplementing and neutralizing the dehydrating effect of the isopropyl alcohol.

6. A non-toxic topical mucosal disinfectant according to claim 5, wherein said composition has an effective treatment life of at least four hours.

7. A non-toxic topical mucosal disinfectant according to claim 5, wherein said isopropyl alcohol is at least 91% pure isopropyl alcohol.

8. A non-toxic topical disinfectant comprising a non-toxic topical disinfectant comprising a composition of a solution of isopropyl alcohol ranging from 50 to 75% by weight, sesame oil ranges from about 24.9% to 49.9% by weight and lemon oil ranging from about 0.1% to 0.5% by weight, said sesame oil supplementing and neutralizing the dehydrating effect of the isopropyl alcohol.

9. A non-toxic topical disinfectant composition for treatment of an infection comprising a non-toxic topical disinfectant according to claim 8, with about 50% by weight of said disinfectant being 91% isopropyl alcohol and about 50% by weight of said disinfectant consisting of sesame oil and lemon oil.

10. A non-toxic topical disinfectant according to claim 8, wherein said isopropyl alcohol is present in a range of about 65 to 75%.

11. A non-toxic topical disinfectant according to claim 8, wherein said lemon oil is present in said composition at about 0.5%.

12. A non-toxic topical disinfectant according to claim 8, wherein said isopropyl alcohol is at least 91% pure isopropyl alcohol.

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2. The second part of the document is a report from the Secretary of the Treasury, dated January 1, 1861. It contains a detailed account of the financial state of the country at the beginning of the year.

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ANTIMICROBIAL AGENTS ISOLATED FROM ALOE VERA

This application claims the benefit under 35 U.S.C. §119(e) of U.S. provisional application Ser. No. 60/077,145, 5
Filed Mar. 6, 1998.

TECHNICAL FIELD OF THE INVENTION

The present invention relates in general to the field of antimicrobial agents, and more particularly, to the characterization and isolation of agents that are responsible for antimicrobial activity of *Aloe vera* and its gel. 10

BACKGROUND OF THE INVENTION

Without limiting the scope of the invention, its background is described in connection with the identification of novel anti-microbial agents isolated from *Aloe vera*, as an example. 15

Heretofore, in this field, organisms that cause infectious disease, namely, viruses, bacteria, fungi and multicellular parasites, humankind has sought to control their morbidity and mortality. With the isolation and characterization of powerful antibiotics, beginning over half a century ago, the balance of power between humans and microbes has been shifted toward humankind. For several decades after the introduction of penicillin in the 1940's, for example, the conquest of infectious disease appeared imminent. The widespread use of antibiotics, added to the evolutionary flexibility of microbes, has made that victory less than certain. 20

An increasing number of bacteria, fungi and other microbes are developing resistance to antibiotics. A number of factors have contributed to the increase in microbes that are resistant to antimicrobial agents. The use of combinations of anti-microbial agents to treat nosocomial infections, particularly among patients whose immune systems are compromised by AIDS, chemotherapy, or immunosuppressive drugs, has led to a dramatic increase in multiple drug resistant (MDR) infections. Unfortunately, the future does not look bright in the war against infectious disease, as MDR strains of microbes continue to proceed at an alarming rate. In fact, MDR strains are adapting faster than the introduction of new, more potent antibiotics. 25

Microbes have been developing strategies to cope with change for hundreds of millions of generations. In fact, some bacteria have generation cycles of 20 minutes, with each cycle providing the opportunity to evolve and adapt. Bacteria have adapted to an extraordinary range of conditions and developed defenses against all sorts of environmental threats, environmental and artificial. To a microbe the human body is just another environment to colonize. While antibiotics are just another toxic environmental agent against which the microbe must develop an escape strategy. For organisms with populations that have already adapted to such extreme environments as boiling underwater hot-springs, learning to cope and evade antibiotics was only a matter of time and evolution. 30

Overuse of antibiotics has contributed to the problem of MDR microbial strains. The indiscriminate use of antibiotics throughout the world contributes to the continued emergence of MDR strains of bacteria such as *Pseudomonas*, *Streptococcus* and *Staphylococcus*. MDR strains have evolved in large part because many patients fail to complete the required course of antibiotic treatment, allowing stronger members of the microbial pool to be selected for in the next round of treatment. Increases in ear and sinus infections in 35

children have been caused by the use of antibiotics to treat viral infections, infections that are not susceptible to antibiotic treatment. The current trend in medicine is to prescribe second-line and even last-resort antibiotics in place of first-line antibiotics. Even when there is no reason to suspect resistance to first-line antibiotics, the drive toward using stronger, faster drugs is inevitable when faced with a sick patient. In the case of recurrent lung infections in cystic fibrosis patients, physicians have had no choice but to escalate to antibiotic treatment with second-line antibiotics, eventually causing the infecting bacteria to become resistant to all available antibiotics. 40

The emergence of MDR microbes has changed the balance between host and parasite, from a position in which the medical community seemed poised to achieve a conquest has lost ground in achieving a permanent conquest of microbial infection. But much has been learned in the process. Using a deeper understanding of microbes and their mechanisms of resistance, the biomedical community can continue to mount a broad array of defenses against them. The microbes growing resistance to traditional antibiotics has renewed the attention to medical basics, such as public health measures, that include a renewed effort to stem infectious diseases by increasing hygiene. For example, in HIV-infected populations, which have become breeding grounds for resistant microbes, renewed educational outreach efforts focus on the use of prophylactics. 45

Nosocomial infections present the greatest threat to immuno-compromised patients, because MDR microbes infect the most vulnerable patients. It is the increase in MDR of microbes, and in particular bacteria, that has led to a resurgence of interest in revitalizing and improving basic techniques (like hand-washing) for preventing the spread of infection. It has also increased the need for alternative, next-generation, anti-microbial agents. These anti-microbial agents, viz., anti-viral, anti-bacterial, anti-fungal and anti-parasitic, must also be safe for use in humans and other animals. 50

Antimicrobial-drug resistance is an increasingly important factor and poses a serious international challenge to public health in community and institutional settings. The list of resistant bacteria of major public health importance includes those causing tuberculosis, gonorrhea, pneumococcal infections, and hospital-acquired enterococcal and staphylococcal infections. Antimicrobial-drug resistance has resulted in prolonged and more serious illness, the use of more expensive and often more toxic drugs and drug combinations, and increased fatality rates. 55

While pharmaceutical and biotechnology companies are constantly developing novel products based on presently known antibiotics to overcome resistance, the next-generation of anti-microbial agents must break from the known approaches to isolate and characterize these activities. A better understanding of the microbiology and molecular genetics of microbial resistance is leading to the development of a new generation of anti-microbial agents that use an approach that is intended to attack standard mechanisms of action to kill bacteria or fungi. These so called new approaches to fighting microbial infections rely on variations of existing drugs having longer half-lives and more potent effects, but rely on the existing database of pharmaceuticals to attempt to outpace the microbes ability to evolve. 60

SUMMARY OF THE INVENTION

Both competition for nutrients and bacteriocin production play a role in determining the establishment of microbial 65

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The following is a list of the names of the persons who have been appointed to the various positions in the various departments of the Government of the State of New York, for the year 1900.

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1. The first step is to identify the problem or question that needs to be answered. This involves understanding the context and the specific requirements of the task.

1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the situation.

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1. The first step in the process is to identify the problem. This involves gathering information about the situation and understanding the needs of the stakeholders involved.

2. Once the problem is identified, the next step is to develop a plan. This involves setting goals and determining the steps that need to be taken to achieve those goals.

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4. Finally, the fourth step is to evaluate the results. This involves assessing the effectiveness of the plan and making adjustments as needed.

communities in nature. When analyzing symbiotic associations this may be further influenced by the presence of antimicrobial chemicals produced by the host. In the case of *Aloe vera barbedensis*, the plant has been shown for centuries to exert broad spectrum healing activities. The source of the antimicrobial agents isolated herein were determined, as were the distinct populations of bacteria, and their dynamics within the indigenous microflora of *Aloe vera*. Localization of specific microbial populations was assessed using both direct culture of dissected plant material and immunological detection within tissue sections. Relative size and population diversity were determined through direct culture. Immunological detection demonstrated discrete populations within specific plant structures.

Bacterial identification was accomplished using standard staining and biochemical analysis. To more completely identify the specific species of bacterium isolated and their role in the antibacterial activity isolated herein, the environmental specimens were further analyzed using restriction fragment length polymorphism (RFLP) analysis. RFLP analysis was used to differentiate the various species of *Bacillus* found within the plant as well as definitive identification of *Aeromicrobium* species and *Curtobacterium* species.

The efficacy of aloe liquid (see e.g., Coats, *Aloe Vera: The Inside Story*) as an antimicrobial agent is shown herein to have a wide range of gram negative and gram positive bacteria. The antimicrobial agents of the present invention are shown herein to effectively kill, or greatly reduce or eliminate the growth rate of the following bacteria: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *E. coli*, *Propionibacterium acne*, *Helicobacter pylori*, and *Salmonella typhi*.

The anti-bacteriocidal activity demonstrated herein is not due to the preservatives used in the preparation of the clear gel or the isolation of the antibacterial components, as the antimicrobial agents isolated herein were isolatable from liquid collected directly from freshly cut whole leaves prior to used in the same killing assays.

In addition to the antimicrobial activities of its liquid, it also has been shown to be nontoxic even when taken internally. These properties combined provide the impetus for the use of the antimicrobial agents isolated herein when used alone or in combination. The present invention demonstrates the isolation and identification of new, non-toxic, FDA approved antimicrobial agents that have been identified and isolated from the Aloe plant. These agents are efficacious and nontoxic, with a broad spectrum of antimicrobial properties.

Generally, and in one form of the invention, a composition isolated from the gel liquid of *Aloe vera* including, at least one antimicrobial agent isolated from the clear gel isolated from the whole leaf of *Aloe vera*, wherein the antimicrobial agent is an agent produced by the *Aloe vera* or indigenous bacteria that colonize the *Aloe vera*, is disclosed.

Furthermore, a method of decreasing the growth of a broad spectrum of bacteria including the steps of, isolating at least one antibacterial agents from the clear gel of an *aloe vera* plant and directly contacting the bacteria with at least one antibacterial agent from *aloe vera*, is also disclosed.

The present invention is based on the recognition that *aloe vera* isolated have been used to treat, and increase the healing rate of, wounds and other infectious diseases. The present invention is also based on the recognition that antibacterial agents secreted by *aloe vera* and the bacteria that grow in the gel and rind of *aloe vera* and which exhibit

a wide range antimicrobial activity when exposed directly to the target microbe can be isolated. The antibacterial agents isolated herein have molecular weights of about: 555,000; 470,000; 240,000; 160,000; 25,000 and 4,000 Daltons.

Also, the antibacterial agents isolated herein, are partially secreted by bacteria that grow in the gel and rind of *Aloe vera*. The secreted products of these species of bacteria exhibit a wide range antimicrobial activity when exposed directly to target microbes and each other and include: *Aerobacterium*, *Bacillus*, *Curtobacterium*, *Arthrobacter*, *Sporosarcina*, and *Clavibacter*.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying FIGURE in which:

FIG. 1 is a taxonomic chart of the DNA fingerprint analysis of bacterial isolates according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts which can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

Competition for nutrients and bacteriocin production play a role in determining the establishment of microbial communities in nature. When analyzing symbiotic associations between plants and bacteria, the presence of chemicals produced by the host as well as the bacteria play a role in the interaction. In the case of *Aloe vera barbedensis*, a tropical or subtropical plant of the genus *Aloe*. These plants are characterized by having lance shaped leaves that contain a viscous clear gel. The leaves are given structural rigidity by hairlike connective fibers. The clear gel of the *aloe vera* is to be distinguished from the thick, mucilaginous yellow juice that occurs about the base of the plant leaves and adjacent the rind of the leaf. This yellow juice, generally known as aloin, has been used for many years as an ingredient in many cathartics and purges.

The therapeutic qualities of the clear gel of *aloe vera* leaves depend to a large extent on the freshness of the gel. For example, the gel of the Aloe plant has been used to stop the pain caused by a jelly fish sting. If the gel has been exposed to air and light for about one and a half hours, however, the ability of the gel to reduce the concomitant immediate type hypersensitivity form of inflammation is greatly reduced.

The Aloe plant has been shown for centuries to exert broad spectrum of disease curing activities, these activities have not been characterized at the molecular biologic level. With the renewed interest in the isolation and characterization of antimicrobial agents, the present inventors recognized that in addition to the ability to promote the healing of animal cells, that Aloe gel may also contain antimicrobial agents.

Aloe Plant Antimicrobial Agents

A screen for antimicrobial activity of the aloe liquid was developed by the present inventors to quantify and qualify the antimicrobial activity of the aloe liquid. A number of strains of bacteria were tested. The following strains of

antimicrobial
screening
method

1. The first step in the process of the investigation is the identification of the problem. This is done by the investigator who is responsible for the study. The investigator must first identify the problem that is being investigated. This is done by the investigator who is responsible for the study. The investigator must first identify the problem that is being investigated.

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A large, dense, black and white photograph of a textured surface, possibly a wall or a piece of fabric, with a complex, organic pattern. The texture is highly irregular, with many small, dark, irregular shapes scattered across a lighter background, creating a mottled or marbled effect. The overall appearance is that of a close-up of a rough, aged, or perhaps painted surface.

1. The first step in the process is to identify the problem. This involves gathering information about the situation and understanding the needs of the stakeholders involved.

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Streptococci pneumoniae were obtained from the American Type Culture Collection: ATCC 51936, ATCC 51937, ATCC 51938, and ATCC 51422. Each of these has been implicated in severe cases of otitis media, and are multi-drug resistant (MDR). Culture of these microorganisms was on Brain Heart Infusion media (BHI) supplemented with 5% sheep red blood cells (SRBC). Incubation was under increased CO₂ conditions to enhance growth.

The organisms were initially grown overnight to log phase. Dilutions were made to obtain cultures of approximately 10⁵ cells/ml. These bacteria were inoculated into the appropriate media to which 90% aloe liquid (Coates Aloe International as well as freshly isolated) was added. The present inventors recognized that the aloe liquid is non-toxic to animals in pure form, be it topical or internal, therefore, studies can be conducted using pure aloe liquid isolated and sterile. Positive and negative controls were used to verify the grown and killing data, as well as the sterility of the components used. Proper sterile technique was used throughout these studies. Inoculations were conducted under a sterile hood or using standard bacteriologic techniques. The final concentration of cells in the aloe liquid cell culture, and control cultures, was 10⁴ cells/ml. Aliquots of the culture were sampled at 0, 6, 12, and 24 hours and plated onto BHI with 5% SRBC, without aloe liquid, to determine the number of viable cells remaining in the culture. The following data were collected (all counts represent the average of triplicate platings):

STRAIN	TIME			
	0 Hrs	6 Hrs	12 Hrs	24 Hrs
ATCC 51936	5 × 10 ⁴	3 × 10 ⁴	1 × 10 ²	0
ATCC 51937	6 × 10 ⁴	4 × 10 ⁴	1 × 10 ²	0
ATCC 51938	5 × 10 ⁴	4 × 10 ⁴	8 × 10 ²	0
ATCC 51422	8 × 10 ⁴	8 × 10 ⁴	7 × 10 ²	0

In addition to broth culture assays, diffusion plates were also used to assess the efficacy of the aloe liquid. BHI plates supplemented with 5% SRBC were swabbed with overnight cultures of one of the strains so that a lawn of growth would result upon overnight incubation. A well was cut into the center of each plate and filled with 100% aloe liquid (Coates Aloe International). The plates were incubated overnight and zones of inhibition measured the following day. Each of the strains of *Streptococcus pneumoniae* exhibited strong susceptibility to aloe liquid as evidenced by rings of inhibition averaging 2 cm in diameter.

The results of the assays of antimicrobial activity on the bacterial strains *Helicobacter pylori* ATCC 49503 and *Streptococcus pneumoniae* ATCC 35088. The assays were run twice in triplicate and the percentages of killing activity compared to control cultures under the same conditions without aloe product added. All cultures contained 5% sheep red blood cells for survival of these bacterial strains and were incubated in atmospheres enriched in CO₂.

% Aloe Product	<i>Helicobacter pylori</i>	<i>Streptococcus pneumoniae</i>
	% Killing	% Killing
90%	33% at 6 hrs 100% at 12 hrs	10% at 6 hrs 80% at 12 hrs 100% 18 hrs
75%	71% at 24 hrs	43% at 24 hrs

-continued

% Aloe Product	<i>Helicobacter pylori</i> % Killing	<i>Streptococcus pneumoniae</i> % Killing
50%	49% at 24 hrs	12% at 24 hrs
25%	34% at 24 hrs	9% at 24 hrs

Source of the Aloe Bactericidal Activity

In order to more closely identify and isolate the source of antimicrobial activity produced and isolated from the Aloe plant, the present inventors have analyzed the population dynamics within the indigenous microflora of *Aloe vera*. Localization and characterization of specific microbial populations was assessed using both direct culture of dissected plant material and immunological detection within tissue sections. Relative size and population diversity were determined through direct culture. In situ hybridization using specific immunological detection techniques demonstrated that discrete populations of bacteria are located within, and are segregate into, specific plant structures.

Most bacterial identification is accomplished using standard staining and biochemical analysis. The identification of bacterial and other microbes in environmental specimens requires attention to the fact that many microorganisms that are closely related members of the same genus or extremely unique organisms may be isolated. Isolation methods are often inadequate providing only broad classification at best. Therefore, in an attempt to clarify the classification of indigenous bacteria from *Aloe vera barbadensis*, Restriction Fragment Length Polymorphism (RFLP) analysis was used to identify variations in the strains of bacteria isolated. RFLP analysis indicated that various species of *Bacillus* are associated with the Aloe plant, and also led to the definitive identification of *Aeromicrobium* species and *Curtobacterium* species.

The efficacy of aloe liquid (prepared according to the Coats process, U.S. Pat. No. 5,356,811, incorporated herein by reference) was used as an antimicrobial agent. A clear aloe vera mixture is a mixture that is substantially aloin free, i.e., the aloin content is less than 1 ppm. Aloe liquid was shown to have antibacterial activity against a wide range of gram negative and gram positive bacteria. The aloe liquid produced by the Coats process, and isolated from whole leaf, was shown to effectively kill: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *E. coli*, *Propionibacterium acne*, *Helicobacter pylori*, and *Salmonella typhi*.

The bactericidal activity of the aloe liquid was not due to the preservatives used in the preparation of this product, as liquid was collected directly from freshly cut whole leaves used in the same killing assays, demonstrated identical results. The inventors recognized that one advantage to the use of aloe liquid as an antimicrobial agent is that it is nontoxic even when taken internally. New antimicrobial agent were identified and isolated from the aloe liquid are efficacious and nontoxic, with broad spectrum properties. Aloe Bactericidal Activity is not from Previously Identified Components

Previously isolated and identified individual aloe products as antimicrobial products were studied to confirm that these compounds were not responsible for the anti-bacterial activity identified herein. Purified extracts of several known Aloe components were provided by Dr. I. Danhoff, including: Albarin, Awbarin, Anthraquinone, Aloin A, Aloin A & B, Aloe emodin, and Yellow sap components were tested in like assays. When used in culture killing assays, only Aloin A

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and Aloe emodin showed any inhibition of bacterial growth, however, neither were comparable to the percent killing achieved using liquid from the whole leaf. Isolation of Novel Aloe Constituents as Antimicrobial Products

Fractionation of fresh aloe liquid from whole leaf was conducted using standard column chromatography on Sephacryl 300. The resulting fractions were collected, peaks combined, the components precipitated to concentrates assayed for antimicrobial activity. Fractions containing molecular weights of approximately 550,000; 470,000; 240,000; 160,000; 25,000; and 4,000 Daltons were found to have varying degrees of bactericidal or bacteriostatic activity.

When these fractions were analyzed using gel electrophoresis, they were found to be mixtures of 1 to 3 components, and are thus considered to be heterogenous mixtures of the same compound whose molecular weight might be affected by glycosylation or association with lipid moieties. Alternatively, the fractions may contain proteins that have undergone varying forms of degradation or cleavage during processing. One the other hand, these fractions might indicate that several different components were isolated.

Chromatographic Separation

A chromatographic separation gel to be used in the procedures of the present invention is a three dimensional network which has a random structure. Molecular sieve gels comprise cross-linked polymers that do not bind or react with the material being analyzed or separated. For gel filtration purposes, the gel material is generally uncharged. The space within the gel is filled with liquid and the liquid phase constitutes the majority of the gel volume. Materials commonly used in gel filtration columns include dextran, agarose and polyacrylamide.

Dextran is a polysaccharide composed of glucose residues and is commercially available under the name Sephadex (Pharmacia Fine Chemicals, Inc.). The beads are prepared with various degrees of cross-linking in order to separate different sized molecules by providing various pore sizes. Alkyl dextran is cross-linked with N,N'-methylenebisacrylamide to form Sephacryl-S300 which allows strong beads to be made that fractionate in larger ranges than Sephadex can achieve.

Polyacrylamide may also be used as a gel filtration medium. Polyacrylamide is a polymer of cross-linked acrylamide prepared with N,N'-methylenebisacrylamide as the cross-linking agent. Polyacrylamide is available in a variety of pore sizes from Bio-Rad Laboratories (USA) to be used for separation of different size particles.

The separation gel material swell in water and in a few organic solvents. Swelling is the process by which the pores become filled with liquid to be used as eluant. As the smaller molecules enter the pores, their progress through the gel is retarded relative to the larger molecules which do not enter the pores. This is the basis of the separation. The beads are available in various degrees of fineness to be used in different applications. The coarser the bead, the faster the flow and the poorer the resolution. Superfine is to be used for maximum resolution, but the flow is very slow. Fine is used for preparative work in large columns which require a faster flow rate. The coarser grades are for large preparations in which resolution is less important than time, or for separation of molecules with a large difference in molecular weights. For a discussion of gel chromatography, see Freifelder, Physical Biochemistry, Second Edition, pages 238-246, incorporated herein by reference.

The most preferred methods of gel filtration for use in the present invention are those using dextran gels, such as

Sephadex, and those using dextran-polyacrylamide gels such as Sephacryl which are able to separate molecules in the 180 to 500 kiloDalton range.

In addition studies, the fractions were compared singly and in various combinations in the bacterial killing assay. The level of killing achieved with whole aloe liquid could only be achieved when all the fractions were combined, indicating that the unconcentrated components together provide for aloe's broad spectrum antibacterial activity.

In order to more closely identify the components responsible for the broad spectrum antimicrobial activity observed for aloe liquid, and having isolated bacteria indigenous to the aloe plant, the present inventors recognized that the bacteria might be the source of some or all of the broad spectrum antimicrobial activity identified herein.

Eight distinct microorganisms were repeatedly been isolated from the rind and gel of the Aloe plant. Population studies have indicated the relative abundance and locale of each of these representatives. Since many antimicrobial substances are known to be produced by other bacteria, the production of such bacteriocins from each aloe isolate was analyzed. Three bacteria produced secreted products that were capable of killing one or more of the following indicator organisms, including: *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Enterococcus* (Streptococcus) *faecalis*.

Using gel electrophoresis, comparison of the secreted products and whole aloe liquid, three bands appear to be shared. Those of molecular weight 470,000; 160,000; and 25,000. Using antibody staining techniques, two of the bacteria displaying antimicrobial activity were localized within the rind of the plant; one at the gel rind interface, the other just below the outer cuticle of the plant, and within the parenchymal tissue. The distinct locations of the bacteria were identified by the present inventors to be in prime locations for the products secreted by the bacteria to enter the veins of the plant and become constituents of the plant liquid along with those of plant origin.

Contributions from these bacteriocins provide part of the answer for the broad spectrum activity of the aloe liquid isolated from the whole leaf. None of the bacteriocins, alone or in combination, were capable of killing all of individually killing the microbes previously shown to be affected. The secreted products of the individual bacteria contribute some of the components identified in the fractionation studies, but not all.

Aloe Plant Defense Proteins Capable of Antimicrobial Activity

As the bacterial secretion products did not fully explain the plants full antimicrobial activity, mixed fractions, rather than clean single component fractions, were studied. Plant defense proteins appear to make up, or be responsible for, the last anti-bacterial components.

Most bacterial identification is accomplished using standard staining and biochemical analysis. In the identification of environmental specimens, where many microorganisms are closely related members of the same genus or extremely unique organisms, these methods are often inadequate providing only broad classifications at best. To further the classification of indigenous bacteria from *Aloe vera* previously isolated, RFLP (Restriction Fragment Length Polymorphism) analysis was initiated. These results have been helpful in differentiating the various species of *Bacillus* found within the plant as well as assisting in the identification of *Aeromicrobium*, *Curtobacterium*, and *Clavibacter* isolates.

Microbiologists have historically relied on cultural and biochemical analysis for the identification of microorgan-

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Journal of Management Studies, 19(6), 701-718.

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isms. While this provides adequate information for classification to the genus and sometimes species level, it lacks the sensitivity required to detect strain variations in many groups of bacteria. Such identifications are of particular importance when dealing with environmental samples where selection pressures favor subtle variations within species. Both morphological and biochemical characters are often strongly influenced by the environment, and thus special procedures are required to distinguish genotypes from phenotypic variations.

Using standard bacteriological procedures, a variety of bacteria were isolated from two different *Aloe vera* plants. These isolates were identified to species level. Representatives of the genus *Clavibacter*, *Curtobacterium*, and *Arthrobacter* (established plant symbiotes) and multiple members of the genus *Bacillus* comprised the majority of the indigenous flora. There are over 100 different species of *Bacillus* established in Bergey's Manual of Systemic Bacteriology (many with minor biochemical variations). *Clavibacter* and *Curtobacterium* were originally placed in the genus *Corynebacterium*, but have been separated based on rRNA patterns. Because the members of these groups are biochemically similar, DNA fingerprinting was used to provide more definitive classification information including DNA polymorphisms.

Materials and Methods

PLANTS. *Aloe vera* plants were obtained from two sources: Coats Aloe International (Mexico) and Calloway Nurseries (Texas). Additional work was also conducted on a plant from Irving, Tex. (original origin unknown) with similar results. The plants were maintained in the Haggerty Science building on the University of Dallas campus. Natural light exposure was obtained by placing the plants in a hallway floor to ceiling window on the east side of the building. Temperatures were maintained around 25° C. Plants were watered weekly or as needed, allowing the soil to dry in between.

MICROBIAL ISOLATION. Leaves were cut at the base near the stalk with a sterile scalpel. They were washed with soap (Dial Antibacterial Soap) to remove surface contaminants, rinsed thoroughly with sterile water followed by a rinse with 70% ethanol, and allowed to air dry under a sterile hood. Gloves were used through out the isolation process to prevent possible contamination from other sources.

The leaves were cut into strips and finely minced in preparation for tissue homogenization. One gram of plant tissue was suspended in 2 mls of sterile saline and homogenized (Dounce vessel). The resultant suspension was plated using serial dilutions onto Tryptic Soy Agar. Plates were incubated at 37° C., and inspected for growth at 24 and 48 hours. Pure cultures were established from the individual, distinctive colonies which grew from the suspension.

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION. Gram stains and acid fast stains were done on 18 hour cultures. The spore stains were done on five day cultures. Standard microbiological media (Difco and BBL) and formulations thereof, were used to determine both morphological and biochemical characteristics. Enterotubes in place of individual tube media for some of the assays. Resulting data was used in the classification of the various isolates using Bergey's Manual of Determinative Bacteriology.

ISOLATION OF DNA. Cultures were grown overnight at 37° C. in 30 ml Tryptic Soy Broth. Cells were collected by centrifugation, resuspended and lysed in 0.2 M glucose with lysozyme (10 ug/ml) and subsequently digested with pro-

teinase K. Proteins, carbohydrates, and lipids were removed from the suspension by phenol:chloroform:Isoamyl Alcohol extraction. DNA was precipitated by addition of cold 95% ethanol at -20° C. The recovered DNA was resuspended in sterile Tris-EDTA buffer and contaminating RNA removed by overnight digestion with RNase. This was followed by a second proteinase K digestion, phenol:chloroform:Isoamyl alcohol extraction and ethanol precipitation. Purified DNA was quantified spectrophotometrically.

DNA FINGERPRINTING. Five to 10 ug of DNA was completely digested with restriction enzymes (Promega) under the recommended conditions. Restriction fragments were separated from each other by electrophoresis on horizontal agarose gels using 1% in Tris:Acetate:EDTA buffer. Hind III digested phage lambda fragments were used as molecular weight markers. Digestion patterns were compared and used as additional classification information.

Morphological and Biochemical Characterization

A total of 20 bacterial cultures isolated from two separate *Aloe vera* plants. Each was assayed using standard biochemical media. Isolates were tentatively placed in a specific genus according to Bergey's Manual of Determinative Bacteriology.

Of the ten isolates from each plant, five from each of the two plants were classified as members of the genus *Bacillus*. These isolates were gram positive spore forming rods which were catalase positive. Minor variations in sugar utilization and exoenzyme production demonstrated a wide variety of species present within the plants. Two isolates which were catalase negative were also classed as members of the *Bacillus* genus due to other biochemical reactions. Catalase positive non-spore forming irregular rods which were oxidase negative represented members of *Curtobacterium*. Those which were oxidase positive isolates represented members of *Clavibacter*, *Arthrobacter*, or *Aeromicrobium*. All bacteria in these groups exhibited pleomorphic morphology, some with distinct rod-cocci cycle. Catalase negative nonendospore formers were placed in the genus *Listeria*. Only a few cocci were isolated; all were gram positive. One sporeformer was isolated from Plant 1 and was classed as *Sporosarcina*. The remaining cocci were catalase positive nonsporeformers and were placed in the genus *Micrococcus*.

DNA Fingerprinting Analysis

FIG. 1 is a taxonomic analysis and chart that was developed using DNA fingerprint analysis and biochemical testing of the isolates. Both gram positive rods and gram positive cocci were identified using gram staining followed by biochemical and DNA fingerprint analysis. The isolates identified included members of the *Bacillus*, *Arthrobacter*, *Curtobacterium*, *Clavibacter*, *Aeromicrobium*, *Sporosarcina* and *Micrococcus* species.

TABLE 3

Characteristics	ISOLATES									
	1AI	3A	3B	3E	4A	4B	5A	5B	6Ai	6Ao
Gram Stain	+	+	+	+	+	+	+	+	+	+
Morphology	R	R	R	R	C	P	R	P	P	R
Spore	+	+	+	-	+	-	-	-	+	+
Catalase	+	+	+	SI	+	+	+	+	+	+
Oxidase	+	-	-	+	-	+	+	-	+	+
Glucose	+	+	+	SI	+	+	+	+	+	+
Lactose	SI	-	SI	-	SI	+	+	-	+	+
Arabinose	+	-	SI	-	+	+	-	-	SI	+

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TABLE 3-continued

Characteristics	BIOCHEMICAL CHARACTERISTICS									
	ISOLATES									
	1AI	3A	3B	3E	4A	4B	5A	5B	6Ai	6Ao
Adonitol	+	-	SI	+	-	+	+	+	+	+
Sorbitol	-	-	+	-	-	-	-	-	SI	-
Lysine	-	-	SI	SI	-	-	+	+	+	+
Ornithine	-	-	SI	SI	-	-	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-
Citrate	+	-	+	-	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+	+	+
VP	-	+	+	-	-	-	-	-	+	+
Nitrate	+	+	+	+	+	+	-	+	+	+
Starch	+	+	+	-	+	+	-	-	+	+
DNAse	+	-	+	+	+	+	+	+	+	+
MSA	-	G	FG	-	G	G	-	G	FG	FG

***R—bacilli,
C—cocci,
P—pleomorphic,
SI—slight reaction,
G—growth,
FG—fermentative growth

Population Dynamics Among Indigenous Microflora Isolated from *Aloe Vera*

Both competition for nutrients and bacteriocin production play a role in determining the establishment of microbial communities. Symbiotic associations may be influenced by the presence of plant defense compounds produced by the host. In the case of *Aloe vera*, the plant has shown to secrete many anthraquinones (produced by a large number of plants), which have been shown to effectively kill microorganisms. Studies were conducted to determine the population dynamics within the indigenous microflora of *Aloe vera*. The localization of specific microbial populations was assessed using both direct culture of dissected plant material and immunological detection within tissue sections. Relative size and population diversity were determined through direct culture while the immunological detection demonstrated discrete populations within specific plant structures.

Isolation revealed ten different microorganisms indigenous to the *Aloe* plant. These studies were concentrated on determining the population dynamics of the ten isolates. Comparative numbers of the microorganisms at different levels within the plant were first determined using standard bacteriological plating techniques. Microbial interactions were examined using spot plates to determine competition and tissue printing to determine the exact location within the plate of each of the microorganisms.

MICROBIAL ISOLATION. Leaves were cut at the base near the stalk with a sterile scalpel. They were then washed with soap (Dial Antibacterial Soap) to remove surface contaminants, rinsed thoroughly with sterile water followed by a rinse with 70% ethanol, and allowed to air dry under a sterile hood. Gloves were used through out the isolation process to prevent possible contamination from other sources.

The leaves were cut into three segments: top, middle, and bottom. Each of these were processed separately. The segments were cut into strips then finely minced in preparation for tissue homogenization. In addition, the rind was separated from the internal 'gel' of the plant for some strips in each segment and processed. One gram of plant tissue was suspended in 2 mls of sterile saline and homogenized (Dounce vessel). The resultant suspensions were plated using serial dilutions onto Tryptic Soy Agar. Plates were incubated at 37° C., and inspected for growth at 24 and 48 hours.

POPULATION ENUMERATION. The number of similar colonies from each of the suspension was counted and recorded.

SPOT PLATES. Overnight cultures of each of the isolates were swabbed onto Tryptic Soy Agar (TSA) plates to produce a lawn of growth. The growth of each of these was challenged by spotting the other symbiotes on top of the lawn and allowing the organisms to grow overnight at 37° C. A clear area around the spot indicated exclusive competition between the two organisms. A zone of inhibition which appeared to have been there initially but had been filled in at a later time was interpreted as a static competition, where increasing population levels over came the initial exclusion.

TISSUE PRINTING. Leaves from the plants were cut and cleaned as for the homogenization procedure. They were then cut into strips approximately 5 mm in length. These were placed onto nitrocellulose filters and pressure was applied to facilitate the transfer of bacteria from the plant tissue onto the filter. The filters were either used immediately or stored dry at -4° C.

ANTIBODY PREPARATION. Cultures of each of the microorganisms were grown up overnight in 10 mls of Tryptic Soy Broth. The cells were pelleted, resuspended in 1 ml of saline, and heated at 70° C. for 1 hour to inactivate the cells. Further inactivation was achieved by addition of formaldehyde to the cells at a concentration of 0.6%. Inactivation was checked by plating both aerobically and anaerobically for 48 hours. No growth indicated complete inactivation.

Lowry protein assays were done to determine the antigen concentration. Two hundred micrograms (μ g) of antigen was administered to Sprague Dawley rats in the presence of complete Freund's adjuvant. The second administration of antigen was fourteen days later in the presence of incomplete Freund's adjuvant. The rats were bled ten days later and the serum isolated. Antibodies were purified using hydroxyapatite.

IMMUNOBLOTTING. The tissue blots were blocked with Phosphate Buffered Saline (PBS) containing skim milk. Primary antibody was applied to the blot for 1 hour. Subsequent washings with PBS removed unbound antibody. Second antibody (anti-rat IgG-peroxidase) was applied for 1 hour. Unbound second antibody was washed off and the peroxidase substrate 4-chloro-1-naphthol was used to stain the blot. Areas of intense blue-black indicated areas of microbial localization.

TABLE 4

POPULATION ENUMERATION											
SECTION/ SITE	MICROORGANISM (per 50 ug tissue)										
	1	2	3	4	5	6	7	8	9	10	Total
Bottom-Whole	1	15	1.5	9	58	1	0	0	0	0	84.5
Bottom-Gel	0	0	0	40	0	0	1	0	300	0	340
Bottom-Rind	2.5	19	1	4	61	0	0	1	0	0	87.5
Mid-Whole	2.5	21	1	2.5	0	3.5	1	0	0	1	1.5
Mid-Gel	0	0	0	41	0	0	0	0	500	0	541
Mid-Rind	6	24	2	1	0	4	1	0	0	1	39
Top-Whole	1.5	13	2.5	0	35	3	1	20	0	1	77
Top-Gel	4	19	1.5	0	0	2.5	0	0	0	5	32
Top-Rind	1	25	2	1	25	15.5	1	21	0	1	95.5

Microbial Key
1 - Aeromicrobium - 3E
2 - Bacillus - 3A

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the 1990s, the number of people in the United States who are 65 years of age or older is projected to increase from 20 million to 30 million, and the number of people 75 years of age or older is projected to increase from 10 million to 15 million (U.S. Census Bureau, 1996). The number of people 85 years of age or older is projected to increase from 2 million to 4 million (U.S. Census Bureau, 1996). The number of people 90 years of age or older is projected to increase from 500,000 to 1 million (U.S. Census Bureau, 1996). The number of people 95 years of age or older is projected to increase from 100,000 to 200,000 (U.S. Census Bureau, 1996). The number of people 100 years of age or older is projected to increase from 10,000 to 20,000 (U.S. Census Bureau, 1996).

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the 1990s, the number of people in the world who are under 15 years of age is expected to increase from 1.1 billion to 1.5 billion. The number of people aged 65 and over is expected to increase from 250 million to 450 million. The number of people aged 15 and over is expected to increase from 3.5 billion to 4.5 billion. The number of people aged 15 and over is expected to increase from 3.5 billion to 4.5 billion. The number of people aged 15 and over is expected to increase from 3.5 billion to 4.5 billion.

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 3. *Scirpus setaceus* (L.) Link.
 4. *Scirpus robustus* (L.) Link.
 5. *Scirpus tabernaemontani* (Cav.) Trin. ex Steud.
 6. *Scirpus torreyana* (L.) Link.
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TABLE 4-continued

<u>POPULATION ENUMERATION</u>											
SECTION/ SITE	MICROORGANISM (per 50 ug tissue)										
	1	2	3	4	5	6	7	8	9	10	Total
3 - Bacillus - 6Ai											
4 - Curtobacterium - 5B											
5 - Arthrobacter - 4B											
6 - Bacillus - 3B											
7 - Bacillus - 1A											
8 - Sporosarcina - 4A											
9 - Clavibacter - 5A											
10 - Bacillus - 6Ao											

TABLE 5

LAWN	MICROBIAL COMPETITION									
	SPOT ISOLATE									
	1A	3A	3B	3E	4A	4B	5A	5B	6Ai	6Ao
1A		+	++	+	++	++	++	-	++	-
3A	-	-	-	-	-/+	-	-/+	-	-	-/+
3B	-	-	-	-	-	-	-	-	-	+/-
3E	-	-	+	-	++	-	++	-	++	-
4A	-	-	-	-	-	-	-	-	-	-
4B	-	-	++	-	++	-	++	-	++	-
5A	-	-	-	-/+	-	-	-	-	-	-
5B	-	-	-	Not Available				-	-	-
6Ai	-	-	-	-	-	-	-	-	-	-
6Ao	-	+/-	++	+/-	++	-	++	-	++	-

Microbial Key

1A - Bacillus
3A - Bacillus
3B - Bacillus
3E - Aeromicrobium
4A - Sporosarcina/Micrococcus
4B - Arthrobacter
5A - Clavibacter
5B - Curtobacterium

6Ai - Bacillus

6Ao - Bacillus

Growth Key

+ = mild inhibition
++ = strong inhibition
-/+ = static inhibition
- = no inhibition

Representatives of all the isolates except Clavibacter were found present within the rind. Clavibacter dominated the gel region. A few Bacillus species, Aeromicrobium and Curtobacterium species were found in low numbers within the gel. The majority of the isolates were associated with or immediately surrounding the tubule containing rind (Table 4). On the surface this result is surprising since the rind is the site of yellow sap, comprised primarily of anthraquinones. These compounds have been classified as plant defense compounds. They are also extremely unstable. Not only are anthraquinones readily inactivated through oxidation, it is conceivable that many of the Bacillus species present within the rind secrete enzymes which might assist their inactivation. One of the characteristics of Bacillus species is the prolific secretion of a wide variety of exoenzymes.

Location in the rind/tubules may also be a protective measure by those microorganisms sensitive to some bacteriocin produced by Clavibacter and thus reflect competitive exclusion between endosymbionts. A review of population numbers reveals that although the variety of microbes is small within the gel filler, the population density of those found in the gel is at least five times more dense than even

the most prominent member of the rind microenvironment (Table 4). Members of the rind population, specifically Aeromicrobium, do show inhibitory activity against the gel isolates (Table 5). These organisms could establish the barrier between the rind and the secretions of the gel allowing the various rind organisms to live undisturbed.

Bacteria are known to produce substances known as bacteriocins which are capable of killing closely related species, a process termed competitive exclusion. The data show that various members of the indigenous microflora do produce bacteriocins setting up regions of exclusive regions within the plant.

Because of the possibility that these bacteriocins interfered with the identification of certain microorganisms within the gel and rind, immunoblotting of tissue prints was chosen as a more specific method for localization of the plant's microflora. Only four microorganisms have successfully been localized using this method. The inability to develop antibodies may be due either to low antigenic properties of the remaining microorganisms or to the low population numbers within the plant itself making it difficult to transfer sufficient numbers required for immunoblots techniques.

Bacillus 1A shows distinctive localization within the inner tubules of the rind while Bacillus 6Ai and 3B appear to inhabit the more exterior regions, even outside of the plant itself in the skin. These results place the sporeforming coccus Sporosarcina (4A) at the interface between the rind and the gel and within the gel itself. These results confirm the population enumeration studies.

A wide range of bacteria are adapted to various microenvironments at the soil and air interface and are important in nutrient uptake, frost damage, and biological control of plant pathogens. There are also those associated with the plant surfaces, both root and aerial. The location of these organisms reflects their ecology. Physiologically some may be extracellular, multiplying within intercellular spaces but not penetrating plant cell walls or entering protoplasts. Others may be able to penetrate the higher plant cell. These associations ultimately lead to symbiotic interactions: commensalistic, mutualistic, or in some instances parasitic. Parasitic conditions, however, are considered to be rare with respect to plant-bacteria associations. The internal environment of healthy plants is not conducive to bacterial growth in general, however, the acquisition and development of cell wall degrading enzymes and toxins leading to disease. Alternatively, the secretion of compounds with the ability to inhibit plant defense compounds may assist in the establishment of certain symbioses.

Aqueous compositions (inocula) of the bacteriocins isolated from the Aloe plant as described herein, comprise an effective amount of the antimicrobial agent dissolved or dispersed in a pharmaceutically acceptable aqueous medium. As used herein the terms "contact", "contacted", and "contacting", are used to describe the process by which an effective amount of a pharmacological agent, e.g., pure or dilute aloe gel, comes in direct juxtaposition with the target cell. The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human, such as the gel isolated for Aloe vera as described herein.

The preparation of an aqueous composition that contains a protein or proteoglycan, such as the active components derived from the bacteria and aloe plant, is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid

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forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

Proteoglycans, for example, can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like.

For parenteral administration in an aqueous solution, for example, the aloe liquid or gel can be used directly without any toxic effects to the animal. Alternatively, the aloe solution and the antimicrobial agents identified herein, can be dissolved or resuspended, in a suitable buffer, if necessary. Liquid diluents can first be rendered isotonic with sufficient saline or glucose.

To kill a cell in accordance with the present invention, one would generally contact the cell with pure or diluted aloe gel in a combined amount effective to kill the microbial cell. The term "in a combined amount effective to kill the cell" means that the amount of bacteriocins are sufficient so that, when combined within the cell, the cell is induced to undergo apoptosis or another form of cell death. Although not required in all embodiments, the combined effective amount of the isolated aloe gel compounds will preferably be an amount that induces significantly more cell death than the use of either element alone, and most preferably, the combined effective amount will be an amount that induces synergistic cell death in comparison to the effects observed using either element alone.

While these particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration, the aloe solution of the present invention can be administered directly at full concentration. In this connection, sterile aqueous procedures to produce aloe gel can be employed, as will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

While this invention has been described in reference to illustrative embodiments, this description is not intended to be construed in a limiting sense. Various modifications and combinations of the illustrative embodiments, as well as other embodiments of the invention, will be apparent to persons skilled in the art upon reference to the description.

It is therefore intended that the appended claims encompass any such modifications or embodiments.

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 - Harlow E and Lane D. 1988. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press.
 - Holt C A. 1992. Detection and localization of plant pathogens, *Tissue Printing*. Reid PD, et al, editors. Academic Press.
 - Reid P D. 1992. Physical Tissue Printing. In: *Tissue Printing*, Reid P D, et al, editors. Academic Press.
- What is claimed is:
1. A composition derived from a clear *aloe vera* mixture which has less than 1 ppm aloin content and which has been prepared from *aloe vera* gel, consisting essentially of a fraction obtained by gel filtration of said clear mixture, wherein the fraction has an average molecular weight of approximately 550,000 Daltons and has bactericidal or bacteriostatic activity.
 2. A composition derived from a clear *aloe vera* mixture which has less than 1 ppm aloin content and which has been prepared from *aloe vera* gel, consisting essentially of a fraction obtained by gel filtration of said clear mixture, wherein the fraction has an average molecular weight of approximately 470,000 Daltons and has bactericidal or bacteriostatic activity.
 3. A composition derived from a clear *aloe vera* mixture which has less than 1 ppm aloin content and which has been prepared from *aloe vera* gel, consisting essentially of a fraction obtained by gel filtration of said clear mixture, wherein the fraction has an average molecular weight of approximately 240,000 Daltons and has bactericidal or bacteriostatic activity.
 4. A composition derived from a clear *aloe vera* mixture which has less than 1 ppm aloin content and which has been prepared from *aloe vera* gel, consisting essentially of a fraction obtained by gel filtration of said clear mixture, wherein the fraction has an average molecular weight of approximately 160,000 Daltons and has bactericidal or bacteriostatic activity.
 5. A composition derived from a clear *aloe vera* mixture which has less than 1 ppm aloin content and which has been prepared from *aloe vera* gel, consisting essentially of a fraction obtained by gel filtration of said clear mixture, wherein the fraction has an average molecular weight of approximately 25,000 Daltons and has bactericidal or bacteriostatic activity.
 6. A composition derived from a clear *aloe vera* mixture which has less than 1 ppm aloin content and which has been prepared from *aloe vera* gel, consisting essentially of a fraction obtained by gel filtration of said clear mixture, wherein the fraction has an average molecular weight of approximately 4,000 Daltons and has bactericidal or bacteriostatic activity.
 7. A method of decreasing the growth of a bacteria comprising: preparing a composition derived from a clear *aloe vera* mixture which has less than 1 ppm aloin content and which has been prepared from *aloe vera* gel, consisting essentially of one fraction obtained by gel filtration of said clear mixture, wherein the fraction has an average molecular weight of either approximately 550,000 or 470,000 or 160,000 or 25,000 or 4,000 Daltons and contacting said bacteria with said fraction.

* * * * *

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1. The first step in the process is to identify the problem. This involves gathering information about the situation and understanding the needs of the stakeholders involved.

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1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

2. Once the problem is identified, the next step is to define the objectives and goals of the project. This helps to clarify what needs to be achieved and provides a clear direction for the team.

3. The third step is to develop a plan or strategy to address the problem. This involves breaking down the problem into smaller, manageable tasks and determining the resources needed to complete each task.

4. The fourth step is to implement the plan. This involves putting the strategy into action and monitoring progress regularly to ensure that the project is on track.

5. Finally, the fifth step is to evaluate the results of the project. This involves assessing the outcomes against the objectives and goals and identifying any areas for improvement or further action.

[illegible][illegible]

the 1990s, the number of people in the world who are under 15 years of age is expected to increase from 1.1 billion to 1.5 billion. The number of people aged 65 and over is expected to increase from 200 million to 400 million. The number of people aged 15 and over is expected to increase from 3.5 billion to 4.5 billion. The number of people aged 15 and over is expected to increase from 3.5 billion to 4.5 billion. The number of people aged 15 and over is expected to increase from 3.5 billion to 4.5 billion.

1. The first step in the process is to identify the problem. This involves gathering information about the situation and understanding the needs of the stakeholders involved.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

2020
Bogotá, D.C.

Abogado
MARIBEL HERNANDEZ QUINTERO

ASUNTO	Radicación	06-005321
	Trámite	011
	Evento	378
	Actuación	430
	Oficio	6274
	Folios	019

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☒	Cap. I	☒
No. Sol. Internacional		PCT/US03/24598	Cap. II
Fecha de Presentación Internal.		06-08-2003	☐

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción:	Folios 22-37 y 42 como se radicó inicialmente
Reivindicaciones:	Folios 37-40 como se radicó inicialmente
Prioridad:	Folio 1 21-07-2003, US, 10/623.816

2. Objeto de la invención

Título de la solicitud: DESINFECTANTE DE MUCOSA NO TOXICO QUE CONTIENE ALCHOLO ISOPROPILICO, ACEITE DE AJONJOLÍ, SÁBILA Y ACEITE DE LIMON (folio 1).

El problema técnico planteado en esta solicitud está relacionado con la necesidad de proporcionar una composición tópica desinfectante de la mucosa de la nariz no toxica, que destruya efectivamente los organismos patógenos eliminados durante la exhalación, estornudo y/o tos, que no se evapore rápidamente ni interfiera con la identificación sensorial de olores y tenga un mayor tiempo de acción que las composiciones existentes (folio 24).

Para solucionar este problema técnico la solicitud en estudio proporciona en: Reivindicaciones 1-17: una composición tópica desinfectante de mucosa que comprende alcohol isopropílico (por lo menos 50% por peso), aceite de ajonjolí (no excede el 45% por peso), aceite o bálsamo de limón (no excede el 3% por peso) y sábila o derivado de sábila (no excede el 10% por peso).

Clasificación internacional de Patentes (IPC): A61K35/78, A61K9/08, A61P31/02.

3. De la solicitud de Patente

3.1. Descripción

No es claro a que se refieren las letras "ok" (folio 26, lín. 12; folio 32, lín. 17 y 19), es necesario revisar, corregir y cambiar las respectivas páginas.

No es claro cuáles son las composiciones específicas de las muestras 1, 2 y 3 mencionadas en el estudio clínico (folios 32-36).

Por lo anterior, el capítulo descriptivo no cumple con el requisito de tener claridad, de acuerdo con las exigencias de los artículos 30 de la Decisión 486.

3.2. Reivindicaciones (artículo 30 D 486)

- ◇ Existen términos ambiguos que no permiten dar claridad y concisión como "de por lo menos, que no excede, aproximadamente, por lo menos, varía entre cerca de", los cuales deben ser eliminados o cambiados por rangos concretos.
- ◇ No es clara ni concisa la expresión "un componente de sábila o derivado de sábila", ya que no permite reconocer una interpretación concreta de cuál es el componente de sábila ni cuál es el derivado de sábila al que se refiere.
- ◇ Las reivindicaciones 6 y 7 dependientes de la 1 caracterizan una composición por tener extracto de semilla de toronja y/o gluconato de clorhexidina entre sus componentes, pero la reivindicación 4 es la que establece estos dos componentes como adicionales, por lo que la dependencia debe ser de la 4.
- ◇ La reivindicación 9 caracteriza inapropiadamente una composición por un resultado a obtener (un tiempo dado de vida de tratamiento efectiva y de vida útil); una composición debe caracterizarse sólo por los elementos o ingredientes que la comprenden y sus proporciones.
- ◇ Las reivindicaciones 10-17 caracterizan una composición por los mismos elementos que los de la composición de las reivindicaciones 1-7 y 9, siendo de esta manera un duplicado de la materia solicitada en las reivindicaciones 1-7 y 9, por lo que deben ser eliminadas.

Por lo anterior, el capítulo reivindicatorio no cumple con los requisitos de tener claridad y concisión de acuerdo con las exigencias de los artículos 30 de la Decisión 486.

4. Determinación del Estado de la Técnica

Considerando que la fecha de presentación de la primera solicitud de patente de la que se reivindica prioridad es 21-07-2003, se buscaron documentos del estado de la técnica relacionados, con fecha de publicación anterior a ésta.

1. The first part of the report deals with the general situation of the country and the results of the work done during the year.

2. The second part of the report deals with the results of the work done during the year.

3. The third part of the report deals with the results of the work done during the year.

4. The fourth part of the report deals with the results of the work done during the year.

5. The fifth part of the report deals with the results of the work done during the year.

6. The sixth part of the report deals with the results of the work done during the year.

7. The seventh part of the report deals with the results of the work done during the year.

8. The eighth part of the report deals with the results of the work done during the year.

9. The ninth part of the report deals with the results of the work done during the year.

10. The tenth part of the report deals with the results of the work done during the year.

11. The eleventh part of the report deals with the results of the work done during the year.

Nº	Documento	Título	Fecha de publicación
D1	US6296882	Non-toxic mucosal disinfectant containing isopropyl alcohol, sesame oil, and lemon oil	02-10-2001
D2	US6290964	Antimicrobial agents isolated from aloe vera	18-09-2001

D1 en folios 63-67, D2 en folios 68-77.

5. Análisis de patentabilidad (artículo 14 D486)

5.1 Nivel inventivo (artículo 18 D486)

Se solicita en las reivindicaciones 1-17 una composición tópica desinfectante de mucosa nasal como se presenta a continuación:

SOLICITUD	D1	D2
Composición tópica desinfectante de mucosa nasal	Composición tópica desinfectante de mucosa nasal (folio 65; col. 4)	Composición desinfectante (folios 76-77; col. 14-15)
Alcohol isopropílico por lo menos 50%	Alcohol isopropílico (50-75)%	---
Aceite de ajonjolí no excede el 45%	Aceite de ajonjolí (24,5-49,5)%	----
Aceite o bálsamo de limón no excede el 3% por peso	Aceite de limón (0,1-0,5)%	---
Sábila o derivado de sábila no excede el 10%	----	Gel de aloe vera (extraído de las hojas de sábila folio 71; col 3, lln. 51) o fracciones o compuestos aislados de la planta de aloe (folio 76; col. 14)
EFECTO: desinfectante no toxico, que destruye diversos organismo patógenos, no se evapora rápidamente y no interfiere con la identificación sensorial de olores (folio 24) Destruye gérmenes por lo menos por 8 horas (folios 24, 36,37)	desinfectante no toxico, que destruye diversos organismo patógenos, no se evapora rápidamente y no interfiere con la identificación sensorial de olores (folio 65; col. 3). Destruye gérmenes por cerca de 4-6 horas (folios 66; col 5-6).	Desinfectante no toxico de amplio espectro de actividad antibacterial, adicional a su habilidad para aumentar la velocidad de curación de heridas y otras enfermedades infecciosas (folio 71; col. 3-4). Destruye gérmenes incluso hasta las 24 horas usando solución de gel aloe al 25% (folio 72; col 5-6).

En el estado de la técnica se encuentra D1 que divulga una composición tópica desinfectante de mucosa nasal que contiene alcohol isopropílico, aceite de ajonjolí y aceite de limón como se enseña en la tabla comparativa, que no es toxica, destruye diversos organismos patógenos (por cerca de 4-6 horas), no se evapora rápidamente y no interfiere con la identificación de olores (folios 64-66; col. 3-6).

La solicitud se diferencia de D1 en que contiene sábila o un derivado de sábila.

El Efecto Técnico que logra la diferencia es aumentar a 8 horas el tiempo de destrucción de gérmenes de la composición desinfectante.

El Problema Técnico Objetivo de esta solicitud es entonces, aumentar el tiempo de destrucción de gérmenes de la composición desinfectante.

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No obstante D2 señala que composiciones que contienen gel de aloe vera (o compuestos o fracciones aisladas de la planta de aloe vera), tienen un amplio espectro de acción antibacterial, además de su habilidad reconocida para aumentar la velocidad de curación de heridas y otras enfermedades infecciosas, destruyendo gérmenes incluso hasta las 24 horas con una solución al 25% de gel de aloe vera (folios 71-72, 76-77; col. 3-6, 14-15).

De manera tal, que un experto en la materia estaría motivado a combinar, en una sola composición, las composiciones de D1 y D2 que comúnmente son usados en composiciones separadas para un mismo propósito (destrucción de organismos patógenos) con la expectativa de que la composición resultante sea útil para el mismo propósito, mejore el espectro de acción al incluir otro desinfectante de amplio espectro antibacterial (gel de aloe vera) y aumente el tiempo de destrucción de gérmenes gracias a la inclusión del gel de aloe vera (que presenta destrucción de gérmenes incluso hasta las 24 horas); por lo que la composición reivindicada no tiene nivel inventivo.

6. Conclusión

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	US6296882	1-17	Nivel inventivo
D2	US6290964	1-17	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2 literal e) del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha
15 JUN. 2009

CONCEPTO TECNICO No. 1422	EXAMINADOR TECNICO Código No. 202079
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UNITED STATES DEPARTMENT OF JUSTICE
FEDERAL BUREAU OF INVESTIGATION
WASHINGTON, D.C. 20535

MEMORANDUM FOR THE DIRECTOR, FBI
SUBJECT: [Illegible]

[The following text is extremely faint and largely illegible due to poor scan quality. It appears to be a memorandum detailing an investigation or report.]

[Illegible text continues...]

Very truly yours,
[Illegible Signature]
Special Agent in Charge

DATED: [Illegible]
AT: [Illegible]

[illegible]

CONFIDENTIAL

1. The first step is to identify the problem or question that needs to be answered. This involves understanding the context and the specific requirements of the task.

100-443881-100

1. The first part of the document is a list of references. The references are listed in a standard format, with the author's name, the title of the work, and the publisher. The references are as follows:

1. J. H. Van Veen, *The History of the Netherlands*, 1910, 1911, 1912, 1913, 1914, 1915, 1916, 1917, 1918, 1919, 1920, 1921, 1922, 1923, 1924, 1925, 1926, 1927, 1928, 1929, 1930, 1931, 1932, 1933, 1934, 1935, 1936, 1937, 1938, 1939, 1940, 1941, 1942, 1943, 1944, 1945, 1946, 1947, 1948, 1949, 1950, 1951, 1952, 1953, 1954, 1955, 1956, 1957, 1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 2440, 2441, 2442, 2443, 2444, 2445, 2446, 2447, 2448, 2449, 2450, 2451, 2452, 2453, 2454, 2455, 2456, 2457, 2458, 2459, 2460, 2461, 2462, 2463, 2464, 2465, 2466, 2467, 2468, 2469, 2470, 2471, 2472, 2473, 2474, 2475, 2476, 2477, 2478, 2479, 2480, 2481, 2482, 2483, 2484, 2485, 2486, 2487, 2488, 2489, 2490, 2491, 2492, 2493, 2494, 2495, 2496, 2497, 2498, 2499, 2500, 2501, 2502, 2503, 2504, 2505, 2506, 2507, 2508, 2509, 2510, 2511, 2512, 2513, 2514, 2515, 2516, 2517, 2518, 2519, 2520, 2521, 2522, 2523, 2524, 2525, 2526, 2527, 2528, 2529, 2530, 2531, 2532, 2533, 2534, 2535, 2536, 2537, 2538, 2539, 2540, 2541, 2542, 2543, 2544, 2545, 2546, 2547, 2548, 2549, 2550, 2551, 2552, 2553, 2554, 2555, 2556, 2557, 2558, 2559, 2560, 2561, 2562, 2563, 2564, 2565, 2566, 2567, 2568, 2569, 2570, 2571, 2572, 2573, 2574, 2575, 2576, 2577, 2578,

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CONFIDENTIAL

1. The first step is to identify the problem. This involves understanding the current situation and the goals that need to be achieved.

1. The first step is to identify the problem or question that needs to be answered. This involves understanding the context and the specific information required.

the 1990s, the number of people in the United States who are 65 years of age or older is projected to increase from 20 million to 30 million, and the number of people 75 years of age or older is projected to increase from 10 million to 15 million (U.S. Census Bureau, 1997).

SECRET

(The following information was obtained from the records maintained by the Bureau of Census, U.S. Department of Commerce, Office of Statistics.)

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10-10-68
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