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Abogado(a)/Señor(a)
LUZ CLEMENCIA DE PAEZ

Asunto:	Radicación:	13-115403- 4
	Trámite:	11
	Evento:	378
	Actuación:	519
	Oficio:	6996
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Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/IB2011/054927				
Fecha de Presentación Internacional	04/11/2011				

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 comunica:

Descripción:	Radicado No 13-115403-00000-0000	8/Mayo/2013
Reivindicaciones:	Radicado No 13-115403-00000-0000	8/Mayo/2013
Prioridad:	FR 1059195	8/Noviembre/2010

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el

presente caso, se tiene en cuenta la totalidad del capítulo reivindicatorio (Radicado No 13-115403-00000-0000) para el examen de patentabilidad.

Título de la solicitud: “Composiciones fluidas aptas para formar un revestimiento que presenta unas propiedades antivíricas”.

El problema planteado en esta solicitud consiste en la necesidad de neutralizar en un plazo corto de tiempo cualquier virus en contacto con un objeto o sustrato dedicado a un uso múltiple.

Para resolver este problema técnico la solicitud en estudio proporciona una composición fluida apta para formar un revestimiento.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A01N 25/04, 37/02, 63/02, 65/00, 65/24; A01P 1/00, 15/00; C09D 5/14, 11/00.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

Las reivindicaciones 15 – 21 se definen por referencia al uso de la composición de las reivindicaciones 1 – 14, al ser aplicada sobre una superficie de un soporte flexible o másico, de manera que no son patentables, de acuerdo con el Art 14.

5. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 – 14 carecen de concisión porque utilizan las expresiones “al menos un viricida de origen natural”, “aceites esenciales que presentan una actividad antivírica”, “uno de sus precursores”, “un biocida bacteriostático y/o bactericida o un biocida fungistático y/o fúngico”, “un agente humectante”, “un poliol”, “un agente ligante”, “un pigmento”, lo cual dificulta determinar la extensión del objeto que se busca proteger. Se recuerda a la solicitante que una composición se caracteriza por sus componentes y proporciones dentro de la misma, de manera que se requiere definir cada componente de la composición fluida por sus características esenciales y sus proporciones dentro de la composición.

La reivindicación 3 no es clara porque utiliza la expresión “(...) dicho viricida es activo frente a virus patógenos respecto a mamíferos y más particularmente al ser humano”, que es un resultado a alcanzar y no define al viricida, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se requiere reemplazarlo por las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto.

La reivindicación 20 no es clara porque define el objeto por referencia al uso de un procedimiento de revestimiento de la superficie con una composición de las reivindicaciones 1 – 14. Se requiere a la solicitante definir el objeto por sus características técnicas esenciales.

Se requiere al solicitante hacer las aclaraciones necesarias.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: noviembre 8 de 2010 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	US 5,968,538	“Anti-bacterial/Anti-viral coatings, coating process and parameters thereof”	19/Octubre/1999

D2	Alternative & Complementary Therapies, Vol. 12	"A review of monolaurin and lauric acid"	11/Diciembre/2006
D3	Toxicology Mechanisms and Methods, Vol. 15, Págs. 279-285	"Effects of essential oils and monolaurin on Staphylococcus aureus: <i>in vitro</i> and <i>in vivo</i> studies"	2005

D1¹ divulga una composición fluida apta para formar un revestimiento, caracterizada porque contiene una cantidad eficaz de al menos un viricida (Resumen y Col. 29, reivindicación 1).

D2² enseña que la monolaurina tiene efectos bactericidas, virucidas y antifúngicos (Resumen).

D3³ divulga que los ácidos aromáticos volátiles y los ácidos grados de cadena media tienen propiedades antimicrobianas (Resumen).

7 . Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 1 consiste en una composición fluida apta para formar un revestimiento, caracterizada porque contiene una cantidad eficaz de al menos un viricida de origen natural seleccionado entre el ácido láurico, la mono laurina, la lactoferrina y los aceites esenciales que presentan una actividad antivírica y/o uno de sus precursores, poseyendo dicha composición una viscosidad comprendida entre 30 mPa.s y 40 Pa.s, a temperatura y presión ambiente.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2	D3
Una composición fluida apta para formar un revestimiento, caracterizada porque contiene una cantidad eficaz de al menos un viricida de origen natural seleccionado entre el ácido láurico, la mono laurina, la lactoferrina y los aceites esenciales que presentan una actividad antivírica y/o uno de sus precursores, poseyendo dicha composición una viscosidad comprendida entre 30 mPa.s y 40 Pa.s, a temperatura y presión ambiente.	Una composición fluida apta para formar un revestimiento, caracterizada porque contiene una cantidad eficaz de al menos un viricida, poseyendo dicha composición una viscosidad comprendida entre 90 y 130 mPa.s. (Resumen y Col. 29, reivindicación 1)	La monolaurina tiene efectos bactericidas, virucidas y antifúngicos. (Resumen)	Los ácidos aromáticos volátiles y los ácidos grados de cadena media tienen propiedades antimicrobianas. (Resumen)

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque enseña una composición fluida apta para formar un revestimiento, caracterizada porque contiene una cantidad eficaz de al menos un viricida, poseyendo dicha

1 [http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=5968538A&KC=A&FT=D&ND=&date=19991019&DB=&locale=en_EP)
CC=US&NR=5968538A&KC=A&FT=D&ND=&date=19991019&DB=&locale=en_EP

2 <http://online.liebertpub.com/doi/abs/10.1089/act.2006.12.310?journalCode=act>

3 <http://informahealthcare.com/doi/abs/10.1080/15376520590968833>

composición una viscosidad comprendida entre 90 y 130 mPa.s (Resumen y Col. 29, reivindicación 1). El documento D1 también divulga que el viricida corresponde a PVP-I y nonoxinol-9 (Resumen), el cual se encuentra en una concentración de 1-12% (Col. 9, líneas 28-30) y comprende un agente humectante como polietilenglicol (Col. 10, línea 1), un agente ligante como ceras (Col. 18, líneas 40-42) y que se presenta en forma de tinta o barniz que comprende pigmentos (Col. 16, línea 37 a Col. 17, línea 40). El documento D1 divulga un proceso para la producción de sustratos recubiertos con la composición que contiene el componente anti-patogénico, de manera que luego del contacto del sustrato recubierto con un material que contiene el patógeno, el componente anti-patogénico es liberado para eliminar el patógeno (Col. 2, líneas 25-30).

La diferencia entre la invención, definida en la reivindicación 1 y la composición del documento D1 consiste en que el viricida de origen natural es seleccionado entre el ácido láurico, la mono laurina, la lactoferrina y los aceites esenciales que presentan una actividad antivírica y/o uno de sus precursores.

Estas diferencias no parecen conceder un efecto técnico inesperado o una ventaja a la composición reclamada porque no presenta una mayor actividad viricida, según lo establecido en la descripción.

El problema técnico objetivo de esta solicitud consiste en la necesidad de una composición fluida de un viricida apta para formar un revestimiento alternativa a la ya conocida en el documento D1.

Teniendo en cuenta que el documento D1 ya divulgaba una composición fluida apta para formar un revestimiento que comprende un agente como PVP-I y nonoxinol-9 con actividad viricida, y que los documentos D2 (Resumen) y D3 (Resumen) enseñan sobre las propiedades bactericidas, virucidas y antifúngicas de compuestos naturales como la monolaurina y los aceites esenciales, la persona del oficio medianamente versada en la materia habría inferido sin mucho esfuerzo de su parte, preparar composiciones fluidas aptas para revestimiento como las reclamadas en la presente solicitud.

De manera que la solución propuesta por esta solicitud en la reivindicación 1 y en las reivindicaciones 2 – 14 es obvia y no tiene nivel inventivo.

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US 5,968,538	1 – 14	Nivel inventivo
D2	Alternative & Complementary Therapies, Vol. 12	1 – 14	Nivel inventivo
D3	Toxicology Mechanisms and Methods, Vol. 15, Págs. 279-285	1 – 14	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 (60) días contados a partir del día siguiente 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.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-06-17

Elaboró: Emilse Cano Urrego
Revisó: Gloria Jacqueline Alfonso Roza

A Review of Monolaurin and Lauric Acid

Natural Virucidal and Bactericidal Agents

**Shari Lieberman, Ph.D., C.N.S., F.A.C.N.,
Mary G. Enig, Ph.D., C.N.S., M.A.C.N., and
Harry G. Preuss, M.D., C.N.S., M.A.C.N.**

Antibiotic resistance has become a worldwide problem for treating infections caused by numerous organisms. Safe, effective antimicrobials that are not easily subject to resistance are desperately needed. The Food and Drug Administration (FDA) states on its website: "Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem. Tuberculosis, gonorrhea, malaria, and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotic drugs."¹ The FDA website also notes:

- Though food-producing animals are given antibiotic drugs for important therapeutic, disease prevention, or production reasons, these drugs can cause microbes to become resistant to drugs used to treat human illness, ultimately making some human sicknesses harder to treat.
- About 70 percent of bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used to treat infections.
- Some organisms are resistant to all approved antibiotics and must be treated with experimental and potentially toxic drugs.
- Some research has shown that antibiotics are given to patients more often than guidelines set by federal and other health care organizations recommend. For example, patients sometimes ask their doctors for antibiotics for a cold, cough, or the flu, all of which are viral and do not respond to antibiotics. Also, patients who are prescribed antibiotics but do not take the full dosing regimen can contribute to resistance.
- Unless antibiotic resistance problems are detected as they emerge, and actions are taken to contain them, the world could be faced with previously treatable diseases that have again become untreatable, as in the days before antibiotics were developed.

Not only bacteria develop antibiotic resistance—viruses develop resistance as well. Accordingly, the need for safe, effective antivirals is also becoming paramount. For example, virucidal resistance has occurred in *Herpes simplex* virus type 1 as a result of acyclovir use.²

In a site-directed study of mutagenesis, researchers investigated the role of six mutations of *Herpes simplex* virus type 1 thymidine kinase (TK) gene in the acquisition of resistance to acyclovir (ACV).² TK activity was not impaired by substitutions located at codons 17, 161, and 374, and these mutations were thus related to TK gene polymorphism. Mutations His105Pro, Leu364Pro, and Asp162Ala lead to the loss of TK activity that could result in ACV-resistance. However, there are very limited antiviral treatments in conventional medicine against the vast majority of viruses that infect humans.

Monolaurin

Monolaurin, a monoester formed from lauric acid (medium-chain fatty acids), has profound antiviral and antibacterial activity.

Recognition of the antimicrobial activity of the monoglyceride of lauric acid (monolaurin) has been reported since 1966. A large body of the research can be credited to Jon J. Kabara, B.S., M.S., Ph.D., a professor emeritus of Michigan State University in East Lansing.³ His early pioneer work focused on the virucidal effects of monolaurin (Lauricidin,[®] Med-Chem Laboratories, Inc., Galena, Illinois) on enveloped RNA and DNA viruses. This work continues to be investigated by numerous researchers because of the potential benefits related to food preservation.

A recent study that investigated the sanitizing effects of monolaurin in a laboratory scale system, partially reproduced dairy plant conditions. The study focused on the effectiveness of chlorine and alternative sanitizers for reducing the number of viable bacteria attached to stainless-steel surfaces.⁴

Bacteria-contaminated stainless-steel tubes in a continuous-flow reactor were exposed to a standard clean-in-place regimen (water rinse, 1 percent sodium hydroxide at 70°C for 10 minutes, a second water rinse, 0.8 percent nitric acid at 70°C for 10 minutes, and a third water rinse) followed by exposure to either chlorine (200 ppm) or combinations of nisin (500 ppm), Lauricidin (100 ppm), and the lactoperoxidase system (LPS) (200 ppm) for 10 minutes or 2, 4, 8, 18, or 24 hours.⁴

There was significant variation in the effectiveness of the alkaline-acid wash steps in reducing cell numbers (log reduction between 0 and 2). Following a 10-minute treatment, none of the

sanitizers significantly reduced the number of attached cells. Two (2) hours of exposure to chlorine, nisin + the LPS, or Lauricidin + the LPS achieved 2.8, 2.2, and 1.6 log reductions, respectively. Exposure times > 2 hours did not decrease the number of viable bacteria attached to the stainless steel further. The effectiveness of combinations of nisin, Lauricidin, and the LPS was similar to that of chlorine ($P > 0.05$); thus, according to these study results these sanitizers could be used to decontaminate the surfaces of small-volume or critical hard-to-clean milk-processing equipment.⁴ (Additional studies of monolaurin's antibacterial effects are discussed in the section entitled Bactericidal Effects.)

Monolaurin Chemistry in the Human Body

Coconut oil and certain coconut products contain approximately 50 percent lauric acid and approximately 6–7 percent capric acid. The question arises frequently whether ingestion of lauric acid can lead to significant endogenous production of monolaurin. There do not appear to be any clear data concerning how much monolaurin is actually formed from lauric acid in the human body. Nevertheless, there is evidence that some is formed. Lauric acid is the main antiviral and antibacterial substance found in human breast milk.

Comparatively speaking, lauric acid (C12) has a greater antiviral and antibacterial activity than other medium-chain triglycerides such as caprylic acid (C8), capric acid (C10), or myristic acid (C14). Monolaurin is many times more biologically active than lauric acid in killing viruses and bacteria, leading to the interesting question concerning the conversion rate in the human body. Unlike these medium-chain fatty acids, diglycerides and triglycerides are inactive against microorganisms.⁵

Research has suggested that monolaurin exerts virucidal and bactericidal effects by solubilizing the lipids and phospholipids in the envelope of the pathogen causing the disintegration of its envelope. Recent evidence has also indicated that the antimicrobial effect is related to its interference with signal transduction in cell replication.⁶

Bactericidal Effects

Other studies, in addition to the abovementioned one with the milk-processing equipment, indicate that monolaurin has antibacterial activity. This monoester has been shown to be effective against both susceptible and antibiotic-resistant *Staphylococcus aureus*.⁷

Generally, monolaurin is more effective against gram-positive bacteria such as staphylococcus and streptococcus. In a study conducted by Preuss et al., monolaurin was shown to be bactericidal to *S. aureus* and *Mycobacterium terrae* but not *Escherichia coli* and *Klebsiella pneumoniae*, which are both gram-negative, confirming prior work on monolaurin, and was shown to be static to a variant of the virulent anthrax pathogen, *Bacillus anthracis* Sterne.⁸

Monolaurin also inhibits production of staphylococcal toxic shock toxin-1 effectively.⁶ The monoester is effective against cytomegalovirus and the expression of virulence factors includ-

Table 1. Lipid-Coated Bacteria Inactivated by Monolaurin

- *Listeria monocytogenes*
- *Helicobacter pylori* (gram-negative)
- *Hemophilus influenzae* (gram-negative)
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- Groups A, B, F, and G streptococci
- Gram-positive organisms
- Gram-negative organisms if pretreated with a chelator

Sources: Refs. 14 and 17.

ing protein A, alpha-hemolysin, B-lactamase, and the induction of vancomycin resistance in *Enterococcus faecalis*.^{8–10} Monolaurin has also been shown to inactivate *Listeria monocytogenes*, *Streptococcus agalactiae*, and Groups A, F, and G streptococci.^{10–12} (See Table 1.)

Unlike conventional antibiotics, monolaurin does not appear to have an adverse effect on gut probacteria.

Antifungal Effects

In addition, a number of fungi, yeasts, and protozoa are reported to be inactivated or killed by monolaurin. These fungi include several species of ringworm. *Candida albicans* and the protozoan parasite *Giardia lamblia* were both reported to be killed by monolaurin.^{11–14}

Virucidal Effects

Some of the viruses inactivated to some extent by monolaurin include HIV, measles, *Herpes simplex-1*, vesicular stomatitis, visna virus, and cytomegalovirus.¹⁴ Results of an extremely promising trial with HIV-positive male and female patients compared the effectiveness of monolaurin at two doses (2.4 g versus 7.2 g) with 50 mL of coconut oil in 15 patients.¹⁵ The objective was to document if the patients' viral loads could be lowered by any of these treatments.

The patients were divided into 3 groups of 5 patients. They were seen daily with laboratory values determined at the beginning of the study and after 3 months and 6 months. At the onset of the trial the viral load of the patients measured by PCR, ranged from 1.96×10^3 to 1190.0×10^3 copies.

One (1) male had a viral count too low to measure and was not included in the final statistics. Seven (7) of the remaining 14 patients had reduced loads at 3 months (2 males and 5 females), and 8 patients of the 14 patients had reduced viral loads at 6 months.

However, the reduction was significant in only 3 patients (2 males and 1 female). Two (2) of these 3 patients were in the coconut-oil group and 1 was in the lower dose (2.4 g) monolaurin group.¹⁵

Significant funding is being sought in order to carry out a larger investigation. It is significant that several of the viruses inacti-

Table 2. Lipid-Coated Viruses Inactivated by Monolaurin

- Human immunodeficiency virus HIV-1, HIV+
- Measles virus
- *Herpes simplex virus-1*
- *Herpes simplex virus-2*
- *Herpes viridae* (all)
- Human lymphotropic viruses (type 1)
- Vesicular stomatitis virus
- Visna virus
- Cytomegalovirus
- Epstein-Barr virus
- Influenza virus
- Pneumovirus
- Sarcoma virus
- Syncytial virus
- Rubeola virus

Sources: Refs. 14 and 17.

vated by monolaurin are responsible for opportunistic infections in HIV-positive individuals and may also be implicated in other illnesses such as chronic fatigue syndrome and immune dysfunction syndrome. (See Table 2.)

Loss of Lauric Acid from the American Diet

Over the past 40 years, the American diet has undergone drastic changes, few of which appear to be improving human health. Many of these changes have involved fats and oils. Humans have increased intake of partially hydrogenated vegetable oils and trans fatty acids as well as dramatically increasing intake of linoleic acid. These types of changes and many others have set the stage for a proinflammatory state in the human body. In contrast, there is very little intake of healthy fats, such as omega-3 fatty acids from certain fish and plants, as well as almost a total loss of antimicrobial fatty acids such as lauric acid from coconut oil that could produce monolaurin with all its described benefits.

Lauric acid has also been shown to be virucidal and bactericidal although monolaurin has even greater activity. Enteral feedings have been reformulated without coconut oil and formulas such as Ensure and Nutren are made with hydrogenated oils. Both humans and animals can metabolize some monolaurin from lauric acid.¹⁴ However, it is worth repeating that the level of this

Table 3. Lauric Acid Content of Selected Foods

Food	Grams/cup
Coconut cream, raw	37.0
Coconut cream, canned	23.3
Fresh grated coconut, packed	19.4
Fresh grated coconut, loose	11.9
Granola cereal (with coconut oil and coconut)	6.05
Coconut cream pudding	1.29
Whole milk	0.23

Source: U.S. Department of Agriculture Handbook Nos. 8-1, (1976), 8-8 (1982), 8-12 (1984), and 8-19 (1991).

metabolizing has not been quantified. Dr. Kabara has postulated that, if monolaurin is formed from lauric acid in coconut fat, the level is no greater than 3 percent.¹⁶

Mothers' milk, a rich source of lauric acid, may also provide a lipase that converts the triglycerides to monoglycerides by the infant. Even small amounts of monolaurin converted from lauric acid in coconut fat or mothers' milk and lauric acid are still virucidal and bactericidal. Coconut fat also provides caprylic acid, capric acid, and myristic acid, which are virucidal and bactericidal as well.¹⁵

In the past, infant formulas were good sources of lauric acid, because a greater amount of coconut oil was used. However, they have been reformulated with little or no coconut oil, thus losing their similarity to mothers' milk, which provides antimicrobial and antiviral fats. Human milk provides approximately 3.5 percent of calories as lauric acid for the human infant. Mature human milk has been noted to have up to 12 percent of the total fat as lauric acid (6.6 percent of calories).

Discussion

Unlike monolaurin, which has been patented as a product, lauric acid is not, and cannot be, patented. This clearly limits the funding available for research. It is hoped that the companies that manufacture coconut-based products will see the need to extend the research and start funding studies.

In the meantime, there is an excellent body of research demonstrating that monolaurin is a safe and effective virucidal and bactericidal agent. One of the authors of this review (SL) has used it

Table 4. Three Foods with Lauric Acid and Capric Acid (for Your Patients)

Brand	Origin	Lauric acid/capric acid
Jennies Macaroons ^a	United States	7.0 g per 2 oz
Grace Coconut Milk ^b	Sri Lanka	3.5 g per 2 oz or 0.4 g per 2 oz
Taste of Thai ^c	Thailand	3.5 g per 2 oz or 0.4 g per 2 oz

Source: Refs 14 and 17; ^aRed Mill Farms Inc., Brooklyn, NY.; ^bGrace, Kingston, Jamaica; ^cAndre Prost, Inc., Old Saybrook, CT.

for more than a decade with excellent results. Given its broad-spectrum activity, it has been used along with glycyrrhizin successfully to treat numerous cases of chronic fatigue syndrome, CMV infection, Epstein-Barr virus, *Herpes simplex*-1 and -2, fungal infections, and *C. albicans*—all common conditions that are seen in an integrative practice. SL has not personally used it for *G. lamblia*, which has become resistant to metronidazole and other antiparasite drugs.

Despite the unanswered question regarding whether substantial amounts of lauric acid are metabolized to monolaurin, it is important to note that lauric acid is also effective against, at least to some extent, the microorganisms listed in Tables 1 and 2. Lauric acid produces greater activity against microorganisms than caprylic acid, capric acid, or myristic acid, all of which are present in coconut oil. Given that coconut oil provides approximately 50 percent lauric acid, a substantial amount of this bactericidal and virucidal fatty acid can be obtained from consuming coconut fat in pure coconut oil, and in many foods and products. (See Tables 3 and 4; Table 4 suggests three coconut-based products.)

In addition, it is important to note that lauric acid appears to have immune-boosting properties as evidenced by feeding coconut oil to laboratory animals in whom the expected immune-factor responses (inhibition of interleukin-1) to endotoxin were induced via corn-oil feeding.¹⁷ Ingesting monolaurin on a daily basis may be an inexpensive way to both treat and prevent infection from microorganisms.¹⁸ This would be especially valuable, given that we are now living under a constant threat of bioterrorism.

The results of the small study examining the effects of monolaurin versus coconut oil were compelling.¹⁵ It is a shame that a larger study has not been funded thus far. Despite the small number of patients in the study, both coconut oil and the lower dose of monolaurin (2.4 g) were both effective in significantly lowering the viral load for several patients. Perhaps a large amount of coconut oil may have a similar effect to a smaller dose of monolaurin. Because monolaurin is more bioactive than lauric acid, a smaller amount is needed for its antimicrobial effects.

Conclusions

Monolaurin has Generally Recognized As Safe (GRAS) status and is considered to be nontoxic. It is effective against many microorganisms and can be taken on a daily basis, given that evidence suggests it does not create antiviral or antibacterial resistance.

The general recommended adult dose of monolaurin is 1–3 g. Higher amounts can be used to achieve desired results if necessary. It is safe for children (ages 3–10) with the recommended dose being smaller—30 mg, one to three times per day. Monolaurin is available as minipellets that should be swallowed and not chewed.¹⁹

Coconut oil, coconut cream, grated coconut, and other products listed in Tables 3 and 4 are excellent sources of lauric acid and the other medium-chain fatty acids as well. Coconut oil provides mostly medium-chain triglycerides that are rapidly absorbed and transported to the mitochondria where they are utilized for fuel and may be less likely to be stored as body fat.²⁰

Coconut oil does not have a deleterious effect on cholesterol or other blood lipids. In fact, it may raise high-density lipoprotein cholesterol. Coconut oil is rich in “good” saturated fatty acids that conserve the elongated omega-3 fatty acid. Animal studies have shown that some omega-3 fatty acids can be formed as a result of ingestion of coconut oil. Coconut oil does not contain trans fatty acids that have deleterious effects on blood lipids and insulin binding.¹⁷

Both monolaurin and coconut oil are excellent choices for combating a host of microorganisms—both therapeutically and preventively. More human studies are needed to elucidate the best therapeutic dose of monolaurin and coconut for addressing specific microorganisms and conditions.

It is also important to quantify how much monolaurin is metabolized from a specific quantity of coconut oil. Finally, it is equally important to compare large doses of coconut oil to specific doses of monolaurin for their antimicrobial action as was done in the HIV study population. Dietary supplements such as monolaurin may be unavailable to some populations while coconut oil may be a less-costly alternative. □

References

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Shari Lieberman, Ph.D., C.N.S., F.A.C.N., is a research scientist and industry consultant based in New York City and Pompano Beach, Florida. She is also the Founding Dean of New York Chiropractic College's (Seneca Falls, New York) M.S. Degree Program in Applied Clinical Nutrition. **Mary G. Enig, Ph.D., C.N.S., M.A.C.N.**, is the director of the Nutrition Division of Enig Associates, Inc., Silver Spring Maryland. **Harry G. Preuss, M.D., C.N.S., M.A.C.N.**, is a professor of physiology, medicine, and pathology at Georgetown University Medical Center, Washington, D.C.

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Effects of Essential Oils and Monolaurin on *Staphylococcus aureus*: *In Vitro* and *In Vivo* Studies

Harry G. Preuss, MD, MACN, CNS, Bobby Echard, MT, Azad Dadgar, BS,
Nadeem Talpur, MD, and Vijaya Manohar, PhD

Department of Physiology and Biophysics, Georgetown University Medical Center,
Washington, DC 20057

Mary Enig, PhD

Enig Associates, Silver Springs, MD 20904

Debasis Bagchi, PhD, FACN, CNS

Department of Pharmacy Sciences, Creighton University Medical Center, Omaha, NE 68178

Cass Ingram, DO

North American Herb and Spice, Waukegan, IL 60089

The antimicrobial properties of volatile aromatic oils and medium-chain fatty acids derived from edible plants have been recognized since antiquity. To give examples, Origanum oil, used as a food-flavoring agent, possesses a broad spectrum of antimicrobial activity due, at least in part, to its high content of phenolic derivatives such as carvacrol and thymol. Similarly, lauric acid, present in heavy concentrations in coconuts, forms monolaurin in the body that can inhibit the growth of pathogenic microbes. Using *Staphylococcus aureus* in broth cultures and a microdilution method, comparative efficacy of Origanum oil, and a constituent carvacrol, other essential oils and monolaurin were examined. Origanum oil was the most potent of the essential oils tested and proved bactericidal in culture to two strains of *Staphylococcus aureus* (ATCC #14154 and #14775) at 0.25 mg/mL. *In vitro*, monolaurin's effects mirrored Origanum oil. The combination of both was bactericidal at the 0.125 mg/mL concentration of each. In two separate *In vivo* experiments, injected *Staphylococcus aureus* (ATCC #14775) killed all 14 untreated mice within a 1-week period. In treated mice, over one third survived for 30 days when given oral Origanum oil daily for 30 days (6/14). Fifty percent of the mice survived for 30 days when receiving daily vancomycin (7/14) and monolaurin (4/8). Over 60% of mice survived when receiving a daily combination of Origanum oil and monolaurin (5/8). Origanum oil and/or monolaurin may prove to be useful antimicrobial agents for prevention and therapy of *Staphylococcus aureus* infections.

Keywords Origanum Oil, Monolaurin, *Staphylococcus aureus*, Natural Antimicrobials

INTRODUCTION

Microbial resistance to antibiotics, especially among staphylococcal strains, is a major threat to public health (U.S. Congress 1985). Since resistance by certain strains of *Staphylococcus* to multiple antibiotics like methicillin emerged in the late 1970s (Ayliffe 1997), many strategies to control antibiotic resistance have been proposed (Cunha 2002). Considering current therapeutic regimens, vancomycin usage has proven to be the most reliable to treat resistant Staphylococcal infections (Edmond et al. 1996; Waldvogel 1999; Burnie et al. 2000). However, some Staphylococcal strains have become resistant, at least to some extent, even to vancomycin—indicating a dire need for new alternative therapeutic approaches (Burnie et al. 2000; Hiramatsu et al. 1997; Sieradzki et al. 1999). One specific strategy has been to use multiple antibiotics of the same spectrum and low resistance potential when single antibiotic substitutions are not effective. However, we have examined yet another strategy—use of natural products with low potential for the development of resistance.

Essential oils have been used to preserve foods since the days of the early explorers (Kim et al. 1995). Therefore, it is not too surprising that they are effective against many pathogenic organisms (Kim et al. 1995; Carson et al. 1995; Ismaiel and Pierson 1990; Mansour et al. 1999; Kivanc et al. 1991; Hammer et al. 1999; Sivropoulos et al. 1996). Certain fatty acids and monoglycerides are also recognized to have antimicrobial potential (Kabara, 1980). The optimum antimicrobial activity for fatty acids and their corresponding monoglycerides is a

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Address correspondence to Harry G. Preuss MD, Georgetown University Medical Center, Department of Physiology and Biophysics, Basic Science Building, Room 231B, 4000 Reservoir Road, N.W., Washington, DC 20057. E-mail: preussgh@georgetown.edu

chain length of C12 (Kabara and Vrabie 1977). Lauric acid (C12) has a greater antiviral activity than caprylic acid (C8), capric acid (C10), or myristic acid (C14) (Kabara and Vrabie 1977).

The purpose of the present study was to determine the efficacy of a group of essential oils and the fat monolaurin alone or combined to treat *Staphylococcus aureus*. Of the 12 essential oils tested, Origanum oil proved to be the best for treating staphylococcal infections. We also found that the fat monolaurin inhibits the growth of *Staphylococcus aureus in vitro* and *in vivo* to an extent similar to Origanum oil. We conclude that the daily oral administration of these natural products alone or combined may be highly effective in preventing and treating *Staphylococcus aureus* infections and theoretically may even be effective against antibiotic-resistant strains.

MATERIAL AND METHODS

Animals and Treatment

Female BALB/c mice (15 to 20 g) were obtained from Taconic Farms (Germantown, NY) at approximately 4 to 6 weeks of age. The rodents were maintained in a controlled environment at 24°C with a 12-h light and 12-h dark cycle and were acclimatized in the animal facility for 3 to 5 days before use. The mice were housed in groups of five, fed commercial rodent pellets, and given water *ad libitum* throughout the experiments. The Animal Welfare Board at Georgetown University Medical Center approved the protocol (#99-098).

Plant Oils and Chemicals

Origanum (P73 Oreganol™), other essential oils, and olive oil were provided by North American Herb and Spices, Inc., Waukegan, IL, USA. Monolaurin was obtained from the Center for Research on Lauric Oils, Bethesda, MD (www.lauric.org). Sabouraud's glucose (S.g.) broth and agar media were obtained from Difco Laboratories (Detroit, MI, USA). Carvacrol, antibiotics, and all other chemicals used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade or the highest commercial grade available.

Organisms

Standard strains of *Staphylococcus aureus* (ATCC #14154 and #14775) were obtained from ATCC, Fairfax, VA, USA, and were grown and maintained on S.g. agar slants.

Susceptibility Testing

A microbroth dilution technique was employed to determine the susceptibility of the strains of *Staphylococcus aureus* to oil of Origanum, carvacrol, other essential oils, and monolaurin (Shadomy et al. 1985; Rex et al. 1997; Manohar et al. 2001). Susceptibility was expressed as minimum inhibitory (MIC) and/or minimum bactericidal (MBC) concentration. The stock solutions of Origanum oil, other essential oils, olive oil, and carvacrol

were dissolved in 50% ethanol-Tween 80 solvents. Antibiotics were dissolved in 50% ethanol and used as positive controls. Solvent controls (addition of carrier without essential oil and/or monolaurin) were also included for reference in addition to regular controls.

The Sabouraud's glucose (S.g.) broth containing varying amounts (logarithmic, serially and twofold diluted) of Origanum oil, other essential oils, carvacrol, monolaurin, and various controls were inoculated with actively dividing *Staphylococcus aureus* cells. The cultures were incubated for 24 h and 48 h at 30°C on a metabolic rotary shaker (220 rev/min), and the growth was monitored both visually and colorimetrically (at 540 nm). The minimum inhibitory concentration (MIC) was defined as the lowest concentration required to arrest the growth of the bacteria at the end of 24 h of incubation. Minimum bactericidal concentration (MBC) was determined by subculturing a 0.01 mL aliquot of the medium drawn from the culture tubes after 48 h on S.g. agar plates and incubated further for bacterial growth. The plates were scored for growth of the bacteria colonies. The lowest concentration of the antimicrobial agent causing negative growth (fewer than three colonies) was considered as MBC. In every case, the MIC and MBC were virtually the same, so we will only report the MBC in the results.

In Vivo Protocol

In two separate experiments, groups of mice (six and eight, respectively) infected with *Staphylococcus aureus* (ATCC #14775) ($5 \times \text{LD}_{50}$) were gavaged daily with Origanum oil, carvacrol, monolaurin, or combined Origanum oil/monolaurin in 0.2 mL of olive oil for 30 days. The amount of bacterial agents administered was calculated based on the body weight of the mice. Control mice received daily gavages of either olive oil alone (negative control) or olive oil orally plus vancomycin (400 µg) *i.p.* (positive control). In the first *in vivo* experiment, the daily dose of Origanum oil was either 2.0 µL (1.6 mg) or 4.0 µL (3.2 mg). The mice gavaged with carvacrol received doses comparable to the phenol content in the two doses of Origanum oil. In the second *in vivo* study, the daily dose of Origanum oil was the same as the higher dose used before, that is, 4.0 µL (3.2 mg). Monolaurin was given at the same dose, and when both agents were combined, the same individual doses were used, that is, 3.2 mg of each. Both experiments were terminated at the end of 30 days. The body weight, the disease status, and the overall health of the mice during the experiments were recorded. The pathological status of the mice was determined by visual examination of the internal organs after their death or sacrifice at the completion of the experiment. Culturing aliquots of kidney homogenates on S.g. agar plates further tested the renal burden of *Staphylococcus aureus*.

RESULTS

Table 1 shows results from a microdilution study on *Staphylococcus aureus* grown in broth over 48 hours (ATCC #14154).

TABLE 1
Comparative effects of various antibiotics and essential oils at the selected concentrations on *Staphylococcus aureus* (ATCC#14154)

Concentration (mg)	Olive oil	Pumpkin	Fenugreek	Myrtle	Allspice	Sage	Lavender	Bay leaf	Cumin	Cinnamon	Cassia	Oregano	Carvacrol
8	4+	4+	3+	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
4	4+	4+	4+	3+	NG	NG	NG	NG	NG	NG	NG	NG	NG
2	4+	4+	4+	4+	4+	3+	1+	NG	NG	NG	NG	NG	NG
1	4+	4+	4+	4+	4+	4+	3+	3+	NG	NG	NG	NG	NG
0.5	4+	4+	4+	4+	4+	4+	4+	4+	2+	NG	NG	NG	NG
0.25	4+	4+	4+	4+	4+	4+	4+	4+	4+	NG	NG	NG	4+
0.125	4+	4+	4+	4+	4+	4+	4+	4+	4+	2+	2+	2+	4+

See Materials and Methods section for details: solvent control 4+; growth control 4+; streptomycin (20 µg) 2+; vancomycin (40 µg) NG; penicillin (64 µg) 4+.

In examining the comparative effects of various antibiotics and essential oils at the selected concentrations, penicillin had essentially no effect, and streptomycin did not completely kill the bacteria. However, vancomycin completely destroyed the bacteria. Addition of the carrier alone to the plates did not alter growth. Of the oils examined, olive oil (the diluent), pumpkin, fenugreek, and myrtle had virtually no effect at any concentration. Allspice, sage, lavender, and bay leaf were bactericidal only at the higher concentrations. Cumin had an intermediate effect, while cinnamon, cassia, and Origanum oil were quite effective at lower concentrations. Carvacrol was added at a concentration equivalent to the total phenol content in a similar volume of Origanum oil (63% v/v). While Origanum oil proved completely bactericidal at a concentration of 0.25 mg, the carvacrol was effective only at the concentration comparable to the 0.5-mg dose of Origanum oil.

Table 2 shows data from a microdilution study performed with another strain of *Staphylococcus aureus* (ATCC #14775). Although the results from the two strains were generally similar, there were some minor differences. Myrtle appeared relatively more effective against the second strain. Cumin seemed to work as well as cassia. Origanum oil again inhibited down to 0.25 mg,

and addition of carvacrol once more inhibited at a phenol content equivalent to that in 0.5 mg% Origanum oil.

Table 3 shows data from an *in vitro* experiment on *Staphylococcus aureus* (ATCC #14775) where the effects of monolaurin alone and combined with Origanum oil were compared with the effects of Origanum oil alone. In this experiment, a few colonies grew after 2 days of incubation in 0.125 mg of Origanum oil or 0.125 mg of monolaurin. However, a combination of 0.125 mg% Origanum oil and 0.125 mg% monolaurin prevented any growth.

The *in vivo* therapeutic effects of the natural edible Origanum oil and one of its major constituents, carvacrol, were examined in a murine systemic bacteremia model (Table 4). Preliminary studies showed that *Staphylococcus aureus* (ATCC #14154) did not cause mortality or death in mice, whereas *Staphylococcus aureus* (ATCC #14775) caused mortality rapidly. Groups of six mice infected with *Staphylococcus aureus* (ATCC #14775) ($5 \times LD_{50}$) were gavaged with varying amounts of Origanum oil (2.0 µL [1.6 mg] and 4.0 µL [3.2 mg]) or injected i.p. with vancomycin (400 µg) daily for 30 days. A dose-dependent survival was observed in mice receiving Origanum oil. Fifty percent of the mice (3/6) receiving Origanum oil (3.2 mg) survived over the 30 days, while only one of six receiving 1.6 mg Origanum oil survived

TABLE 2
Comparative effects of various antibiotics and essential oils at the selected concentrations on *Staphylococcus aureus* (ATCC#14775)

Concentration (mg)	Olive oil	Lavender	Sage	Bay leaf	Myrtle	Cumin	Cassia	Carvacrol	Oregano
8	4+	NG	NG	NG	NG	NG	NG	NG	NG
4	4+	2+	NG	NG	NG	NG	NG	NG	NG
2	4+	3+	1+	1+	NG	NG	NG	NG	NG
1	4+	4+	3+	3+	2+	NG	NG	NG	NG
0.5	4+	4+	4+	4+	4+	NG	NG	NG	NG
0.25	4+	4+	4+	4+	4+	2+	1+	1+	NG
0.125	4+	4+	4+	4+	4+	4+	2+	3+	3+

See Materials and Methods section for details: solvent control 4+; growth control 4+; streptomycin (20 µg) NG; penicillin (63 µg) NG.

TABLE 3

Comparative effects of various antibiotics and essential oils at the selected concentrations on *Staphylococcus aureus* (ATCC#14775)

Concentration (mg)	Olive oil	Oregano	Monolaurin	Combination
8	4+	NG	NG	NG
4	4+	NG	NG	NG
2	4+	NG	NG	NG
1	4+	NG	NG	NG
0.5	4+	NG	NG	NG
0.25	4+	NG	NG	NG
0.125	4+	2+	1+	NG

See Materials and Methods section for details: solvent control 4+; growth control 4+; streptomycin (20 µg) NG; vancomycin (40 µg) NG; penicillin (62 µg) NG.

for 30 days. Also, 50% of those receiving vancomycin (400 µg) survived for 30 days, while all in the control group died within a 3-day period. Although prolonged survival was noted in rats gavaged with carvacrol, none of the 12 at the two doses equivalent to the total phenol content in 1.6 and 3.2 mg of Origanum oil survived beyond 21 days. In all 30-day survivors, no internal abscesses were noted at *postmortem*, and renal cultures showed no bacterial growth. In contrast, numerous abscesses were noted and renal cultures were positive in all dying mice.

In a separate *in vivo* study, the effects of monolaurin alone and combined with Origanum oil were compared to the effects

TABLE 4

In vivo study examining effects of vancomycin, Origanum oil, and carvacrol on mice challenged with *Staphylococcus aureus* (ATCC#14775)

Agent concentration	# Mice Tested/ survived 30 days	Comment
Olive oil (0.2 mL)	0/6	None survived more than 3 days
Vancomycin (400 µg)	3/6	No renal burden in survivors
Origanum oil (1.6 mg)	1/6	No renal burden in survivors
Origanum oil (3.2 mg)	3/6	No renal burden in survivors
Carvacrol (1.6 mg equiv*)	0/6	
Carvacrol (3.2 mg equiv*)	0/6	All survived beyond 3 days, but none survived more than 27 days

Staphylococcus grew from kidneys of all mice that died.

Equiv* indicates that amount of carvacrol was given equal to the calculated amount of phenol content in the doses of Origanum oil.

TABLE 5

In vivo study examining effects of Origanum oil, monolaurin, and a combination of both on mice challenged with *Staphylococcus aureus* (ATCC#14775)

Agent concentration	# Mice tested/ survived 30 days	Comment
Olive oil (0.2 mL)	0/8	None survived more than 3 days
Vancomycin (400 µg)	4/8	No renal burden in survivors
Origanum oil (3.2 mg)	3/8	No renal burden in survivors
Monolaurin (3.2 mg)	4/8	No renal burden in survivors
Combined (3.2 mg of each)	5/8	No renal burden in survivors

All dead mice grew *Staphylococcus* from kidneys.

of Origanum oil alone (Table 5). Groups of eight mice were infected with five times the LD₅₀ of *Staphylococcus aureus* (ATCC #14775). Fifty percent of the mice (4/8) injected daily with vancomycin (400 µg i.p.) survived for the 30 days of study in contrast to the control group, where all eight mice died within a week's time. In the three test groups, 3/8 survived for 30 days in the Origanum oil group (3.2 mg), 4/8 survived in the monolaurin group (3.2 mg), and 5/8 survived in the group receiving the combination of Origanum oil (3.2 mg) and monolaurin (3.2 mg). Again, no abscesses were seen on inspection, and no bacterial growth was found in the kidney tissue of the 30-day survivors.

DISCUSSION

The carriage and subsequent dissemination of antibiotic-resistant *Staphylococcus aureus* by hospital staff and patients is a recognized risk for nosocomial infections (Carson et al. 1995). Although use of antibiotics has generally been tempered to avoid antibiotic resistance, the development of resistance was inevitable (Ayliffe 1997; Cunha 2002; Edmond et al. 1996; Waldvogel 1999; Burnie et al. 2000; Hiramatsu et al. 1997; Sieradzki et al. 1999; Denis et al. 2002). Therefore, what alternatives now exist to treat antibiotic-resistant organisms? Essential oils, especially Origanum oil, and the fat monolaurin are natural substances reported to have the ability to kill *Staphylococcus aureus* and other microbes in culture (Carson et al. 1995; Hitokoto et al. 1980; Ismaiel and Pierson 1990; Mansour et al. 1999; Kim et al. 1995; Kivanc et al. 1991). Nevertheless, *in vivo* studies designed to test the antimicrobial effects of various essential oils and monolaurin are lacking.

We found that Origanum oil, a constituent carvacrol, certain other essential oils, and monolaurin killed *Staphylococcus aureus* effectively *in vitro* (Tables 1 to 3). More importantly, however, we also found that Origanum oil and monolaurin are

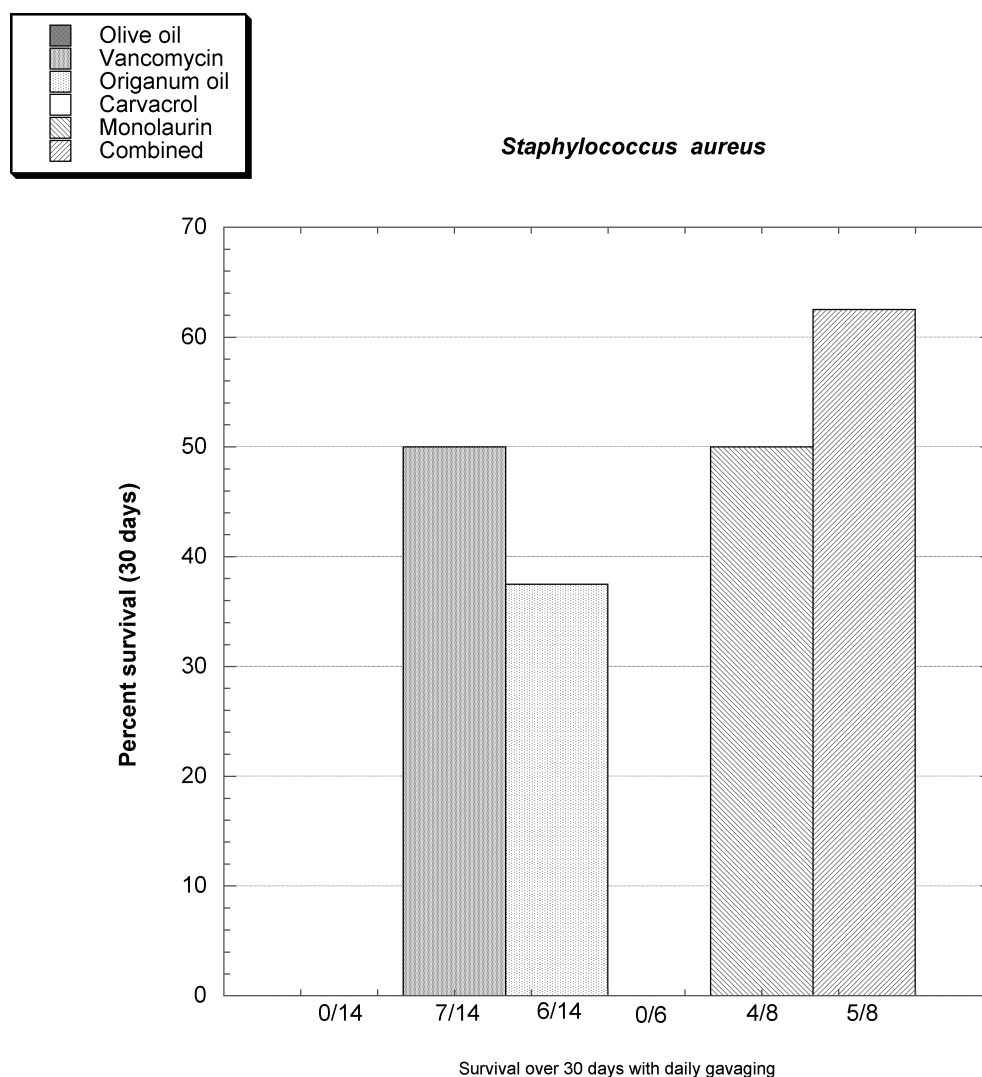


FIG. 1. Combined data from two *in vivo* studies. See Materials and Methods for details.

protective *in vivo* (Tables 4 and 5). Figure 1 combines the data from Tables 4 and 5. In the 14 rats used as control, *Staphylococcus aureus* (ATCC #14775) killed all the mice within 7 days. In contrast, 37.5 to 50% of the mice survived for 30 days after receiving daily gavages of vancomycin (7/14), Origanum oil (6/14), and monolaurin (4/8). The survival with combined Origanum oil and monolaurin was slightly better—62.5% (5/8). Obviously, more studies are needed to establish whether a combination of natural products is superior to individual use, especially if higher doses of individual agents are used. As a final point, no abscesses were found at postmortem, and *Staphylococcus aureus* could not be grown out of the kidneys of the 30-day survivors, suggesting a cure.

Interestingly, carvacrol gavaged in a dose calculated to be equivalent to the phenol content in the gavaged Origanum oil delayed death but could not cure the mice. In the first *in vivo* study where carvacrol was examined, all untreated mice died within 3 days, whereas all mice receiving carvacrol lived be-

yond 3 days, but died by 21 days. *Staphylococcus aureus* was grown out of their kidneys after death. Thus, carvacrol alone could not duplicate, to the same extent, the beneficial effects of Origanum oil. Either the addition of thymol and/or other phenols to carvacrol is necessary or there are other significant factors present in the Origanum oil.

What do we really know about essential oils and their antimicrobial properties? The benefits of essential oils to preserve various foods have been known since the days of the explorers (Kim et al. 1995). The previous results of studies using broth microdilution techniques support the notion that plant essential oils and extracts may have a role as nutraceuticals and preservatives (Hammer et al. 1999). An advantage of essential oils over antibiotics may be that bacteria do not develop resistance to essential oils (Hitokoto et al. 1980). In addition, some oils actually stimulate immune function. The major components of oregano with antibiotic properties are two phenols (carvacrol and thymol) (Ingram 2000; Sivropoulos et al. 1996). Phenols

are antiseptic substances found in Lysol, Pinesol, Chloroseptic, and throat lozenges.

Many essential oils have been shown to be effective against a number of organisms. Origanum oil, cinnamon, and clove were judged "very active" by examining their inhibitory effects on *Clostridium botulinum* 33A (Ismail and Pierson 1990). In addition to effects against *Klebsiella pneumonia* and *Staphylococcus aureus*, Origanum oil is fungicidal (Manohar et al. 2001; Hitokoto et al. 1980). Also, antiviral actions of Origanum and clove oils against RNA and DNA viruses have been reported (Siddiqui et al. 1996). As a potential mechanism of action, the outer protective membrane of the viruses disintegrated after exposure to the Origanum oil when viewed by electron microscopy (Siddiqui et al. 1996). Importantly, most essential oils of spices are classified as GRAS (generally recognized as safe), indicating that consumers can eat them reasonably without fear (Ismail and Pierson 1990). Accordingly, the benefits/risks ratio of essential oils in treating microbes would seem to be very high.

Considering other natural products with antimicrobial properties, Kabara championed the use of certain lipids (Kabara 1980). He measured the antimicrobial activity of fatty acids and their corresponding monoglycerides and reported that the optimum chain length was C12 (Kabara and Vrable 1977). Of the saturated fatty acids, lauric acid (C12) has greater antiviral activity than caprylic acid (C8), capric acid (C10), or myristic acid (C14). In contrast to monolaurin, the dilaurin derivative was inactive. It is now generally accepted that monoglycerides are active; diglycerides and triglycerides are inactive.

A broth dilution method was used to determine the minimal inhibitory concentration of a series of fatty acid ester of polyhydric alcohols against Gram-negative and Gram-positive organisms (Conley and Kabara 1973). Gram-negative organisms were not affected. Gram-positive organisms were affected to the greatest extent by monolaurin (Conley and Kabara 1973). Monolaurin is effective in blocking or delaying the production of exotoxins by pathogenic Gram-positive bacteria (Schlievert et al. 1992) and inhibits the synthesis of most staphylococcal infections and other exoproteins; it does so at the level of transcription (Projan et al. 1994). Monolaurin also inhibits the expression of virulence factors in *Staphylococcus aureus* and the induction of vancomycin resistance in *Enterococcus faecalis* (Ruzin and Novick 1998, 2000). Monolaurin also inhibits spore growth in milk (Projan et al. 1994).

When the antichlamydial effects of several fatty acids and monoglycerides were studied by incubating *Chlamydia trachomatis* bacteria, the results indicated that the lipid kills the bacteria, possibly by disrupting the membranes(s) of the elementary body (Bergesson et al. 1998). Corroborating evidence is available from viral studies, suggesting that the bactericidal effects are via disintegration of membranes by fatty acids (Thormar et al. 1987; Isaacs et al. 1991; Isaacs and Thormar 1991), similar to a report of the action of Origanum oil on viruses (Siddiqui et al. 1996).

This pilot study raises a number of important considerations. The bactericidal effects of Origanum oil, other essential oils,

and monolaurin *in vivo* indicate a need for further studies to establish the importance of these natural products in combating pathogenic microbes, especially those resistant to antibiotics. Because of the potential for fewer side effects with natural products compared to pharmaceuticals, the health care provider might eventually recommend taking them prophylactically to prevent future infections. While combining Origanum oil and monolaurin produces additive effects, more studies are necessary to determine if synergy exists. It is also possible that these agents might also work well in the presence of antibiotics and make them more effective.

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