Cassandra QUAVE, *et al.*
Herbs vs MRSA

http://preventdisease.com/news/17/021317_Peppertree-Disarms-Most-Dangerous-Bacteria.shtml
Prevent Disease

Common weed found to disarm MRSA superbug
by Karen Foster

Superbugs are without a doubt a major threat affecting all health care systems. Methicillin-resistant Staphylococcus aureus (MRSA) infection is caused by a type of staph bacteria that's become resistant to many of the antibiotics used to treat ordinary staph infections. Despite attempts by new antibiotics to neutralize the effects of MRSA, none have succeeded. But nature did.

The red berries of the Brazilian peppertree -- a weedy, invasive species common in Florida -- contain an extract with the power to disarm dangerous antibiotic-resistant staph bacteria, scientists at Emory University have discovered.

The journal Scientific Reports is publishing the finding, made in the lab of Cassandra Quave, an assistant professor in Emory's Center for the Study of Human Health and in the School of Medicine's Department of Dermatology.

"Traditional healers in the Amazon have used the Brazilian peppertree for hundreds of years to treat infections of the skin and soft tissues," Quave says. "We pulled apart the chemical ingredients of the berries and systematically tested them against disease-causing bacteria to uncover a medicinal mechanism of this plant."

The researchers showed that a refined, flavone-rich composition extracted from the berries inhibits formation of skin lesions in mice infected with MRSA. The compound works not by killing the MRSA bacteria, but by repressing a gene that allows the bacteria cells to communicate with one another. Blocking that communication prevents the cells from taking collective action, a mechanism known as quorum quenching.

"It essentially disarms the MRSA bacteria, preventing it from excreting the toxins it uses as weapons to damage tissues," Quave says. "The body's normal immune system then stands a better chance of healing a wound."

While infections are becoming increasingly difficult to beat, no new class of antibiotic has been discovered since 1987. In contrast, a new infection emerges on an almost yearly basis.

The discovery may hold potential for new ways to treat and prevent antibiotic-resistant infections, a growing international problem. Antibiotic-resistant infections annually cause at least two million illnesses and 23,000 deaths in the United States, according to the Centers for Disease Control and Prevention. The United Nations last year called antibiotic-resistant infections a "fundamental threat" to global health and safety, citing estimates that they cause at least 700,000 deaths each year worldwide, with the potential to grow to 10 million deaths annually by 2050.

Bacteria are rife in conventionally grown US meat including antibiotic-resistant bacteria also known as superbugs. Almost half of beef, chicken, pork and turkey in samples tested from US grocery stores contained staph bacteria reported the Los Angeles Times in 2011 including the resistant MRSA staph bacterium (methicillin-resistant S. aureus). Pork tested by Consumer Reports in 2013 also contained MRSA and four other kinds of resistant bacteria.

Blasting deadly bacteria with drugs designed to kill them is helping to fuel the problem of antibiotic resistance. Some of the stronger bacteria may survive these drug onslaughts and proliferate, passing on their genes to offspring and leading to the evolution of deadly "super bugs."

In contrast, the Brazilian peppertree extract works by simply disrupting the signaling of MRSA bacteria without killing it. The researchers also found that the extract does not harm the skin tissues of mice, or the normal, healthy bacteria found on skin.

"In some cases, you need to go in heavily with antibiotics to treat a patient," Quave says. "But instead of always setting a bomb off to kill an infection, there are situations where using an anti-virulence method may be just as effective, while also helping to restore balance to the health of a patient. More research is needed to better understand how we can best leverage anti-virulence therapeutics to improve patient outcomes."

Quave, a leader in the field of medical ethnobotany and a member of the Emory Antibiotic Resistance Center, studies how indigenous people incorporate plants in healing practices to uncover promising candidates for new drugs.

The Brazilian peppertree (Schinus terebinthifolia) is native to South America but thrives in subtropical climates. It is abundant in much of Florida, and has also crept into southern areas of Alabama, Georgia, Texas and California. Sometimes called the Florida holly or broad leaf peppertree, the woody plant forms dense thickets that crowd out native species.

"The Brazilian peppertree is not some exotic and rare plant found only on a remote mountaintop somewhere," Quave says. "It's a weed, and the bane of many a landowner in Florida."

From an ecological standpoint, it makes sense that weeds would have interesting chemistry, Quave adds. "Persistent, weedy plants tend to have a chemical advantage in their ecosystems, which help may protect them from diseases so they can more easily spread in a new environment."

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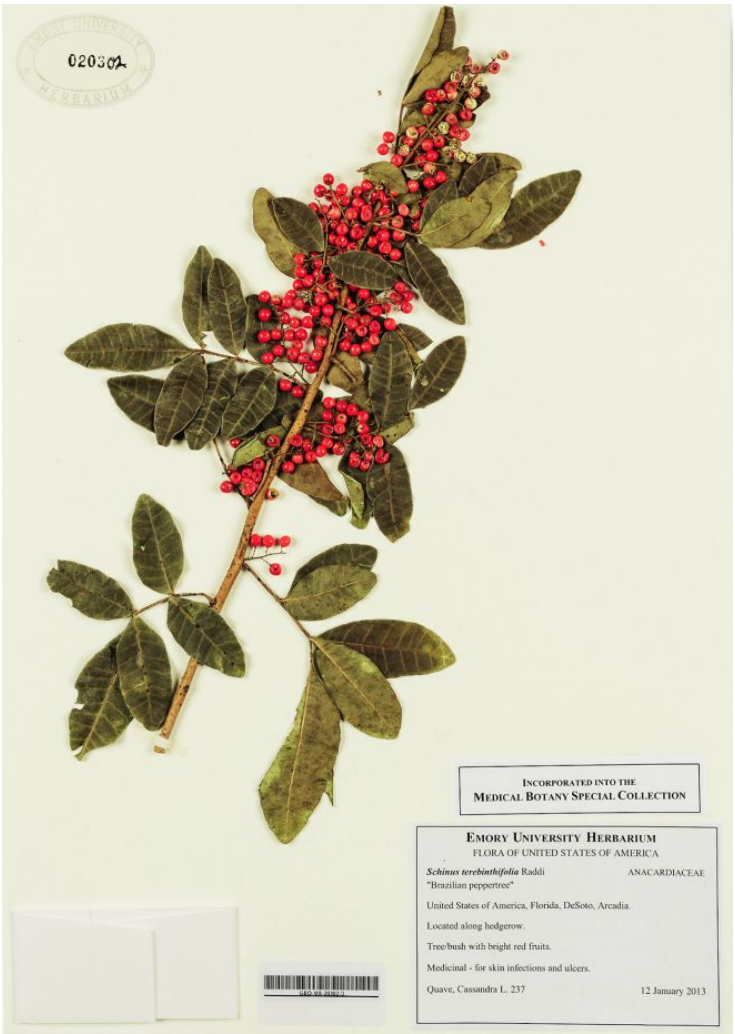
https://en.wikipedia.org/wiki/Schinus_terebinthifolius

Schinus terebinthifolius



Schinus terebinthifolius
Starr 041018-0009 Schinus terebinthifolius.jpg
Scientific classification
Kingdom: Plantae, Angiosperms, Eudicots, Rosids, Order: Sapindales; Family: Anacardiaceae; Genus: Schinus ; Species: S. terebinthifolia

Binomial name : Schinus terebinthifolia Raddi, 1820[1]

Schinus terebinthifolia is a species of flowering plant in the cashew family, Anacardiaceae, that is native to subtropical and tropical South America (southeastern Brazil, northern Argentina, and Paraguay). It is found in these states of Brazil: Alagoas, Bahia, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Pernambuco, Paraná, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo, and Sergipe. Common names include Brazilian peppertree,[2] aroeira, rose pepper, broadleaved pepper tree,[3] wilelaiki (or wililaiki),[4] and Christmasberry.[5]

Description

Brazilian peppertree is a sprawling shrub or small tree, with a shallow root system, reaching a height of 7–10 m. The branches can be upright, reclining, or nearly vine-like, all on the same plant. Its plastic morphology allows it to thrive in all kinds of ecosystems: from dunes to swamps, where it grows as a semiaquatic plant.[6] The leaves are alternate, 10–22 cm long, pinnately compound with (3–) 5–15 leaflets; the leaflets are roughly oval (lanceolate to elliptical), 3–6 cm long and 2–3.5 cm broad, and have finely toothed margins, an acute to rounded apex and yellowish veins. The leaf rachis between the leaflets is usually (but not invariably) slightly winged. The plant is dioecious, with small white flowers borne profusely in axillary clusters. The fruit is a drupe 4–5 mm diameter, carried in dense clusters of hundreds.

The two varieties are:

S. terebinthifolius var. acutifolius, leaves to 22 cm, with 7–15 leaflets, fruit pink

S. terebinthifolius var. terebinthifolius, leaves to 17 cm, with 5–13 leaflets, fruit red

Cultivation and uses

Brazilian pepper is widely grown as an ornamental plant in frost-free regions of South America for its foliage and fruit. It is considered as a melliferous flower[6] and is the main source of food for the bee Tetragonisca angustula, which is an important honey producer.[7]

Although it is not a true pepper (Piper), its dried drupes are often sold as pink peppercorns, as are the fruits from the related species Schinus molle (Peruvian peppertree). The seeds can be used as a spice, adding a pepper-like taste to food. They are usually sold in a dry state and have a bright pink color. They are less often sold pickled in brine, where they have a dull, almost green hue.

In the United States, it has been introduced to California, Texas, Hawaii, Arizona, Nevada, Louisiana,[8] and Florida. Planted originally as an ornamental outside of its native range, Brazilian pepper has become widespread and is considered an invasive species in many subtropical regions with moderate to high rainfall, including parts or all of Australia, the Bahamas, Bermuda, southern China, Cuba, Fiji, French Polynesia, Guam, Hawaii, Malta, the Marshall Islands, Mauritius, New Caledonia, New Zealand, Norfolk Island, Puerto Rico, Réunion, South Africa, and the United States. In drier areas, such as Israel and southern California, it is also grown, but has not generally proved invasive. In California, it is considered invasive in coastal regions by the California Invasive Plant Council (www.cal-ipc.org.)

Brazilian pepper is hard to control because it produces basal shoots if the trunk is cut. Trees also produce abundant seeds that are dispersed by birds and ants. This same hardiness makes the tree highly useful for reforestation in its native environment, but enables it to become invasive outside of its natural range.[6]
Toxicity

Like many other species in the family Anacardiaceae, Brazilian pepper has an aromatic sap that can cause skin reactions (similar to poison ivy burns) in some sensitive people – although the reaction is usually weaker than that induced by touch of the closely related Lithraea molleoides, known in Brazil as "wild" aroeira (aroeira brava). Conversely, Schinus terebinthifolius is commonly known as "tame" aroeira (aroeira mansa).

In a paper on triterpenes, the ingested fruits are noted to have a “paralyzing effect” on birds.[9] The narcotic and toxic effects on birds and other wildlife has also been noted by others, e.g., Bureau of Aquatic Plant Management. The AMA Handbook of Poisonous and Injurious Plants reports that the triterpenes found in the fruits can result in irritation of the throat, gastroenteritis, diarrhea, and vomiting in man.[10] Like most other members of the Anacardiaceae, Brazilian pepper contains active alkenyl phenols, e.g., urushiol, cardol, which can cause contact dermatitis and inflammation in sensitive individuals.[11][12] Contact with the “sap” from a cut or bruised tree can result in rash, lesions, oozing sores, severe itching,reddening and swelling (especially of the eyes), and welts.[13]

The burning of plant matter releases many airborne irritants, so is not an effective means of control. It is said to have a "mace-like" effect upon nearby people and is highly advised against.

History

"Florida holly" was introduced to Florida by at latest 1891, probably earlier,[14] where it has spread rapidly since about 1940,[15] replacing native plants, like mangroves, with thousands of acres occupied. It is especially adept at colonizing disturbed sites and can grow in both wet and dry conditions. Its growth habit allows it to climb over understory trees and invade mature canopies, forming thickets that choke out most other plants.

Legal status

The species, including the seed, is legally prohibited from sale, transport, or planting in Florida, according to the Florida Department of Agriculture and Consumer Services Noxious Weed List.[16] It is classified as a Category I pest by The Florida Exotic Pest Plant Council (FL EPPC).[17] To keep the plant from spreading into native plant communities and displacing them, local regulations and environmental guidelines require eradication of Brazilian pepper wherever possible. The plant and all parts are also illegal for sale or transfer in Texas.[18] As one of the two species sold as pink peppercorn, the other being Schinus molle, it lacks generally recognized as safe (GRAS) status with the FDA.[19]

It is a declared weed in several states of Australia.[20][21][22] In South Africa, it is classified as a category 1 invader in KwaZulu-Natal province, where any plants are to be removed and destroyed, and a category 3 invader in all other provinces, meaning it may no longer be planted.[23]

Control

Two herbicides are approved for use in the United States to exterminate Brazilian pepper: Triclopyr, using the basal bark method; and glyphosate. Picloram can be used if the stump has been freshly cut, but this is not the preferred nor most effective means of eradication.

Medicinal uses

Peppertree is the subject of extensive folk medicinal lore where it is indigenous. Virtually all parts of this tropical tree, including its leaves, bark, fruit, seeds, resin, and oleoresin (or balsam) have been used medicinally by indigenous peoples throughout the tropics. The plant has a very long history of use and appears in ancient religious artifacts and on idols among some of the ancient Chilean Amerindians.[citation needed]

Throughout South and Central America, Brazilian peppertree is reported to be an astringent, antibacterial, diuretic, digestive stimulant, tonic, antiviral, and wound healer. In Peru, the sap is used as a mild laxative and a diuretic, and the entire plant is used externally for fractures and as a topical antiseptic. The oleoresin is used externally as a wound healer, to stop bleeding, and for toothaches, and it is taken internally for rheumatism and as a purgative. In South Africa, a leaf tea is used to treat colds, and a leaf decoction is inhaled for colds, hypertension, depression, and irregular heart beat. In the Brazilian Amazon, a bark tea is used as a laxative, and a bark-and-leaf tea is used as a stimulant and antidepressant. In Argentina, a decoction is made with the dried leaves and is taken for menstrual disorders and is also used for respiratory and urinary tract infections and disorders.[citation needed]

Brazilian peppertree is still employed in herbal medicine today in many countries. It is used for many conditions in the tropics, including menstrual disorders, bronchitis, gingivitis, gonorrhea, gout, eye infections, rheumatism, sores, swellings, tuberculosis, ulcers, urethritis, urogenital disorders, venereal diseases, warts, and wounds. In Brazilian herbal medicine today, the dried bark and/or leaves are employed for heart problems (hypertension and irregular heart beat), infections of all sorts, menstrual disorders with excessive bleeding, tumors, and general inflammation. A liquid extract or tincture prepared with the bark is used internally as a stimulant, tonic, and astringent, and externally for rheumatism, gout, and syphilis. [24]

Recently, the fruit of the plant has been studied and shows promise as a treatment for MRSA. A chemical in the berry appears to stop bacteria from producing a toxin which breaks down tissue. It also appears to suppress the way the bacteria communicate. [25]

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Could Ancient Remedies Hold the Answer to the Looming Antibiotics Crisis?
One researcher thinks the drugs of the future might come from the past: botanical treatments long overlooked by Western medicine.
By FERRIS JABR

...Recently, Quave and her research team have discovered that an extract of Brazilian peppertree berries — an invasive species common in many warmer parts of the United States — prevents MRSA from forming skin lesions in mice and shrinks biofilms formed by the bacteria. “I really believe these kind of inhibitors are a major part of the solution to antibiotic resistance,” Quave says. “We can shut down bacteria’s most dangerous machinery without killing them.” She envisions using such drugs as prophylactics in surgeries with a high infection risk, or in combination with other antimicrobials if a serious infection is already established...

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https://www.researchgate.net/profile/Cassandra_Quave/publications
https://www.researchgate.net/publication/313592977_Virulence_Inhibitors_from_Brazilian_Peppertree_Block_Quorum_Sensing_and_Abate_Dermonecrosis_in_Skin_Infection_Models
Scientific Reports 7:42275 (February 2017)
DOI: 10.1038/srep42275

Virulence Inhibitors from Brazilian Peppertree Block Quorum Sensing and Abate Dermonecrosis in Skin Infection Models
Amelia Muhs, et al.

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Chestnut leaves yield extract that disarms deadly staph bacteria
Extract shuts down staph without boosting its drug resistance

Leaves of the European chestnut tree contain ingredients with the power to disarm dangerous staph bacteria without boosting its drug resistance, scientists have found.

PLOS ONE is publishing the study of a chestnut leaf extract, rich in ursene and oleanene derivatives, that blocks Staphlococcus aureus virulence and pathogenesis without detectable resistance.

The use of chestnut leaves in traditional folk remedies inspired the research, led by Cassandra Quave, an ethnobotanist at Emory University.

"We've identified a family of compounds from this plant that have an interesting medicinal mechanism," Quave says. "Rather than killing staph, this botanical extract works by taking away staph's weapons, essentially shutting off the ability of the bacteria to create toxins that cause tissue damage. In other words, it takes the teeth out of the bacteria's bite."

The discovery holds potential for new ways to both treat and prevent infections of methicillin-resistant S. aureus, or MRSA, without fueling the growing problem of drug-resistant pathogens.

Antibiotic-resistant bacteria annually cause at least two million illnesses and 23,000 deaths in the United States, according to the Centers for Disease Control and Prevention. MRSA infections lead to everything from mild skin irritations to fatalities. Evolving strains of this "super bug" bacterium pose threats to both hospital patients with compromised immune systems and young, healthy athletes and others who are in close physical contact.

"We've demonstrated in the lab that our extract disarms even the hyper-virulent MRSA strains capable of causing serious infections in healthy athletes," Quave says. "At the same time, the extract doesn't disturb the normal, healthy bacteria on human skin. It's all about restoring balance."

Quave, who researches the interactions of people and plants -- a specialty known as ethnobotany -- is on the faculty of Emory's Center for the Study of Human Health and Emory School of Medicine's Department of Dermatology. She became interested in ethnobotany as an undergraduate at Emory.

For years, she and her colleagues have researched the traditional remedies of rural people in Southern Italy and other parts of the Mediterranean. "I felt strongly that people who dismissed traditional healing plants as medicine because the plants don't kill a pathogen were not asking the right questions," she says. "What if these plants play some other role in fighting a disease?"

Hundreds of field interviews guided her to the European chestnut tree, Castanea sativa. "Local people and healers repeatedly told us how they would make a tea from the leaves of the chestnut tree and wash their skin with it to treat skin infections and inflammations," Quave says.

For the current study, Quave teamed up with Alexander Horswill, a microbiologist at the University of Iowa whose lab focuses on creating tools for use in drug discovery, such as glow-in-the-dark staph strains.

The researchers steeped chestnut leaves in solvents to extract their chemical ingredients. "You separate the complex mixture of chemicals found in the extract into smaller batches with fewer chemical ingredients, test the results, and keep honing in on the ingredients that are the most active," Quave explains. "It's a methodical process and takes a lot of hours at the bench. Emory undergraduates did much of the work to gain experience in chemical separation techniques."

The work produced an extract of 94 chemicals, of which ursene and oleanene based compounds are the most active.

Tests showed that this extract inhibits the ability of staph bacteria to communicate with one another, a process known as quorum sensing. MRSA uses this quorum-sensing signaling system to manufacture toxins and ramp up its virulence.

"We were able to trace out the pathways in the lab, showing how our botanical extract blocks quorum sensing and turns off toxin production entirely," Quave says. "Many pharmaceutical companies are working on the development of monoclonal antibodies that target just one toxin. This is more exciting because we've shown that with this extract, we can turn off an entire cascade responsible for producing a variety of different toxins."

A single dose of the extract, at 50 micrograms, cleared up MRSA skin lesions in lab mice, stopping tissue damage and red blood cell damage. The extract does not lose activity, or become resistant, even after two weeks of repeated exposure. And tests on human skin cells in a lab dish showed that the botanical extract does not harm the skin cells, or the normal skin micro-flora.

The Emory Office of Technology Transfer has filed a patent for the discovery of the unique properties of the botanical extract. The researchers are doing further testing on individual components of the extract to determine if they work best in combination or alone.

"We now have a mixture that works," Quave says. "Our goal is to further refine it into a simpler compound that would be eligible for FDA consideration as a therapeutic agent."

Potential uses include a preventative spray for football pads or other athletic equipment; preventative coatings for medical devices and products such as tampons that offer favorable environments for the growth of MRSA; and as a treatment for MRSA infections, perhaps in combination with antibiotics.

"It's easy to dismiss traditional remedies as old wives' tales, just because they don't attack and kill pathogens," Quave says. "But there are many more ways to help cure infections, and we need to focus on them in the era of drug-resistant bacteria."

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Mechanistic Studies on Staphylococcal Quorum Quenching Natural Products
Quave, Cassandra Leah
Emory University, Atlanta, GA, United State

Abstract
Staphylococcus aureus is a highly problematic pathogen. Rates of infection in both the community and healthcare setting are on the rise, and coupled with its highly antibiotic-resistant nature, this makes S. aureus a top public health concern. In fact, invasive methicillin-resistant S. aureus (MRSA) is responsible for more deaths in the USA than AIDS. Nevertheless, the number of new antibiotic leads in the pipeline is diminishing, and many scientists have put out a call for the discovery and development of a new class of drugs which could mediate microbial pathogenicity rather than growth and survival. The staphylococcal quorum-sensing pathway, controlled by the accessory gene regulator (agr) system, is a potential target for such anti-pathogenic drug discovery efforts, as it serves as a global regulator of staphylococcal virulence. Following extensive studies on the complementary and alternative medical (CAM) practices of southern Italians in the treatment of skin and soft tissue infection, over 100 plant samples were identified, collected, extracted, and examined for their anti-staphylococcal potential. Among the tests included was a screen for the inhibition of??-hemolysin, a translational protein product of RNAIII, whose production is regulated through the agr quorum-sensing pathway. Extract 134, which is derived from a popular tree with edible fruits and medicinal leaves and bark, was found to exhibit a strong dose-dependent inhibition of ?-hemolysin at sub-inhibitory concentrations for growth. The dose-dependent quorum-quenching effects of Extract 134 were confirmed through the use of fluorescent genetic reporters for agr (types I-IV). This activity is important based upon previous animal studies with agr knockout mutants that show a diminished capacity to initiate and persist in a skin infection model. In the proposed study, we seek to improve our understanding of the mechanistic basis for Extract 134's quorum-quenching effects and evaluate the therapeutic relevance of such an anti-virulence therapy using in vivo models. The study will address four specific aims: 1) identification and structural elucidation of the active constituent(s) (or marker compounds for standardization) in Extract 134;2) elucidation of the mechanism of action for the quorum-quenching effects observed;3) determination of drug metabolism and pharmacokinetic parameters (DM/PK) of the bioactive constituent(s);and 4) evaluation of efficacy in treating S. aureus skin infection in a murine model.

PLoS ONE 11(9):e0163655 (September 2016)
DOI: 10.1371/journal.pone.0163655

Correction: Castanea sativa (European Chestnut) Leaf Extracts Rich in Ursene and Oleanene Derivative...
Hospital superbug MRSA could be beaten with an unusual cure
Researchers used a Mediterranean folk remedy to obtain an extract that stopped staphylococcus aureus producing harmful toxins in mice
By John von Radowitz

Chemicals from sweet chestnut tree leaves can help fight the MRSA superbug, scientists have found.

They used a Mediterranean folk remedy to obtain an extract that stopped staphylococcus aureus producing harmful toxins in mice.

Cassandra Quave of Emory University, Atlanta, Georgia, said it did not kill the bug but “takes the teeth out of the bacteria’s bite”.

The compounds "disarm" Staphylococcus aureus bacteria and stop them producing harmful toxins.

Yet they do not appear to boost levels of drug resistance.

Dr Quave said: "Rather than killing staph, this botanical extract works by taking away staph's weapons, essentially shutting off the ability of the bacteria to create toxins that cause tissue damage."

For years the Emory team had investigated the traditional remedies of rural people in southern Italy and other parts of the Mediterranean.

Detective work by the researchers led them to the European sweet chestnut tree, Castanea sativa.

"Local people and healers repeatedly told us how they would make a tea from the leaves of the chestnut tree and wash their skin with it to treat skin infections and inflammations," said Dr Quave.

In the laboratory, the scientists steeped chestnut leaves in solvents to extract 94 chemicals including the anti-bacterial ursene and oleanene compounds.

A single 50 microgram dose of the extract cleared up MRSA skin infections in laboratory mice, halting damage to tissue and red blood cells.

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http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0136486
PLOS ONE, 2015; 10 (8): e0136486
DOI: 10.1371/journal.pone.0136486

Castanea sativa (European Chestnut) Leaf Extracts Rich in Ursene and Oleanene Derivatives Block Staphylococcus aureus Virulence and Pathogenesis without Detectable Resistance.
Cassandra L. Quave, James T. Lyles, Jeffery S. Kavanaugh, Kate Nelson, Corey P. Parlet, Heidi A. Crosby, Kristopher P. Heilmann, Alexander R. Horswill.

Abstract

The Mediterranean is home to a rich history of medical traditions that have developed under the influence of diverse cultures over millennia. Today, many such traditions are still alive in the folk medical practices of local people. Investigation of botanical folk medicines used in the treatment of skin and soft tissue infections led us to study Castanea sativa (European Chestnut) for its potential antibacterial activity. Here, we report the quorum sensing inhibitory activity of refined and chemically characterized European Chestnut leaf extracts, rich in oleanene and ursene derivatives (pentacyclic triterpenes), against all Staphylococcus aureus accessory gene regulator (agr) alleles. We present layers of evidence of agr blocking activity (IC50 1.56–25 μg mL-1), as measured in toxin outputs, reporter assays hemolytic activity, cytotoxicity studies, and an in vivo abscess model. We demonstrate the extract's lack of cytotoxicity to human keratinocytes and murine skin, as well as lack of growth inhibitory activity against S. aureus and a panel of skin commensals. Lastly, we demonstrate that serial passaging of the extract does not result in acquisition of resistance to the quorum quenching composition. In conclusion, through disruption of quorum sensing in the absence of growth inhibition, this study provides insight into the role that non-biocide inhibitors of virulence may play in future antibiotic therapies.

A series of studies by Quave et al. [4–6] investigated the bioactivity of plant extracts used in the traditional treatment of skin and soft tissue infections (SSTI) in Italy. Extracts were screened for activity against multiple targets, including S. aureus biofilms, communication (quorum-sensing) and growth. As a result of this work, three potential leads (Castanea sativa, Ballota nigra, and Sambucus ebulus) for the inhibition of quorum sensing in the absence of growth-inhibitory effects were identified [4]. Here, we continue to explore other mechanisms by which anti-infective traditional botanical medicines may function, and report the discovery of quorum quenching natural products extracted from Castanea sativa (European Chestnut) leaves, which are used in traditional therapies for treating skin inflammation SSTIs in the Mediterranean [7]. Notably, we report the ability of C. sativa leaf extracts to attenuate virulence by quenching S. aureus agr-mediated quorum sensing, effectively blocking production of harmful exotoxins at sub-inhibitory concentrations for growth. We also report the lack of cytotoxicity to human skin cells, lack of growth inhibitory activity against the normal skin microflora, lack of resistance development, and efficacy in a skin abscess animal model...

Extraction and purification of QSI-containing fractions

Crude methanol extracts (Extract 224) of the ground leaves were created by maceration of the plant materials at room temperature using a ratio of 1g dry leaves:10 mL MeOH for two successive periods of 72 hours, with daily agitation. Filtered extracts were combined, concentrated at reduced pressure and a temperature <40°C with rotary evaporators, and lyophilized before being re-suspended in water and partitioned in succession with hexane, ethyl acetate and butanol (all solvents acquired from Fisher Chemical, Certified ACS). The resulting non-aqueous partitions were dried over anhydrous Na2SO4, concentrated in vacuo, and lyophilized before testing for activity.

The most active partition (ethyl acetate, extract 224C) was subjected to further fractionation using a CombiFlash Rf+ (Teledyne ISCO) flash chromatography system using a RediSep Rf Gold silica column. Extract 224C was bonded to Celite 545 (Acros Organics) at a 1:4 ratio and dry-loaded using a RediSep dry load cartridge. The mobile phase consisted of (A) hexane, (B) EtOAc, and (C) MeOH. The linear gradient begins with 100% A for 6.3 column volumes (CV), and then increased to 50:50 A:B by25.3 CV, and increased to 100% B at 63.3 CV, which was held until 69.6 CV, and then to 70:30 B:C at 88.6 CV, which was held until 94.9 CV. The chromatography was monitored at 254 and 280 nm, as well as via ELSD. The resulting fractions were combined into 5 fractions. Following further bioassay testing, it was determined that the fraction which eluted from 30–40 CV (224C-F2) was most active. The full extract fractionation scheme is presented in Fig 2...

Discussion

The ethnobotanical approach to drug discovery [55] was used here to identify Castanea sativa leaves as a potential source new anti-infective agents. Through design of a bioactivity-guided fractionation strategy based on limited growth-impact coupled to quorum sensing inhibition, we were successful in creating a highly efficacious botanical composition with universal quenching activity for all agr alleles. To the best of our knowledge, the present work represents the first in-depth investigation of European Chestnut leaf extract for its quorum quenching and anti-virulence effects since its identification as a potential quorum quenching lead [4]. Furthermore, this is the first report of the quorum quenching effects of a botanical composition rich in ursene and oleanene derivatives (Fig 10) against S. aureus. Additional compounds identified in the most active region (at <1% relative abundance each) included putative gallotannins, which share a tri-galloyl structure with varying core sugars (32, 33, 34), and a putative ellagitannin (39). It is possible that in addition to the pentacyclic triterpenes present in 224C-F2, hydrolysable tannins also contribute to the extract's quorum quenching activity.

European Chestnut leaf extracts have been the focus of a number of studies centered on evaluation of its activity in scavenging reactive oxygen species [42, 56] and cytoprotective effects, specifically with regards to protection from UV-damage in skin cells [57]. The examination of European Chestnut leaf extracts with a patch test revealed that with respect to irritant effects, such extracts can be considered as safe for topical applications [58]. The integration of C. sativa leaf extracts into cosmetic compositions has also been patented, and is based on the antibacterial and reactive oxygen species (ROS) scavenging effects of the extract [59]. Our safety studies in both human keratocytes (HaCaT cells) and murine skin (Fig 6) have reconfirmed that this version of European Chestnut leaf extract (224C-F2) can be considered safe for topical applications based on its lack of cytotoxic and irritant effects.

Several layers of evidence in support of the efficacy of C. sativa leaf extracts in blocking S. aureus virulence have been presented. Specifically, we have demonstrated that European Chestnut leaf extracts are effective in blocking production of the translational products of RNAIII, including a number of exotoxins. Overall virulence was quenched as demonstrated by the lack of cytotoxic effects elicited by supernatants of cultures treated with the extract. Importantly, using an in vivo model, we have demonstrated efficacy in attenuating dermonecrosis, even in the absence of adjuvant antibiotics.

This inhibition of virulence and pathogenesis was accomplished without posing growth inhibitory pressures on not only S. aureus, but also a panel of common members of the human cutaneous microbiome. A robust skin microflora is critical to skin barrier health and prevention of disease onset. The majority of the bacterial cutaneous microbiome is represented by Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes [60]. Much like cases of dysbiosis in gut microflora, broad-spectrum activity against the skin microflora also holds the potential for fostering an environment amenable to the proliferation of pathogenic bacteria [61]. The presence of commensals, like Staphylococcus epidermidis, is essential to state of host innate immunity [62]. Thus, it is noteworthy that 224C-F2 specifically blocks S. aureus virulence without adding selective pressures on major representatives of the cutaneous microbiome.

The mechanistic basis for 224C-F2's quorum quenching activity remain unclear. Multiple lines of evidence suggest that components within 224C-F2 directly target the core machinery of the agr system, such as our observation of agr P3 promoter reduction (Fig 3) and reduced levels of d-toxin production (Fig 4), which is encoded within RNAIII transcript regulated by P3. If 224C-F2 only targeted downstream factors regulated by quorum sensing, such as a-hemolysin, inhibition of agr P3 or d-toxin production would not have been expected. Potential targets within the agr system include inhibition of AIP docking with AgrC, prevention of AIP production through AgrB, or reduction of AgrA activation (Fig 1). Future studies will seek to resolve the mechanism, and this will be facilitated by the isolation of individual active components for incorporation in structure-activity relationship (SAR) studies.

We hypothesized that use of a complex mixture that targets an indirect pathway to pathogen success (rather than direct targeting for growth and survival) would be unlikely to result the generation of resistant mutations. In fact, following 15 days of sequential passaging with 224C-F2 in vitro, no resistance was detected. This is not surprising; recent findings comparing individual natural products to complex botanical compositions in other targets, such as multidrug-resistant malaria, have demonstrated that single-compound drugs may not be the best answer. For example, in the face of growing artemisinin resistance for malaria, more chemically complex whole plant therapies (Artemisia annua L., Asteraceae) have demonstrated superior efficacy to the single compound in preventing drug resistance [63]. Indeed, complex botanical compositions that meet the FDA standards for safety and efficacy are eligible for an alternative regulatory approval pathway as "botanical drugs", which are distinct from dietary supplements, and are standardized to levels of marker compounds and regulated like other single compound pharmaceuticals once approved [64]. Two examples of successful botanical drugs include Veregen (Camellia sinensis (L.) Kuntze, Theaceae, sinecatechin topical formulation for anogenital warts) and Fulyzaq (Croton lechleri Müll. Arg., Euphorbiaceae, procyanidin and prodelphinidin oral formulation for HIV/AIDS-related diarrhea).

While it is debatable whether virulence inhibitors will ever serve as stand-alone therapeutics, many agree that their application as adjuvants to existing lines of antibiotics could be a critical tool in this era of rising antibiotic resistance. Specifically, by inhibiting agr, such a therapy effectively blocks the production of an entire suite of diverse staphylococcal toxins, ranging from immune-attacking PSMs, pore-forming hemolysins, and a number of other proteases and lipases that damage the host tissue and weaken the host immune response. This will be of particular relevance to patients faced with toxin-mediated infection, including staphylococcal scalded skin syndrome (esp. in neonates), abscesses, necrotizing fasciitis, sepsis, atopic dermatitis (eczema) and more.

In conclusion, we have demonstrated that a folk-medical treatment for skin inflammation and SSTIs that does not demonstrate "typical" antibacterial activity (bacteriostatic or bactericidal) nevertheless shows great potential for development as a therapeutic due to its ability to specifically target and quench S. aureus virulence. The results of this study are important not only to future antibiotic discovery and development efforts, but are also vital to the validation of this previously poorly understood traditional medicine as an efficacious therapy, and not simply an unsubstantiated relict of folklore. Importantly, this composition was non-toxic to human keratinocytes and no dermatopathology was noted upon administration to murine skin. Moreover, the composition did not inhibit growth of the normal skin microflora, suggesting that its disruptive action on the cutaneous microbiome would be minimal to nil. Future work will focus on evaluation of individual actives within the composition with the aim of determining whether a complex mixture, such as 224C-F2 or a single compound will prove most effective against all agr alleles and which will be least likely to develop resistance when administered under multiple selective pressures, such as for in vivo administration as an antibiotic adjuvant...

[Excerpts]

Botanical Extracts and Compounds from Schinus Plants and Methods of Use
US2017007652

This disclosure relates to extracts from the Anacardiaceae (cashew plant family) and compositions comprising compounds contained therein. In certain embodiments, the extracts are derived from the fruit of a Schinus plant. In certain embodiments, the disclosure relates to methods of treating or preventing bacterial infections, acne, and other related uses.

BACKGROUND

[0003] Since the widespread introduction of antibiotics in the 1940s, the same storyline has repeated itself over and over again: new antibiotic is introduced and then resistant variants emerge and quickly spread, effectively limiting the utility and lifespan of the drug. Staphylococci are frequently the cause of hospital infections such as infections from implanted medical devices. Many staphylococcal strains have become resistant to many modern day antibiotics. Improved therapies are needed.

[0004] One proposed strategy to overcome the problem of highly virulent and resistant variants is to indirectly attack bacteria by interfering with their means of communication, also known as quorum sensing. Targeting microbial communication makes sense because bacteria coordinate many of their virulence and pathogenesis pathways through these systems. Quave et al., report quorum sensing inhibitors of Staphylococcus aureus from botanical extracts. Planta Med. 2011, 77(02):188-95. See also Quave & Horswill, Front Microbiol, 2014, 5:706.

[0005] Schinus terebinthifolia Raddi (synonym: Schinus terebinthfolius) is a flowering plant in the family Anacardiaceae, which can be found in Brazil, the Caribbean and across the southern United States. It is considered an invasive species in a number of countries. El-Massry et al. report chemical compositions and antioxidant/antimicrobial activities of various samples prepared from Schinus terebinthifolia leaves cultivated in Egypt. J Agric Food Chem, 2009, 57:5265-5270. Moura-Costa et al. report antimicrobial activity of plants used as medicinals on an indigenous reserve in Rio das Cobras, Parana, Brazil. J Ethnopharmacol, 2012, 143:631-638. Melo et al. report alcohol extract of Schinu sterebinthifolia Raddi (Aanacardiaceae) as a local antimicrobial agent in severe autogenously fecal peritonitis in rats. Acta cirurgica brasileira/Sociedade Brasileira para Desenvolvimento Pesquisa em Cirurgia, 2014, 29 Suppl 1:52-56. See also Martius, Systema de Materia Medica Vegetal Brasileira. Rio de Janeiro, 1854; Moreira, Diccionario de Plantas Medicinaes Brasileiras. Rio de Janeiro, 1862; Chernoviz, Formulario ou Guia Medica. 6 ed. Paris, 1864; Burton, Viagens aos planaltos do Brasil—Tomo I: Do Rio de Janeiro a Morro Velho, 1868

SUMMARY

[0007] This disclosure relates to extracts from the cashew family of plants (Anacardiaceae) and compositions comprising one or more compounds contained therein and related uses reported herein. In certain embodiments, the extracts are derived from the fruit of a Schinus plant such as Schinus terebinthifolia.

[0008] In certain embodiments, the disclosure relates to extracts comprising a fruit derived mixture of compounds from a Schinus plant wherein the extracting process comprises one or more of the following steps of: mixing a fruit with an alcohol, e.g., ethanol, methanol, or aqueous mixtures thereof (ethanol:water or methanol:water, 50-95% alcohol, 80% methanol) under conditions such that fruit compounds dissolves in the methanol and removing the methanol providing a methanol derived mixture of compounds; partitioning the methanol derived mixture of compounds between hexane and water providing a water derived mixture of compounds; partitioning the water derived mixture of compounds between ethyl acetate and water providing a second water derived mixture of compounds; partitioning the second water derived mixture of compounds by mixing the second water derived mixture of compounds with n-butanol under conditions such that fruit compounds dissolve in the n-butanol and removing the n-butanol providing an n-butanol derived mixture of compounds; and purifying the n-butanol derived mixture of compounds by liquid chromatography.

[0009] In certain embodiments, the extract comprises a mixture of compounds having at least one component from each of the following groups a) to d): a) a compound having a molecular formula of C30H17O10; b) a compound having a molecular formula of C30H21O10; c) a compound having a molecular formula of C30H45O4; and d) a compound having a molecular formula of C30H45O4.

[0010] In certain embodiments, this disclosure relates to methods of treating or preventing bacterial infections or acne comprising administering to a subject in need thereof or contacting the skin of a subject in need thereof with a formula comprising an extract or one or more compounds in an extract as disclosed herein. In certain embodiments, the formula is administered in combination with another antibiotic.

[0011] In certain embodiments, this disclosure relates to methods of treating or preventing a toxin-mediated bacterial infection comprising administering an effective amount of an Schinus extract or compounds contained therein to a subject in need thereof, including a subject at risk of, exhibiting symptoms of, or diagnosed with a staphylococcal scalded skin syndrome (esp. in neonates), abscesses, necrotizing fasciitis, sepsis, or atopic dermatitis (eczema).

[0012] In certain embodiments, the subject is at risk of, exhibiting symptoms of, or diagnosed with toxic shock syndrome, scalded skin syndrome, abscesses, furuncles, cellulitis, folliculitis, bloodstream infections, medical device infections, pneumonia, osteomyelitis, staphylococcal food poisoning, skin and soft tissue infections, endocarditis, eczema, atopic dermatitis, psoriasis, impetigo, septic arthritis, brain abscess, burn wounds, venous ulcers, diabetic foot ulcers, surgical wounds, post-operation infections, carbuncles, meningitis, bacteremia, necrotizing pneumonia, or necrotizing fasciitis...

Extracts and Compounds

[0072] In certain embodiments, an extract is made by the process of extracting a mixture of compounds from the leaves, roots, bark, stem, fruit, or branches of a Schinus plant such as Schinus terebinthifolia. Other contemplated plants include: Schinus andina and varieties (andina and subtridentata), Schinus angustifolia, Schinus antiarthritica, Schinus areira, Schinus bituminosa, Schinus bonplandiana, Schinus brasiliensis, Schinus bumelioides, Schinus canrerae, Schinus chebataroffi, Schinus chichita, Schinus crenata, Schinus dentata, Schinus dependens and varieties (alfa, arenicola, brevifolia, crenata, grandifolia, longifolia, obovata, ovata, paraguarensis, parvifolia, patagonica, subintegra, tomentosa), Schinus discolor, Schinus diversifolia, Schinus engleri and varieties (engleri, uruguayensis), Schinus fagara, Schinus fasciculate and varieties (arenaria, arenicola, boliviensis, fasciculata), Schinus ferox, Schinus gracilipes and varieties (gracilipies, pilosus), Schinus huigan, Schinus huyngan and varieties (heterophyllus, longifolius, obovatus, subtridentata, undulate), Schinus indicus, Schinus johnstonii, Schinus latifolius and varieties (tomentosus), Schinus lentiscifolius and varieties (angustifolia, flexuosa, subobtusa), Schinus leucocarpus, Schinus limonia, Schinus longifolia and varieties (longifolia, paraguarensis), Schinus marchandii, Schinus maurioides, Schinus mellisii, Schinus meyeri, Schinus microphylla, Schinus microphyllus, Schinus molle and varieties (areira, argentifolius, hassleri, huigan, huyngan, molle, rusbyi), Schinus molleoides, Schinus montanus and varieties (crenuloides, patagonicus), Schinus mucronulatus, Schinus myricoides, Schinus myrtifolia, Schinus occidentalis, Schinus odonellii, Schinus paraguarensis, Schinus patagonicus and varieties (crenuloides, patagonicus), Schinus pearcei, Schinus pilifera and varieties (boliviensis, cabrerae, pilifer), Schinus polygama and varieties (australis, chubutensis, crenata, fasciculata, heterophylla, ovata, parviflora, patagonica), Schinus polygamus, Schinus praecox, Schinus pubescens, Schinus ramboi, Schinus resinosus, Schinus rhoifolia, Schinus roigii, Schinus sinuatus, Schinus spinosus, Schinus tenuifolius, Schinus terebinthifolius and varieties (acutifolia, damaziana, glaziovana, pohlianus, raddiana, rhoifolia, selloana, terebinthifolia, ternifolia), Schinus terebinthifolius, Schinus ternifolia, Schinus tomentosa, Schinus tragodes, Schinus velutinus, Schinus venturii, Schinus weinmannifolius and varieties (angustifolius, dubius, glabrescens, hassleri, intermedius, pauciflorus, paucijuga, pubescens, riedelianus, ridelianus, weinmannifolius) and hybrids thereof.

[0073] In certain embodiments, the extracting process comprises the step of mixing the fruit from the plant with a polar solvent, such as a liquid comprising methanol, ethanol, ethyl acetate, n-butanol, acetonitrile, acetone, methylene chloride or chloroform, under conditions such that a mixture of compounds in the fruit dissolves in the solvent. In certain embodiments, the process further comprises the step of removing the solvent by evaporation from the mixture of compounds. In certain embodiments, the process further comprises the step of purifying the mixture of compounds by liquid chromatography through a solid absorbent, e.g., wherein the solid absorbent comprises silica gel or alumina...

[0078] In certain embodiments, methods of extraction comprise mixing the fruit of a Schinus plant with an water miscible carbon containing solvent, e.g., such as a protic solvent, an alcohol, methanol, ethanol, 1-propanol, 2-propanol, tetrahydrofuran, acetone, acetic acid, 1,4-dioxane or mixture providing a concentrate with a mixture of compounds and substantially removing the solvent from the concentrate, purifying the solvent derived concentrate to less than 5%, 1%, or 0.5% by weight of the solvent used in the extraction, e.g., evaporating the protic solvent and/or optionally in combination with mixing the concentrate with water, sonicating the water, freezing the water to provide ice, and removing the ice by sublimation (e.g. in a vacuum of low pressure) wherein said purification methods may be repeated in combination. In certain embodiments, the method further comprises suspending the solvent derived concentrate in water and optionally extract impurities in a hydrocarbon solvent such as cyclohexane, heptane, hexane, pentane, 2,2,4-trimethylpentane, separating the hydrocarbon from the water providing a water layer. In certain embodiments, the method further comprises mixing the water layer with a solvent that is immiscible in water (polar and/or aprotic), e.g., such as ethyl acetate, diethyl ether, methyl tertbutyl ether, n-butanol, toluene, methylene chloride, carbon tetrachloride, 1,2-dichloroethant, and/or chloroform, and purifying the solvent to provide a second solvent derived concentrate. In further embodiments, the second derived concentrate is purified one or more times by liquid chromatography, e.g., normal phase chromatography...

EXAMPLES

Collection of Plant Material

[0161] Schinus terebinthifolia Raddi, Anacardiaceae leaves, stems, and fruits were collected in bulk from private lands in DeSoto County, Fla. in November of 2013 and 2014 after obtaining permission from the land owner. Procedures from the 2003 WHO Guidelines for good agricultural and collection practices (GACP) for medicinal plants were followed for the collection and identification of bulk and voucher specimens, specifically excluding any populations that may have prior exposure to herbicides. Vouchers were deposited at the Emory University Herbarium (GEO) (Voucher CQ-400, GEO Accession No. 020063) and were identified using the standard Flora for Florida. Plant leaves, stems, and fruits were separated and manually cleaned of soil and contaminants. Plant material was then dried in a desiccating cabinet at low heat. Once dry, plant material was sealed in paper bags and stored at room temperature until further processing.

Extraction and Separation.

[0162] Crude methanol extracts of fruits were created by blending a ratio of 1 g dry material:10 mL MeOH into a slurry in a Waring commercial blender for 5 min, and sonicating the material for 20 minutes. Following decantation of the extract, plant material was subjected to two more rounds of sonication followed by filtration. Filtered extracts were combined, concentrated at reduced pressure with rotary evaporators (<40° C.), and lyophilized. The dried extract was resuspended in 1:5 MeOH:H2O at 1 g:31 mL and underwent sequential liquid-liquid partitioning three times each with an equal volume of hexane, EtOAc, and H2O saturated n-butanol. The organic partitions were dried over Na2SO4 and filtered. Each partition was concentrated in vacuo at <40° C. The hexane partition was dissolved and transferred to a tared scintillation vial and dried under forced air to yield 430B. The remaining partitions were suspended in dH2O, shell frozen, lyophilized and stored at −20° C. The EtOAc partition was labeled 430C, the n-butanol 430D, and final remaining aqueous partition 430E...

[Excerpts]

Botanical Extracts and Compounds from Castanea Plants and Methods of Use
US2016375074

This disclosure relates to extracts from chestnut plants and compositions comprising compounds contained therein. In certain embodiments, the extracts are derived from the leaves of a Castanea plant. In certain embodiments, the disclosure relates to methods of treating or preventing bacterial infections, acne, and other related uses...

BACKGROUND

[0003] Since the widespread introduction of antibiotics in the 1940s, the same storyline has repeated itself over and over again: new antibiotic is introduced and then resistant variants emerge and quickly spread, effectively limiting the utility and lifespan of the drug. From an evolutionary biology perspective, this is not surprising; indeed, resistant mutants are expected to arise when any lifeform with the ability to rapidly reproduce and mutate is faced with a direct selective pressure, especially when a single drug is used against a single target. Staphylococci are frequently the cause of hospital infections such as infections from implanted medical devices. Many Staphylococci strains have become resistant to many modern day antibiotics. Improved therapies are needed.

[0004] One proposed strategy to overcome the problem of resistant variants is to indirectly attack bacteria by interfering with their means of communication, also known as quorum sensing. Targeting microbial communication makes sense because bacteria coordinate many of their virulence and pathogenesis pathways through these systems. Quave et al., report quorum sensing inhibitors of Staphylococcus aureus from botanical extracts. Planta Med. 2011, 77(02):188-95.

[0005] Castanea sativa (chestnut) is a flowering plant in the family Fagaceae which can be found in Europe. See Braga et al., Nat Prod Res., 2015, 29(1):1-18. Almeida et al. report in vivo skin irritation potential of a Castanea sativa (Chestnut) leaf extract. Basic & Clinical Pharmacol Toxicol, 2008, 103(5):461-7. See also Almeida et al. J Photochem Photobiol B: Biol, 2015, 144(0):28-34. Henry et al. report cosmetic compositions containing an extract of leaves of the Castanea sativa plant and cosmetic treatments. U.S. Pat. No. 8,067,044 (2011).

[0006] Garo et al., report asiatic acid and corosolic acid enhance the susceptibility of Pseudomonas aeruginosa biofilms to tobramycin. Antimicrob Agents Chemother, 2007, 51(5):1813-7. See also Rangasamy et al. South African J Botany, 2014, 93:198-203.

[0007] Wong et al. report aqueous methanolic extracts of Melastoma malabathricum L. exhibited antibacterial activity. Nat Prod Res, 2012,26(7):609-18

[0008] Perioni et al. report a survey on the natural ingredients used in folk cosmetics, cosmeceuticals and remedies for healing skin diseases. J Ethnopharmacol, 2004, 91(2-3):331-44.

[0009] References cited herein are not an admission of prior art.

SUMMARY

[0010] This disclosure relates to extracts from chestnut plants and compositions comprising one or more compounds contained therein and related uses reported herein. In certain embodiments, the extracts are derived from the leaves of a Castanea plant such as Castanea sativa.

[0011] In certain embodiments, the disclosure relates to extracts comprising a leaf derived mixture of compounds from a Castanea plant wherein the extracting process comprises one or more of the following steps of: mixing a leaf with methanol under conditions such that leaf compounds dissolves in the methanol and removing the methanol providing a methanol derived mixture of compounds; partitioning the methanol derived mixture of compounds in hexane and water providing a water derived mixture of compounds; partitioning the water derived mixture of compounds by mixing the water with ethyl acetate under conditions such that leaf compounds dissolve in the ethyl acetate and removing the ethyl acetate providing an ethyl acetate derived mixture of compounds; and purifying the ethyl acetate derived mixture of compounds by liquid chromatography through silica with a mobile phase comprising hexane and ethyl acetate; wherein the mobile phase comprises increasing amounts of ethyl acetate, and a mobile phase fraction is isolated comprising a leaf derived mixture of compounds which does not contain chlorogenic acid, ellagic acid, hyperoside, isoquercitrin, or rutin.

[0012] In certain embodiments, this disclosure relates to methods of treating or preventing a bacterial infections or acne comprising administering to a subject in need thereof or contacting the skin of a subject in need thereof with a formula comprising an extract or one or more compounds in an extract as disclosed herein. In certain embodiments, the formula is administered in combination with another antibiotic...

Chestnut Leaf Extracts Block Staphylococcus aureus Virulence and Pathogenesis

[0063] Quorum quenching activity has been discovered in the natural products extracted from Castanea sativa leaves. The extract is able to attenuate virulence by quenching S. aureus agr-mediated quorum sensing, effectively blocking production of harmful exotoxins at sub-inhibitory concentrations for growth. Experiments indicate a lack of cytotoxicity to human skin cells, lack of growth inhibitory activity against the normal skin microflora, lack of resistance development, and efficacy in a skin abscess animal model.

[0064] Staphylococcus aureus is an abundant, opportunistic pathogen that is the causative agent of numerous infections. Due to its prevalence as a leading cause of healthcare-associated infection, and its highly multidrug resistant nature, S. aureus is a serious threat. It colonizes the nasal passages of approximately 30% of the healthy adult population. S. aureus infections initiate through trauma to the skin or mucosal layer and then progress through an invasive or toxin-mediated process. The prevalence of these infections has increased due to higher rates of immunosuppressive conditions, greater use of surgical implants, and dramatic increases in antibiotic resistance.

[0065] S. aureus produces an extensive array of enzymes, hemolysins, and toxins that are important to its ability to spread through tissues and cause disease. These virulence factors serve a wide scope of purposes in the infection process, including disruption of the epithelial barrier, inhibition of opsonization by antibody and complement, neutrophil cytolysis, interference with neutrophil chemotaxis, and inactivation of antimicrobial peptides. The expression of all of these invasive factors is controlled by cell-density quorum sensing using the autoinducing peptide (AIP) molecule. Like other quorum-sensing signals, AIP accumulates outside the cell until it reaches a critical concentration and then binds to a surface receptor called AgrC, initiating a regulatory cascade. Since AIP controls the expression of accessory factors for S. aureus, this regulatory system has been named the accessory gene regulator (agr), and the majority of the proteins necessary for this quorum-sensing system to function are encoded in the agr chromosomal locus. Applying inhibitors to quench this communication system to attenuate pathogenicity and virulence lies at the core of the quorum quenching approach...

Extracts and Compounds

[0086] In certain embodiments, an extract is made by the process of extracting a mixture of compounds from the leaves, roots, bark, stem, or branches of a Castanea plant e.g., Castanea sativa. Other contemplated plants include: Castanea acuminatissima, Castanea alabamensis, Castanea alnifolia, Castanea americana, Castanea argentea, Castanea argyrophylla, Castanea arkansana, Castanea armata, Castanea ashei, Castanea blaringhemii, Castanea bodinieri, Castanea brevicuspis, Castanea bungeana, Castanea burbankii, Castanea buruana, Castanea californica, Castanea Castanea, Castanea castanicarpa, Castanea castenea var. pubinervis, Castanea chincapin, Castanea chinensis, Castanea chrysophylla, Castanea concinna, Castanea cooperta, Castanea costata, Castanea coudersii, Castanea crenata, Castanea davidii, Castanea dentata, Castanea diversifolia, Castanea dovaricata, Castanea duclouxii, Castanea echidnocarpa, Castanea edonii, Castanea edwii, Castanea endicottii, Castanea eonii, Castanea fagus, Castanea falconeri, Castanea fargesii, Castanea fauriei, Castanea fleetii, Castanea floridana, Castanea formosana, Castanea furfurella, Castanea glomerata, Castanea henryi, Castanea henryi, Castanea hupehensis, Castanea hystrix, Castanea, Castanea inermis, Castanea japonica, Castanea javanica, Castanea kusakuri, Castanea lanceifolia, Castanea latifolia, Castanea margaretta, Castanea martabanica, Castanea microcarpa, Castanea mollissima, Castanea montana, Castanea morrisii, Castanea nana, Castanea neglecta, Castanea ozarkensis, Castanea paucispina, Castanea phansipanensis, Castanea prolifera, Castanea pubinervis, Castanea pulchella, Castanea pumila, Castanea purpurella, Castanea regia, Castanea rhamnifolia, Castanea rockii, Castanea roxburghii, Castanea seguinii, Castanea sempervirens, Castanea sessilifolia, Castanea sinensis, Castanea sloanea, Castanea spectabilis, Castanea sphaeroarpa, Castanea sphaerocarpa, Castanea stricta, Castanea sumatrana, Castanea tribuloides, Castanea tungurrut, Castanea vesca, Castanea vilmoriniana, Castanea vulgaris, Castanea wattii and hybrids thereof.

[0087] In certain embodiments, the extracting process comprises the step of mixing the leaf from the plant with a polar solvent, such as a liquid comprising methanol, ethanol, ethyl acetate, acetonitrile, acetone, methylene chloride or chloroform, under conditions such that a mixture of compounds in the leaf dissolves in the solvent. In certain embodiments, the process further comprises the step of removing the solvent by evaporation from the mixture of compounds. In certain embodiments, the process further comprises the step of purifying the mixture of compounds by liquid chromatography through a solid absorbent, e.g., wherein the solid absorbent comprises silica gel or alumina.

[0088] In certain embodiments, the disclosure relates to extracts comprising a leaf derived mixture of compounds from a Castanea plant wherein the extracting process comprises the steps of: mixing a leaf with methanol under conditions such that leaf compounds dissolves in the methanol and removing the methanol providing a methanol derived mixture of compounds; partitioning the methanol derived mixture of compounds in hexane and water providing a water derived mixture of compounds; partitioning the water derived mixture of compounds by mixing the water with ethyl acetate under conditions such that leaf compounds dissolve in the ethyl acetate and removing the ethyl acetate providing an ethyl acetate derived mixture of compounds; and purifying the ethyl acetate derived mixture of compounds by liquid chromatography through silica with a mobile phase comprising hexane and ethylene acetate; wherein the mobile phase comprises increasing amounts of ethyl acetate, and a mobile phase fraction is isolated comprising a leaf derived mixture of compounds which does not contain chlorogenic acid, ellagic acid, hyperoside, isoquercitrin, or rutin...

[0089] Chromatography refers to the separation of a mixture of compounds dissolved in a fluid called the mobile phase, which carries the compounds through a structure holding another material called the stationary phase. The various compounds or components of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a partition coefficient of each compound result in differential retention on the stationary phase and thus changing the separation.

[0090] In normal-phase chromatography, the stationary phase is polar. In reversed phase, the stationary phase is nonpolar. Typical stationary phases for normal-phase chromatography are silica or organic moieties with cyano and amino functional groups. For reversed phase, alkyl hydrocarbons are the preferred stationary phase. Examples are solid supports containing a surface conjugated with a hydrocarbon chain, e.g., octadecyl (C18), octyl (C8), and butyl (C4).

[0091] In normal-phase chromatography, the least polar compounds elute first and the most polar compounds elute last. The mobile phase typically consists of a nonpolar solvent such as hexane or heptane mixed with a slightly more polar solvent such as isopropanol, ethyl acetate or chloroform. Retention to the stationary phase decreases as the amount of polar solvent in the mobile phase increases. In reversed phase chromatography, the most polar compounds elute first with the most nonpolar compounds eluting last. The mobile phase is generally a binary mixture of water and a miscible polar organic solvent like methanol, acetonitrile or THF.

[0092] In certain embodiments, methods of extraction comprise mixing leaves of a Castanea plant with an water miscible carbon containing solvent, e.g., such as a protic solvent, an alcohol, methanol, ethanol, 1-propanol, 2-propanol, tetrahydrofuran, acetone, acetic acid, 1,4-dioxane or mixture providing a concentrate with a mixture of compounds and substantially removing the solvent from the concentrate, purifying the solvent derived concentrate to less than 5%, 1%, or 0.5% by weight of the solvent used in the extraction, e.g., evaporating the protic solvent and/or optionally in combination with mixing the concentrate with water, sonicating the water, freezing the water to provide ice, and removing the ice by sublimation (e.g. in a vacuum of low pressure) wherein said purification methods may be repeated in combination. In certain embodiments, the method further comprises suspending the solvent derived concentrate in water and optionally extract impurities in a hydrocarbon solvent such as cyclohexane, heptane, hexane, pentane, 2,2,4-trimethylpentane, separating the hydrocarbon from the water providing a water layer. In certain embodiments, the method further comprises mixing the water layer with a solvent that is immiscible in water (polar and/or aprotic), e.g., such as ethyl acetate, diethyl ether, methyl tertbutyl ether, toluene, methylene chloride, carbon tetrachloride, 1,2-di chl oroethant, and/or chloroform, and purifying the solvent to provide a second solvent derived concentrate. In further embodiments, the second derived concentrate is purified one or more times by liquid chromatography, e.g., normal phase chromatography. Typically the solid absorbent is polar such as silica. In certain embodiments, the extract is a portion isolated after the column solvent is more than 50% ethyl acetate in hexane...

Extraction and Purification of QSI-containing Fractions

[0181] Crude methanol extracts (Extract 224) of the ground leaves were created by maceration of the plant materials at room temperature using a ratio of 1 g dry leaves: 10 mL MeOH for two successive periods of 72 hours, with daily agitation. Filtered extracts were combined, concentrated at reduced pressure and a temperature<40° C. with rotary evaporators, and lyophilized before being re-suspended in water and partitioned in succession with hexane, ethyl acetate and butanol. The resulting non-aqueous partitions were dried over anhydrous Na2SO4, concentrated in vacuo, and lyophilized before testing for activity.

[0182] The most active partition (ethyl acetate, extract 224C) was subjected to further fractionation using a CombiFlash® Rf+ (Teledyne ISCO) flash chromatography system using a RediSep Rf Gold silica column. Extract 224C was bonded to Celite 545 (Acros Organics) at a 1:4 ratio and dry-loaded using a RediSep dry load cartridge. The mobile phase consisted of (A) hexane, (B) EtOAc, and (C) MeOH. The linear gradient begins with 100% A for 6.3 column volumes (CV), then 50:50 A:B at 25.3 CV, to 100% B at 63.3 CV, which is held till 69.6 CV, then to 70:30 B:C at 88.6 CV which is held till 94.9 CV. The chromatography was monitored at 254 and 280 nm, as well as via ELSD. The resulting fractions were combined into 5 fractions. Following further bioassay testing, it was determined that the fraction which eluted from 30-40 CV (224C-F2) was most active. The full extract fractionation scheme is presented in FIG. 2...

[Excerpts]

ANTI-BIOFILM COMPOSITIONS AND METHODS FOR USING
US2012088671

FIELD OF THE INVENTION

[0002] The present invention generally relates to biofilms. In particular, it relates to compositions and methods for inhibiting biofilm formation and/or reducing the growth of an established biofilm.

BACKGROUND OF THE INVENTION

[0003] Staphylococcus aureus is arguably the most problematic pathogen faced by modern healthcare systems today, owing in large part to the persistent emergence of antibiotic resistant strains. This is perhaps most evident in the recent appearance of methicillin-resistant strains even among isolates causing community-acquired infection. Moreover, many of these strains, most notably those of the USA300 clonal lineage, have the capacity to cause serious, life-threatening infection even in otherwise healthy individuals. This accounts in large part for the observation that, in the United States alone in 2005, an estimated 94,360 patients suffered from invasive infection caused by methicillin-resistant S. aureus (MRSA), with approximately 18,650 resulting in a fatal outcome.

[0004] The continued emergence of antibiotic-resistant strains has created an urgent need for new antimicrobial agents. However, many S. aureus infections are recalcitrant to antimicrobials even in the absence of issues related to acquired resistance. A primary contributing factor to this recalcitrance is formation of a biofilm on both native tissues and indwelling medical devices. This is due to the fact that the biofilm confers a degree of intrinsic resistance that often necessitates surgical intervention to debride infected tissues and/or remove infected devices. For example, one study found that nearly half of patients with implanted orthopedic devices admitted to a hospital with S. aureus bacteremia had developed an implant-associated infection. Thus, while there is an urgent need for new antibiotics, there is an equally urgent need to develop therapeutic agents that could be used to limit biofilm formation. While such agents would not necessarily function as antibiotics in and of themselves, they could be used as a prophylactic to limit biofilm formation (e.g. coating for implanted devices, surgical lavage, or pre-operative oral prophylaxis) or as a therapeutic to be used in conjunction with more conventional antibiotics to treat an established biofilm-associated infection.

SUMMARY OF THE INVENTION

[0005] Among the various aspects of the present disclosure is the provision of a polyphenolic composition. The polyphenolic composition is prepared by a process comprising (a) partitioning an alcohol extract of a plant with a mixture of water and hexane to form a first water partition and a hexane partition; (b) partitioning the first water partition with a mixture of water and ethyl acetate to form a second water partition and a ethyl acetate partition; (c) partitioning the second water partition with a mixture of water and butanol to form a third water partition and a butanol partition; and (d) fractionating the butanol partition by column chromatography with a mobile phase comprising a mixture of methanol and dichloromethane, wherein the polyphenolic composition is eluted by the mobile phase in which the volume ratio of methanol to dichloromethane is about 40:60.

[0006] Another aspect of the disclosure provides a combination comprising at least one phenolic phytochemical and at least one antimicrobial agent.

[0007] Still another aspect of the disclosure encompasses a method for inhibiting formation of a biofilm. The method comprises contacting a plurality of free floating microorganisms with the polyphenolic composition detailed above or a fraction thereof such that formation of the biofilm is inhibited.

[0008] A further aspect of the disclosure provides a method for inhibiting growth of an established biofilm. The method comprises contacting the biofilm with at least one phenolic phytochemical and at least one antimicrobial agent such that the biofilm has a reduced number of microorganisms.

[0009] Other features and iterations of the invention are described in more detail below...

DETAILED DESCRIPTION OF THE INVENTION

[0023] The present invention provides compositions and methods for inhibiting the formation and growth of biofilms. In one aspect, the disclosure provides a polyphenolic composition comprising ellagic acid and ellagic acid derivatives. The phenolic composition is derived from a plant extract by a process disclosed herein. It has been discovered that the polyphenolic composition inhibits biofilm formation and increases susceptibility of an established biofilm to antimicrobial agents. Another aspect of the disclosure provides a combination comprising at least one phenolic phytochemical and at least one antimicrobial agent, wherein the combination inhibits the growth of established biofilms. Advantageously, the activity of the combination disclosed herein is synergistic, i.e., its activity is more than the sum of the activity of each individual component. Also provided herein are methods for inhibiting the formation of a biofilm, as well as methods inhibiting the growth of an established biofilm.

[0024] (I) Polyphenolic Composition

[0025] In one embodiment a polyphenolic composition is provided. The polyphenolic composition is prepared by a process comprising (a) partitioning an alcohol extract of a plant with a mixture of water and hexane to form a first water partition and a hexane partition; (b) partitioning the first water partition with a mixture of water and ethyl acetate to form a second water partition and a ethyl acetate partition; (c) partitioning the second water partition with a mixture of water and butanol to form a third water partition and a butanol partition; and (d) fractionating the butanol partition by column chromatography with a mobile phase comprising a mixture of methanol and dichloromethane, wherein the polyphenolic composition is eluted by the mobile phase in which the volume ratio of methanol to dichloromethane is about 40:60.

[0026] The method comprises a series of steps such that a fraction enriched with a polyphenolic composition may be isolated from an alcohol extract of a plant.

(a) Alcohol Extract

[0028] The alcohol extract may be derived from a plant belonging to a variety of plant families. Non-limiting examples of suitable plant families include Rosaceae, Fagaceae, Salicaceae, Myrtaceae, Vitaceae, Ericaceae, Combretaceae, Elaeocarpaceae, Lythraceae, Symplocaceae, Hypoxidaceae, Amaranthaceae, Juncaceae, Juglandaceae, Sapindaceae, Lamiaceae, Magnoliaceae, Gentianaceae, Apocynaceae, Moringaceae, Apiaceae, Rutaceae, Aquafoliaceae, Santalaceae, Cornaceae, Asteraceae, Bignoniaceae, and Fabaceae. Preferred plant families include Rosaceae, Fagaceae, Salicaceae, Myrtaceae, Vitaceae, Ericaceae, Combretaceae, and Juglandaceae. In some embodiments, the plant may be Castanea sativa, Quercus cerris, Juglans regia, Vitis vinefera, Crataegus monogyna, Prunus spinosa, Rosa canina, or Rubus ulmifolius. In some embodiments, the plant family may be Rosaceae. In an exemplary embodiment, the plant may be Rubus ulmifolius.

[0029] A variety of plant parts may be used to arrive at the alcohol extract. Suitable plant parts include roots, bulbs, tubers, leaves, basal leaves, stems, stem nodes, stem internodes, galls, stalks, woody parts, flowers, inflorescences, fruits, infructescences, seeds, and combinations thereof. The plant part may be fresh, dried, frozen, or lyophilized. The plant part may be ground or pulverized into a plant material using a homogenizer, a blender, a mortar and pestle, a sonicator, or a similar apparatus.

[0030] The plant extract typically is prepared by contacting the plant material with an alcohol solvent for an appropriate period of time. Non-limiting examples of suitable alcohol solvents include methanol, ethanol, propanol, butanol, or combinations thereof. In preferred embodiments, the solvent may be ethanol such that the alcohol extract is an ethanol extract. The concentration of alcohol that is contacted with the plant material may range from about 1% to about 100%. In embodiments in which ethanol is the solvent, the concentration of ethanol may range from about 1% to about 20%, from about 20% to about 40%, from about 40% to about 60%, from about 60% to about 80%, or from about 80% to about 100%. In an exemplary embodiment, the concentration of ethanol may be about 95%.

[0031] The period of time the plant material is contacted with the alcohol solvent may range from about 1 hour to about 5 days. In various embodiments, the plant material may be contacted with the alcohol solvent for about 1-24 hours, for about 24-48 hrs, for about 48-72 hours, for about 72-96 hours, or for about 96-120 hours. In an exemplary embodiment, the period of time the plant material is contacted with the alcohol solvent may be about 72 hours: Upon removal of the extract from the plant material, the plant material may be extracted one or more additional times with fresh alcohol solvent, essentially as detailed above.

[0032] The alcohol solvent may be removed from the plant alcohol extract to form a dry plant alcohol extract. Those of skill in the art are familiar with suitable techniques to remove the alcohol solvent including, without limit, evaporation, distillation, and lyophillization.

(b) Liquid Extractions

[0034] The process for preparing a fraction rich in the polyphenolic compounds comprises subjecting the plant alcohol extract to a series of liquid extractions such that the polyphenolic compounds are partitioned into one of the phases and the other compounds are partitioned into the other phase. In general, the series of liquid extractions comprises contacting the plant alcohol extract (or partition thereof) with a solvent system, wherein the polarity of one or more of the solvents changes during each successive series of extractions. Those of skill in the art are familiar with liquid extraction protocols and suitable solvent systems. Generally, the liquids are mixed by gentle inversion at room temperature. After separation of the phases, the phase containing the polyphenolic compounds thereof may be extracted one or more times with the solvents of interest.

[0035] For example, the first step of the process may comprise a liquid extraction during which the plant alcohol extract is partitioned in a mixture of water and an alkane to form a first water partition and an alkane partition. Typically, the polyphenolic compounds are partitioned into the water phase upon extraction with a mixture of water and alkane. The alkane typically will comprise from five to ten carbons, and may be linear or branched. Suitable alkanes include, without limit, pentane, hexane, heptane, octane, and combinations thereof. An exemplary alkane is hexane.

[0036] In the next step of the process, for example, the first water partition may be extracted in a mixture of water and a non-polar solvent to form a second water partition and a non-polar solvent partition. Generally, the polyphenolic compounds are partitioned into the water phase upon extraction with a mixture of water and non-polar solvent. In various embodiments, the non-polar solvent may be ethyl acetate, butyl acetate, chloroform, diethyl ether, or combinations thereof. An exemplary non-polar solvent is ethyl acetate.

[0037] The next and final extraction step, for example, may comprise extracting the second water partition with a mixture of water and an alcohol to form a third water partition and an alcohol partition. Typically, the polyphenolic compounds are partitioned into the alcohol phase upon extraction with a mixture of water and alcohol. The alcohol may comprise from one to ten carbons, and may be linear or branched. Non-limiting examples of suitable alcohols include methanol, ethanol, propanol, isopropanol, butanol, pentanol, hexanol, and heptanol. An exemplary alcohol is butanol.

[0038] In a preferred embodiment, the first liquid-liquid extraction comprises water and hexane, the second liquid-liquid extraction comprises water and etliyl acetate, and the third liquid-liquid extraction comprises water and butanol.

(c) Column Chromatography

[0040] The method may further comprise fractionating the polyphenolic-rich fraction by column chromatography. Typically, the column will comprise an inorganic stationary phase. Non-limiting examples of suitable inorganic stationary phase materials include silica-based materials, silica gel, magnetic silica particles, glass powder, diatomaceous earth, zeolites, aluminium oxides, silicon oxides, titanium oxides, zirconium oxides, and hydroxyapatite. In an exemplary embodiment, the column chromatography comprises a silica gel stationary phase.

[0041] The mobile phase may comprise a mixture of methanol and dichloromethane. Those of skill in the art will appreciate that other mobile phases may be used to separate the polyphenolic composition from the other compounds. In embodiments in which the mobile phase comprises methanol and dichloromethane, the concentration of dichloromethane in the mobile phase typically decreases during the fractionation while the concentration of methanol in the mobile phase increases during the fractionation. The phenolic-rich fraction generally elutes from the column with a volume ratio of methanol to dichloromethane from about 30:70 to about 70:30. For example, the volume ratio of methanol to dichloromethane that elutes a phenolic-rich fraction may range from about 30:70, 32.5:67.5. 35:65, 37.5:62.5, 40:60, 42.5:57.5, 45:55, 47.5:52.5, 50:50, 52.5:47.5, 55:45, 57.5:42.5, 60:40, 62.5:37.5, 65:35, 67.5:32.5, or 70:30. In preferred embodiments, the polyphenolic-rich fraction may elute from the column at a volume ratio of methanol to dichloromethane of about 40:60, 50:50, or 60:40. In an exemplary embodiment, the polyphenolic-rich fraction may elute from the column at a volume ratio of methanol to dichloromethane of about 40:60. The polyphenolic-rich fraction may be dried by removing the mobile phase solvents using standard procedures...

Related Patents

CN105267752
Natural plant bacteriostatic agent and preparation method thereof

Inventor(s): WANG ZHENYU +

A natural plant bacteriostatic agent and a preparation method thereof are provided. The natural plant bacteriostatic agent comprises following materials by weight: Flos Lonicerae 300-350 parts, Fructus Forsythiae 300-350 parts, Herba Pogostemonis 200-220 parts, Folium Mori 100-110 parts, mint 100-120 parts, Folium Artemisiae Argyi 100-120 parts, Radix Arnebiae seu Lithospermi 200-210 parts, Radix Glycyrrhizae 100-110 parts, Rhizoma Phragmitis 100-110 parts, sodium benzoate 3-4 parts, and flavoring orange essence 20-24 parts. The natural plant bacteriostatic agent is prepared by: primary decocting, secondary decocting, tertiary decocting, concentrating, alcohol precipitation and separation, concentrating, and preparing. The invention has the advantages that the natural plant bacteriostatic agent is made from herbs, has improved bacteriostatic effect, has little side effect on human body and is nonirritating to the skin; the natural plant bacteriostatic agent is applicable to skin and mouth to play a good role in inhibiting Staphylococcus aureus, Escherichia coli and the like, is nonirritating to the skin and mouth, and is free of drug resistance; compared with market bacteriostatic agents, the natural plant bacteriostatic agent has a wide range of action and is safer and nonirritating.

CN105267273
Natural skin-protecting wound disinfectant and preparation method thereof

Inventor(s): LI QINGYUAN +

The invention relates to a skin-protecting wound disinfectant. The skin-protecting wound disinfectant is prepared from 1%-5% of chitosan oligosaccharide, 30%-40% of medical ethanol, 5%-20% of sweet wormwood herb extracting solution, 5%-30% of moringa leaf extracting solution, 0.5%-5% of moringa seed oil and the balance deionized water. According to the skin-protecting wound disinfectant, the chitosan oligosaccharide is dissolved in the moringa leaf extracting solution and the sweet wormwood herb extracting solution and resists bacteria by cooperating with sweet wormwood herbs, moringa seeds and low-concentration ethanol, the chitosan oligosaccharide does not settle in the low-concentration ethanol, and the antibacterial effectiveness of the disinfectant is guaranteed. Significant sterilization and disinfection effects on pathogenic bacteria such as common staphylococcus, streptococcus and escherichia coli, common cold viruses and the like are achieved without adding antibiotics or other chemosynthetic antibiotic constituents, and moringa leaves and moringa seeds both have significant skin-protecting and moisturizing effects. Therefore, by means of the skin-protecting wound disinfectant, a moistening use feeling is achieved, a protection effect on skins is achieved, the antibacterial and skin-protecting effects are lastingly effective, toxic and side effects to human bodies do not exist, and pollution to the environment does not exist.

CN102600053
Chinese medicinal herb-inorganic antibacterial agent composite sterilization hand sanitizer and preparation

Inventor(s): ZUNLI MO; HAO GOU; XIAOYING MA; JINGXIAN HE; CHAO FENG +

The invention provides a Chinese medicinal herb-inorganic antibacterial agent composite sterilization hand sanitizer, belonging to the field of chemicals for daily use. According to the Chinese medicinal herb-inorganic antibacterial agent composite sterilization hand sanitizer provided by the invention, extractives of Artemisia argyi leaf, Artemisia annua, Scutellaria baicalensis, Punica granatumpericarp and Glycyrriza uralensis are served as a composite Chinese medicinal herb antibacterial agent, and nanometer silver oxide is served as the inorganic antibacterial agent, as a result, the potent sterilization ability of the nanometer silver oxide is combined with the long-term sterilization effect of the Chinese medicinal herbs to form a novel sterilization hand sanitizer with potent, long-term sterilization and bacteriostasis functions.; According to the bacteriostatic tests of Escherichia coli, staphylococcus aureus and Bacillus

subtilis, the Chinese medicinal herb-inorganic antibacterial agent composite sterilization hand sanitizer is good in performance of sterilization and bacteriostasis.

CN1217927
Preparing method and prescription for Chinese medicinal herbs Nongerdan for otopyosis

Inventor(s): SUN SHUNXIAO

A medicine in the form of pill for treating pyogenic tympanitis is prepared from websterite and multiple Chinese-medicinal materials and features high curative effect. It has the suppression action to staphylococcus aureus, proteus, colibacillus, Bacillus anthracis...
