THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 20, Number 5, 2014, pp. 375–382

DOI: 10.1089/acm.2013.0036

A Comparison of the Anti-*Staphylococcus aureus* Activity of Extracts from Commonly Used Medicinal Plants

Rebecca Snowden, ND,[1] Heather Harrington, ND,[1,2] Kira Morrill, ND,[1] LaDeana Jeane, ND,[1] Joan Garrity, ND,[1] Michael Orian, ND,[1] Eric Lopez, ND,[1] Saman Rezaie, ND,[1] Kelly Hassberger, ND,[1] Damilola Familoni, ND,[1] Jessica Moore, ND,[1] Kulveen Virdee, ND,[1] Leah Albornoz-Sanchez, ND,[1] Michael Walker, ND,[1] Jami Cavins, ND,[1] Tonyelle Russell, ND,[1] Emily Guse, ND,[1] Mary Reker, ND,[1] Onyria Tschudy, ND,[1] Jeremy Wolf, ND,[1] Teresa True, ND,[1] Oluchi Ukaegbu, ND,[1] Ezenwanyi Ahaghotu, ND,[1] Ana Jones, ND,[1] Sara Polanco, ND,[1] Yvan Rochon, PhD,[1,3] Robert Waters, PhD,[1,2] and Jeffrey Langland, PhD[1,2]

Abstract

Background: Resurgences of *Staphylococcus aureus* infection continue globally, with antibiotic resistance increasing dramatically, making these infections more difficult to treat. *S. aureus* epidemics impose public health threats, and economic burdens on health care costs worldwide, presenting challenges modern medicine struggles to control.

Objective: In order to answer today's call for effective treatments against *S. aureus*, we evaluated and compared various botanical extracts that have historically been suggested as useful for their antimicrobial properties against *S. aureus.*

Design: Briefly, *S. aureus* cultures were treated with selected botanical extracts and the minimum inhibitory concentration (MIC) determined. In addition, to obtain more quantitative measures on bacterial growth, 24-hour growth studies were done to examine the temporal activity and stability of various botanicals on bacterial replication.

Results: The antimicrobial activity observed for the botanical extracts used in this comparative evaluation of efficacy included both bacteriostatic and bacteriocidal activity against *S. aureus*. Highly effective botanicals including *Salvia officinalis*, *Eucalyptus globulus*, *Coleus forskohlii*, *Coptis chinensis, Turnera diffusa*, and *Larrea tridentata* exhibited MIC values ranging from 60 to 300 μg/mL and a 10^6-fold reduction in bacterial replication. *Arctostaphylos uva-ursi* and *Allium sativum* were slightly less effective, exhibiting MIC values ranging from 90 to 400 μg/mL and a 10^5-fold reduction, while *Anemopsis californica* gave MIC value of 360 μg/mL and a 10^4-fold reduction in bacterial replication. Many botanicals, especially at lower doses, had an initial inhibitory effect followed by a recovery in bacterial replication. Such botanicals included *E. globulus*, *C. chinensis*, *T. diffusa*, *A. californica*, and *Berberis vulgaris*.

Conclusions: Our data demonstrate that *S. officinalis, E. globulus, C. forskohlii, A. uva-ursi, C. chinensis, T. diffusa, A. californica, A. sativum,* and *L. tridentata* all show promising direct antimicrobial activity against *S. aureus.* For many of these botanicals, strong bacteriocidal activity was observed at higher concentrations, but even at lower concentrations, bacteriostatic activity was evident. Other botanicals including *B. vulgaris, Baptisia tinctoria*, and *Glycyrrhiza glabra* showed moderate activity against *S. aureus*, while *Schisandra chinensis, Echinacea angustifolia*, and *Polygonum multiflorum* were shown to be ineffective.

Introduction

Prior to the 1940s, the United States saw an incidence of mortality associated with *Staphylococcus aureus* infections >80%. With the introduction of antibiotics, that percentage dramatically decreased and remained reduced for many years. However, new challenges have evolved to deal with the repeated resurgences of antibiotic-resistant *S. aureus* infections that continue to impose both public health threats and economic burdens on health care costs.[1] Infections commonly caused by *S. aureus* include skin and soft-tissue infections, toxic shock syndrome, endocarditis,

[1]Department of Naturopathic Research, Southwest College of Naturopathic Medicine, Tempe, AZ.
[2]Biodesign Institute, Arizona State University, Tempe, AZ.
[3]Herbal Vitality, Inc., Sedona, AZ.

osteomyelitis, urinary tract infections, and gastrointestinal infections. As many *S. aureus* strains have become resistant to antibiotics over time, we now have essentially two categories of bacterial strains: (1) methicillin-resistant *S. aureus* (MRSA), which are resistant to all ß-lactam antibiotics and (2) methicillin-sensitive *S. aureus* (MSSA), which are strains that have not been able to develop such resistance.[2] Methicillin is a narrow-spectrum ß-lactam antibiotic targeting cell wall synthesis and commonly used for Gram-positive organisms such as *Staphylococcus*. MRSA represents the ever-changing challenges that continue to call for new alternative therapies and ways of management.[1,3] MRSA is a severe pathogen found to be most prevalent in hospitalized patients and is the second most prevalent pathogen found in health care settings outside of the hospital.[2] In a study of children's hospitals in the United States over the period of 1999–2008, the incidence of MRSA-infected children increased 10-fold.[4] In addition, this trend is not limited to the United States, since we see a similar rise in incidence occurring globally.[3,5,6]

The concerns and problems associated with antibiotic resistance are certainly not limited to *S. aureus.* The development of bacterial antibiotic resistance in other microbes such as *Enterobacteriaceae* spp., *Pseudomonas aeruginosa*, and *Neisseria gonorrhea* represent significant emerging public and global health threats that we face beyond MRSA.[7] The development of antibiotic resistance involves genetic mutation and/or the acquisition of resistance genes.[1,6] While pharmacological agents against MRSA and other bacteria have been and continue to be developed, they often have limited success and short shelf lives.[1] Meanwhile, it is the very use of antibiotics, worldwide travel, and lack of precise therapeutic targets that promote a bacteria's ability for resistance and global spread.[1,6]

The emerging global health threats that *S. aureus* and MRSA pose call for the development of new and innovative avenues to control infection.[1,3] In this study, we investigated the anti-*Staphylococcus* activity associated with botanicals historically used in the treatment of infections. The overall goal of these studies was to comparatively evaluate these historical remedies in order to answer today's call for potential therapeutics against *S. aureus* infections. To this end, we investigated the efficacy of ethanol-based extracts of *Salvia officinalis* (sage), *Eucalyptus globulus* (eucalyptus), *Coleus forskohlii* (coleus), *Arctostaphylos uva-ursi* (uva-ursi), *Coptis chinensis* (Chinese goldthread), *Larrea tridentata* (chaparral), *Turnera diffusa* (damiana), *Anemopsis californica* (yerba mansa), *Allium sativum* (garlic), *Baptisia tinctoria* (wild indigo), *Berberis vulgaris* (barberry), *Glycyrrhiza glabra* (licorice), *Schisandra chinensis* (*Wu wei zi*), *Echinacea angustifolia* (echinacea), and *Polygonum multiflorum* (Chinese knotweed) for *in vitro* activity against an MSSA strain of *S. aureus*. Our results demonstrate strong anti-*Staphylococcus* activity associated with certain botanicals, while others demonstrated limited or no direct anti-*Staphylococcus* effects.

Materials and Methods

Botanical extract preparation

Plant material was obtained from reputable sources with documentation of authenticity. All plant material was subsequently verified by qualified botanical specialists using herbal pharmacopoeia monographs and reference keys. A voucher specimen of all plant material was deposited in a repository. For extraction, the botanicals were ground to a fine powder, resuspended in extraction solution, and incubated for 14–21 days at room temperature. The described plant-to-liquid ratio in a mixture of 95% ethanol/distilled water/glycerol: fresh *Salvia officinalis* 1:3 (sage leaf 58/35/07), fresh *Eucalyptus globulus* 1:3 (eucalyptus leaf 53/42/05), dried *Coleus forskohlii* 1:1 (coleus root 47/53/0), fresh *Arctostaphylos uva-ursi* 1:3 (uva-ursi leaf and berry 42/48/10), dried *Coptis chinensis* 1:4 (Chinese goldthread root 58/37/05), fresh *Larrea tridentata* 1:3 (chaparral leaf and flower 79/15/06), dried *Turnera diffusa* 1:4 (damiana leaf 63/27/10), fresh *Anemopsis californica* 1:2 (yerba mansa rhizome and root 63/30/07), fresh *Allium sativum* 1:2 (garlic bulb 53/37/10), fresh *Baptisia tinctoria* 1:3 (wild indigo root 63/32/05), dried *Berberis vulgaris* 1:4 (barberry root 43/47/10), dried *Glycyrrhiza glabra* 1:1 (licorice root 26/64/10), dried *Schisandra chinensis* 1:3 (*wu wei zi* berry 26/74/0), fresh *Echinacea angustifolia* 1:3 (echinacea root 47/48/05), and dried *Polygonum multiflorum* 1:3 (Chinese knotweed root 26/74/0). Following extraction, the liquid was pressed from the solid botanical material, filtered using unbleached paper filters, and pooled. Since the active antibacterial constituent(s) present in these extracts have not been identified, the extracts were normalized based on drying of the extracts and measurement of the remaining material. A sample of each extract was dried, and all extracts were found to contain similar concentrations of nonvolatile solutes (ranging between 19.6 and 37.5 mg/mL extract). The exact concentrations of nonvolatile solutes are noted in Table 1. This value is not meant to imply that the active constituent(s) include only nonvolatile solutes, but rather to provide a value of standardization, normalization, and comparison.

Antibiotic preparation

Antibiotics were obtained from Sigma-Aldrich (St. Louis, MO). The stock solution of tetracycline was prepared at 5 mg/mL in ethanol. A working solution was prepared by dilution in water to 50 μg/mL. Vancomycin was prepared and used as a working solution in water at 500 μg/mL.

Bacterial growth studies

Media and the bacterial culture *Staphylococcus aureus* ATCC 11632 were obtained from Hardy Diagnostics (Santa Monica, CA). For minimum inhibitory concentration (MIC) determination and growth studies, 18-hour cultures (ranging from 1 to 5×10^8 colony-forming units (CFU)/mL) were diluted into media (1:1000 dilution; tryptic soy broth) followed by the addition of indicated concentrations of each botanical extract, antibiotic, or ethanol control. The cultures were incubated at 37°C with aeration (by continuous rotation) for 24 hours. For evaluation, the MIC value was determined as the dose of the botanical extract required to completely inhibit replication of the bacteria (as measured by a lack of turbidity absorbance). For growth studies, at the indicated times, samples were removed and the bacterial concentration was determined by serial dilutions on tryptic soy agar (CFU/mL media).

Table 1. Minimum Inhibitory Concentrations of Anti-*Staphylococcus* Botanical Extracts

Treatment	*MIC (μL/mL)*	*MIC (μg/mL)*	*Concentration (mg/mL)*[a]
Vancomycin	4.5	2.5	0.05
Tetracycline	7.0	0.035	0.005
Salvia officinalis	5.8	150	25.9
Eucalyptus globulus	4.2	120	28.6
Coleus forskohlii	8.0	160	20.0
Arctostaphylos uva-ursi	2.9	90	31.0
Coptis chinensis	4.5	120	26.7
Larrea tridentata	1.7	60	35.3
Turnera diffusa	15.3	300	19.6
Anemopsis californica	18.0	360	20.0
Allium sativum	18.4	400	21.7
Baptisia tinctoria	47	1200	25.5
Berberis vulgaris	80	2400	30.0
Glycyrrhiza glabra	72	2700	37.5
Schisandra chinensis	>100	Ineffect.	28.2
Echinacea angustifolia	>100	Ineffect.	31.3
Polygonum multiflorum	>100	Ineffect.	25.4

Diluted (1000-fold) *S. aureus* cultures were treated with increasing concentrations of the indicated botanical extracts at 0 hours. The cultures were incubated at 37°C with continuous aeration (by rotation) and the MIC was determined at 24 hours. The MIC (noted as both microliters extract/milliliter diluent and micrograms nonvolatile constituents/milliliter diluent) was determined as the dose of the botanical extract required to completely inhibit replication of the bacteria as measured by a lack of turbidity absorbance. All botanical extracts had an average nonvolatile constituent concentration averaging 27.1 mg/mL (ranging from 19.6 to 37.5 mg/mL).

[a]Concentration (milligrams per milliliter) is based on the weight of nonvolatile constituents present in the extract per milliliter of aqueous liquid. MIC (micrograms per milliliter) for botanicals was calculated based on the nonvolatile constituent concentration. This value does not represent the concentration of active constituent(s), which is unknown.

MIC, minimum inhibitory concentrations.

Results

In order to comparatively evaluate the efficacy of botanicals commonly used as therapeutics for *Staphylococcus* infections, we first determined the MIC of each botanical extract against *S. aureus*. Table 1 shows a summary of the MIC (in micrograms per milliliter and microliters per milliliter) of the two control antibiotics (vancomycin and tetracycline), and the botanical extracts compared in these studies. Based on these results, the botanicals were arbitrarily separated into four groups: high activity (MIC $<200\,\mu$g/mL) including *Salvia*, *Eucalyptus*, *Coleus*, *Arctostaphylos, Coptis* and *Larrea*; moderately effective (MIC 200–1000 μg/mL) including *Turnera*, *Anemopsis*, and *Allium*; partially effective (MIC $>1000\,\mu$g/mL) including *Berberis*, *Baptisia* and *Glycyrrhiza*; and ineffective including *Schisandra, Echinacea,* and *Polygonum*. Control experiments done with ethanol and glycerin at the highest doses provided by the extracts did not affect bacterial growth (data not shown).

MIC values expressed in micrograms per milliliter were based on drying of the botanical extracts and measurement of the dried weight. All botanical extracts were reasonably consistent with an average nonvolatile constituent concentration averaging 27.1 mg/mL (ranging from 19.6 to 37.5 mg/mL). These values are not meant to imply that the active constituent(s) present in the extracts are nonvolatile components, but rather are meant to serve solely as a reference standard for comparison and normalization between the different botanical extracts. Since the active constituent(s) for these botanicals have not been identified, these gross values provide a basis for comparison.

MIC values indicate the lowest concentration of an antimicrobial where no visible growth is detected. At the MIC value, minor bacterial growth may still be observed, but may not be visibly detected. In addition, MIC experiments do not allow temporal measurements of an antimicrobial that may affect the growth of a bacteria for a shorter period of time. Bacterial growth curves following treatment with an antimicrobial allow measurement of possible temporal effects as well as precise quantitative valuation of the levels of inhibition of an antimicrobial. Therefore, in order to further evaluate the activity of the botanical extracts against *Staphylococcus*, growth curves were performed where the replication of the bacteria was evaluated every 8 hours for a 24-hour period. As a control, Figure 1 demonstrates proof of concept in illustrating the activity of tetracycline and vancomycin. Tetracycline and vancomycin both began to show strong anti-*Staphylococcus* activity at concentrations of 6 μL/mL. For tetracycline, a slight recovery in replication was observed at 24 hours at the 6 μL/mL dose. For vancomycin at the 6 and 20 μL/mL dose and tetracycline at the 20 μL/mL dose, a 10^5–10^6-fold reduction was observed. Since the botanical extracts were prepared in ethanol, a control experiment was done to assess anti-*Staphylococcus* activity at increasing concentrations of ethanol. Ethanol demonstrated minor anti-*Staphylococcus* activity ($\sim$1 log reduction) at concentrations of 6%. The final ethanol concentrations found in the botanical treatments used in these studies (diluted in media) do not exceed 1%; therefore any antimicrobial activity observed could be attributed to botanical constituents.

The botanical extracts that demonstrated strong and moderate activity against *S. aureus* are shown in Figure 2 and the botanical extracts with weak or no anti-Staphylococcus activity are shown in Figures 3 and 4, respectively. As shown in Figures 2 and 3, bacterial retardation, bacteriostatic, and bacteriocidal activity was observed. Retardation activity is defined in these studies as a slower rate of growth in *S. aureus* colonies as compared to untreated samples. Bacteriostatic is defined as activity that resulted in very little or no bacterial growth related to the initial bacterial concentration (CFU/mL) of the samples. Bacteriocidal is defined as activity that resulted in a marked reduction in the bacterial concentration of the cultures compared to the initial bacterial concentration. As shown in Figure 2, *Salvia*, *Eucalyptus*, *Coleus*, *Coptis, Turnera*, and *Larrea* exhibited a 10^6-fold reduction in bacterial replication as compared to untreated samples. *Arctostaphylos* and *Allium* exhibited a 10^5-fold reduction and *Anemopsis* a 10^4-fold reduction. For *Salvia, Eucalyptus, Coleus, Arctostaphylos, Coptis, Turnera,* and *Larrea,* strong bacteriocidal activity was observed with a 10^2–10^3-fold reduction in bacterial concentration (CFU/mL) compared to the initial starting culture. Many botanicals, especially at lower doses, had initial inhibitory activity followed by growth recovery including *Eucalyptus*, *Coptis*, *Turnera, Anemopsis*, and *Berberis*. At these concentrations, the active constituent(s) present in the extracts may be limiting and are either being metabolized or have a short half-life.

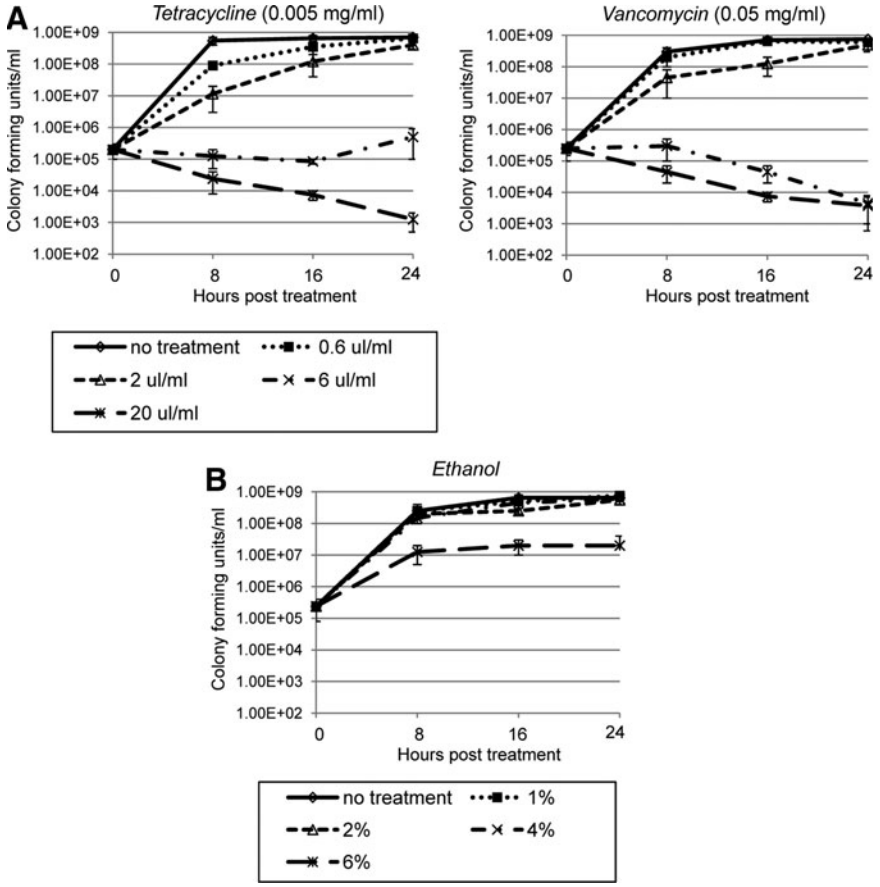

FIG. 1. Anti-*Staphylococcus* activity associated with control substances. Diluted (1000-fold) *S. aureus* cultures were left untreated (solid line), or treated with the indicated concentrations of antibiotic **(A)** or ethanol **(B)** at 0 hours (dashed lines). The tetracycline and vancomycin stocks were prepared at 50 and 500 μg/mL, respectively. The cultures were incubated at 37°C with continuous aeration (by rotation) and colony-forming units per milliliter were determined every 8 hours over a period of 24 hours. Assays were repeated in triplicate.

Figure 3 represents botanicals with slight or moderate activity in our growth curve experiments including *Berberis*, *Baptisia*, and *Glycyrrhiza*. As shown, these botanicals reduced replication by 2–3 log units at the highest concentrations. For *Berberis* and *Glycyrrhiza*, even with this reduction, *Staphylococcus* was able to overcome this inhibitory effect by 24 hours. The botanicals *Schisandra, Echinacea*, and *Polygonum* appeared to be ineffective against *S. aureus* (Fig. 4). As shown, even at the highest concentration, no significant reduction in bacterial replication was observed at any time points in the growth curve.

Discussion

Botanical extracts have long been used as therapeutic agents for bacterial infections. In this study, botanical extracts with historical use in the treatment of *S. aureus*–associated infections were comparatively evaluated for their direct antibacterial activity. Several botanical extracts in this study demonstrated strong antimicrobial activity against *Staphylococcus*, exhibiting as much as a 6-log unit reduction in replication as compared to untreated cultures. These botanical extracts, which include *Salvia, Eucalyptus, Coleus, Arctostaphylos, Coptis, Turnera, Anemopsis, Allium,* and *Larrea*, show promising use against *S. aureus*, particularly for their bactericidal ability, but also for their inhibitory and bacteriostatic actions at lower doses. *Berberis, Baptisia*, and *Glycyrrhiza* also showed limited activity against *S. aureus*, but *Schisandra, Echinacea*, and *Polygonum* were shown to be ineffective. Recommendations regarding evaluatory criteria for botanical extracts suggest that extracts should demonstrate IC_{50} values < 100 μg/mL for *in vitro* studies.[8] For the most active botanicals in our study, MIC values corresponding to a complete lack of growth turbidity of *S. aureus* ranged from 60 to 300 μg/mL. In addition, in our growth studies, the doses of 6–20 μL/mL correspond to approximately 200–600 μg nonvolatile constituents/mL, respectively. Depending on the botanical, these doses resulted in typically 1000–1,000,000-fold reductions in *Staphylococcus* replication (Fig. 2). Therefore, these highly active botanicals in particular meet the recommended stringent endpoint criteria for therapeutic botanical use.

For *Staphylococcus* infections, many of these botanicals represent potentially effective therapeutics for topical, gastrointestinal, or urinary infections. For topical application, the highly active botanicals would likely have strong antimicrobial effects and therapeutic value for an infection. Traditionally, typical oral doses of such botanical tinctures to treat gastrointestinal, urinary, or septic infections range from 1 to 20 mL per day.[9] In our assays, the effective dose for a single treatment ranges between 6 and 20 μL/mL. It is difficult to convert these *in vitro* doses to the same proportion of oral dosing; however, the average human contains approximately 5 L of blood. If our *in vitro* doses are converted to the average human blood volume, it would equate to 30–100 mL extract/5 L. This exceeds the traditional doses of these botanical extracts, but it must be pointed out that our studies were done with a single dose in an *in vitro*

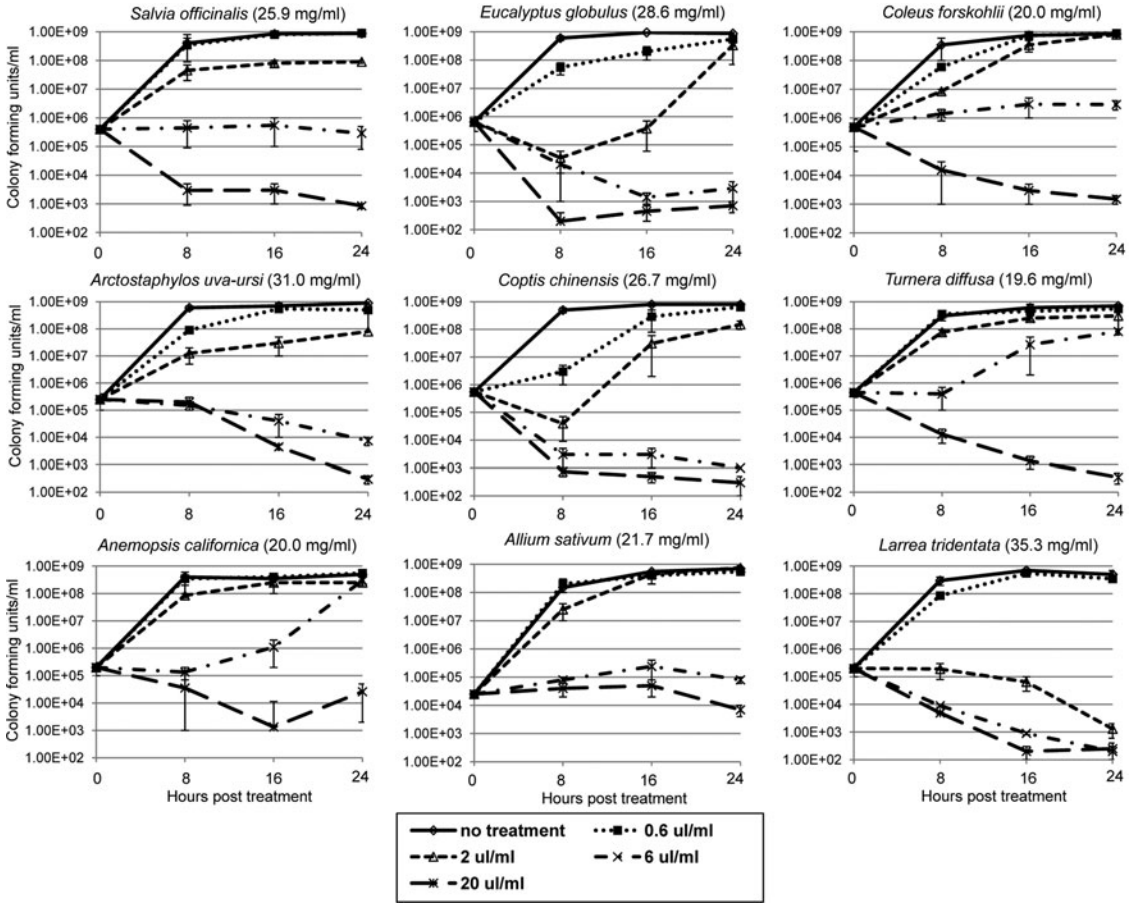

FIG. 2. Highly and moderately active anti-*Staphylococcus* botanical extracts. Diluted (1000-fold) *S. aureus* cultures were left untreated (solid line), or treated with the indicated botanical extracts in the amount of 0.6, 2.0, 6.0, or 20.0 μL/mL at 0 hours (dashed lines). The cultures were incubated at 37°C with continuous aeration (by rotation) and colony-forming units per milliliter were determined every 8 hours over a period of 24 hours. Assays were repeated in triplicate. The milligrams nonvolatile constituents present in the extract per milliliter of aqueous liquid are noted for each botanical for standardization and comparison purposes.

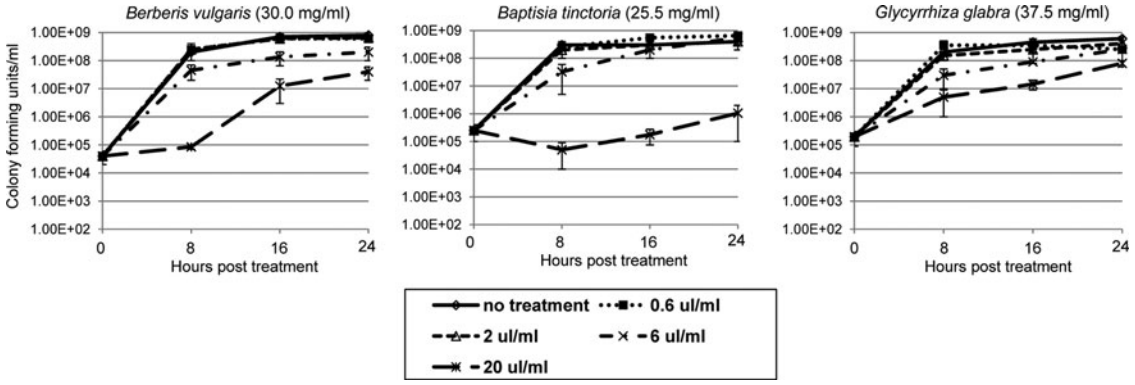

FIG. 3. Weakly active anti-*Staphylococcus* botanical extracts. Diluted (1000-fold) *S. aureus* cultures were left untreated (solid line), or treated with the indicated botanical extracts in the amount of 0.6, 2.0, 6.0, or 20.0 μL/mL at 0 hours (dashed lines). The cultures were incubated at 37°C with continuous aeration (by rotation) and colony-forming units per milliliter were determined every 8 hours over a period of 24 hours. Assays were repeated in triplicate. The milligrams nonvolatile constituents present in the extract per milliliter of aqueous liquid are noted for each botanical for standardization and comparison purposes.

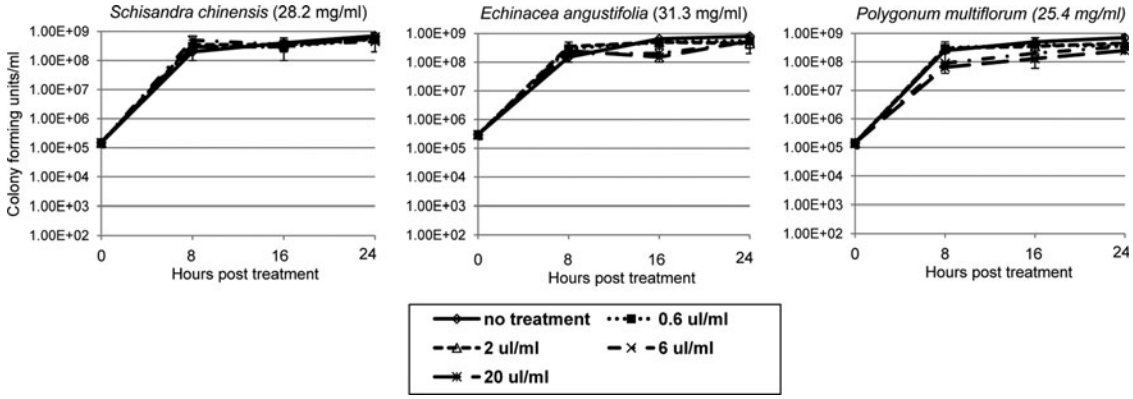

FIG. 4. Nonactive anti-*Staphylococcus* botanical extracts. Diluted (1000-fold) *S. aureus* cultures were left untreated (solid line), or treated with the indicated botanical extracts in the amount of 0.6, 2.0, 6.0, or 20.0 μL/mL at 0 hours (dashed lines). The cultures were incubated at 37°C with continuous aeration (by rotation) and colony-forming units per milliliter were determined every 8 hours over a period of 24 hours. Assays were repeated in triplicate. The milligrams nonvolatile constituents present in the extract per milliliter of aqueous liquid are noted for each botanical for standardization and comparison purposes.

system. As a therapeutic, multiple doses at lower concentrations may have significant antimicrobial effects, even systemically. In addition, bacterial sepsis typically occurs with 1–100 CFU/mL blood, while in our assays, initial bacterial concentrations were approximately 1×10^5 CFU/mL media. Therefore, our assays represent a very high concentration of bacteria, and lower doses of these botanical extracts may have therapeutic value even in a systemically infected patient. However, it is also unclear what the bioavailability and absorption properties of the active constituent(s) present in these extracts are and if they could reach effective concentrations systemically. Therefore, further research is warranted to confirm the efficacy of the highly effective antimicrobial botanicals we have identified for use against *S. aureus* gastrointestinal, urinary, and septic infections.

For most of the botanicals used in our experiments, safety profiles reflect low risk and few side-effects with use within recommended dosages.[10] In our studies, *Salvia*, *Eucalyptus*, *Coleus*, *Arctostaphylos*, *Coptis*, *Turnera*, *Anemopsis*, *Allium*, and *Larrea* demonstrated the strongest anti-*Staphylococcus* activity. Historically, *Salvia officinalis* (sage) has been used as an antiseptic for gingivitis and skin infections, including *S. aureus* infections.[11,12] It has antibacterial effects that appear to be due in part to the constituents carnosol, thujone, 1,8-cineole (an antimicrobial monoterpene that exhibits toxicity against gram-positive bacteria), and rosmarinic acid.[13–15] Its anti-*S. aureus* activity has been shown to be equivalent to commonly used anti-*Staphylococcus* antibiotics.[16] The active constituent carnosol also appears to act synergistically with other antibiotics, including gentamicin, erythromycin and tetracycline.[15] It has been proposed that carnosol may inhibit drug efflux pumps, thereby allowing these antibiotics to have a sustained effect. *E. globulus* has been used historically for bacterial infections including tuberculosis and diphtheria.[11] It has also been used as a general antiseptic for urinary catheters and vaginitis and as a poultice for abscess wounds.[12,17,18] A key constituent, 1,8 cineole, is noted as an antimicrobial monoterpene exhibiting activity against gram-positive bacteria.[13] The antimicrobial properties of *Eucalyptus* are well documented, and some research suggests particularly strong activity against MRSA.[9,14,19–21] *C. forskohlii* has increasingly been shown to be a broad-spectrum antibacterial.[22,23] In limited studies, *Coleus* extracts inhibited the replication of *Haemophilus influenza, S. pneumonia, S. pyogenes* and *S. aureus* in a similar way to standard antibiotics.[24] The presumptive active constituent, forskolin, has been used to reduce urinary tract infections and enhance the antibacterial activity of antibiotics.[25] *A. uva-ursi* is traditionally a urinary antiseptic with action against *Escherichia coli, Proteus vulgaris, Enterobacter aerogenes, Streptococcus faecalis, Salmonella typhi*, and *S. aureus*.[9] Uses have been primarily for urinary tract infections such as pyelonephritis and cystitis, the latter of which is commonly caused by *Staphylococcus. Arctostaphylos* acts a urinary antimicrobial possibly due to constituents including arbutin (a glycosidic ether of hydroquinone), free hydroquinone, and/or aglycone *p*-hydroxyacetophenone.[9,13,14,19] It has also been shown to reduce the MIC of ß-lactam antibiotics against MRSA via the action of corilagin.[19,26] *Coptis chinesis* has traditionally been used for gastrointestinal, eye, and skin infections, including *Staphylococcus*-associated furuncles.[17] Research has shown antimicrobial activity of *Coptis* against several strains of MRSA as well as the potential for *Coptis* to act synergistically when used in combination with ß-lactam antibiotics.[27] *Coptis* extracts contain antibacterial constituents including protoberberine alkaloids such as palmatine and berberine.[13] Berberine is also found in extracts from *Berberis vulgaris*, which in our studies was found to have only weak activity against *S. aureus*. This supports the fact that even though many presumptive active constituents have been identified in many botanical extracts, further research is necessary to confirm their specificity and/or possible synergism with other compounds. *Larrea tridentata* has traditionally been used as a therapeutic for various bacterial infections including tuberculosis, subacute bronchitis, and subacute laryngitis.[18] *Larrea* expresses the antimicrobial constituent, nordihydroguaiaretic acid, which has been shown to have specific activity against MRSA.[14,19,28] *Turnera diffusa* is used as a urinary antiseptic with activity against both gram-positive and gram-negative

bacteria.[14] Research has also demonstrated resistance-modifying activity of *Turnera* extracts, where it can increase the potentiation of various antibiotics.[29] *Anemopsis californica* has traditionally been used for the treatment of skin abscesses commonly associated with *S. aureus* infections and has been shown to have direct antibacterial activity.[30] *Anemopsis* extracts contain antimicrobial constituents including thymol, 1,8 cineole, and lignans.[14,19] *Allium sativum* has antimicrobial actions that are well documented, likely due in large part to the constituents allicin and ajoene.[13,14,19,20] *Allium* has historically been used for a variety of bacterial infections including those caused by *Bordetella pertussis, Salmonella enterica, Mycobacterium tuberculosis, Yersinia pestis, Bacillus anthracis,* and *Corynebacterium diphtheria*.[11,17,31] It has also been used for the treatment of bacterial vaginitis for which *Staphylococcus* is commonly associated.[11] Much research exists on *Allium's* antibacterial properties, and some research shows activity against multidrug-resistant bacteria, including MRSA.[32–37]

The data from this study provide a comparative analysis of the direct antimicrobial activity of various botanicals against *S. aureus*. Further investigation into the role botanical medicines can have in *S. aureus* infections either as stand-alone therapies or as synergists and potentiators of antibiotics is warranted. As noted earlier, previous research suggests that many of these botanical extracts provide synergistic effects when combined with common antibiotics. Future research will focus on whether these synergistic effects may aid in preventing or slowing the development of antibiotic resistance.

Although no or limited direct antibacterial activity was found with some botanicals in this study (including *Berberis*, *Baptisia*, *Glycyrrhiza*, *Schisandra*, *Echinacea*, and *Polygonum*), these botanicals may still have indirect antibacterial effects. Such indirect effects may occur through alternative pathways such as immune modulation. For example, *Echinacea* is a well-known immune modulator commonly used for bacterial infections associated with carbuncles, boils, sepsis, gangrene, syphilis, diphtheria, cholera, and scarlet fever.[14,18,20,38,39]

Identification of the key active constituent(s) present in the effective anti-*Staphylococcus* extracts is of importance toward the development of a standardized pharmaceutical drug. Although presumptive active compounds have been identified for many of the botanicals described in this study, the actual compounds have yet to be confirmed and/or identified. Work is currently under way to fractionate and identify such active constituent(s). However, even though the active constituent(s) have not yet been identified from these extracts, the characterized anti-*Staphylococcus* activity of the total botanical extract is of significant importance since these unfractionated extracts are currently being used in the medical field by naturopathic physicians and by patients seeking alternative medicine therapeutics.

Botanicals offer a repertoire of additional therapeutics for treatment of microbial infections. This study provides a comparative evaluation of the direct antibacterial activity associated with several botanicals historically used for *S. aureus* infections. Future research needs to be done to investigate the ability of *S. aureus* to develop resistance to these botanical extracts, as well as the ability of the botanical extracts to act synergistically and possibly help prevent antibiotic resistance from developing. This global threat demands serious consideration for the complexity of nature's defense mechanisms to be more deeply understood so that appropriate therapies can be developed and utilized accordingly.

Disclosure Statement

Yvan Rochon, PhD, is owner of Herbal Vitality, Inc.

References

1. Lowy F. Antimicrobial resistance: The example of *Staphylococcus aureus*. J Clin Invest 2003;111:1265–1273.
2. Okesola A. Community-acquired methicillin-resistant *Staphylococcus aureus*—A review of literature. Afr J Med Sci 2011;40:97–107.
3. Ohlesen K. Novel antibiotics for the treatment of *Staphylococcus aureus*. Expert Rev Clin Pharmacol 2009;2:661–672.
4. Herigon JC, Hersh AL, Gerber JS, et al. Antibiotic management of *Staphylococcus aureus* infection in US children's hospitals, 1999–2008. Pediatrics 2010;125:e1294–e1300.
5. Kramer A, Wagenvoort H, Ahren C, et al. Epidemiology of MRSA and current strategies in Europe and Japan. GMS Krankenhhyg Interdiszip 2010;5:Doc01.
6. Stefani S. Evolution in the antibiotic susceptibility and resistance. Infez Med 2009;17(suppl 3):5–12.
7. Livermore DM. Has the era of untreatable infections arrived? J Antimicrob Chemother 2009;64(Suppl 1):i29–i36.
8. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof of concept'. J Ethnopharmacol 2006;106:290–302.
9. Mills S, Bone K. Principles and Practice of Phytotherapy. Churchill Livingstone, London, 2000.
10. Brinker F. Herb Contraindications and Drug Interactions. Eclectic Medical Publications. Sandy, OR, 1998.
11. Duke J. Dr. Duke's Phytochemical and Ethnobotanical Databases. March 29, 1995. Online document at: http://ars-grin.gov/duke/ Accessed June 16, 2013.
12. Natural Standard. 2013. Online document at: http://naturalstandard.com Accessed June 16, 2013.
13. Ganora L. Herbal Constituents: Foundations of Phytochemistry. Lisa Ganora, Louisville, CO, 2009.
14. Skenderi G. Herbal Vade Mecum. Rutherford, NJ. 2003.
15. Kumiko H, Sumiko S, Teruo K, et al. Potentiation of antimicrobial activity of aminoglycosides by carnosol from *Salvia officinalis*. Biol Pharm Bull 2007;30:287–290.
16. Shriazi MH, Renjbar R, Eshraghi S, et al. Inhibitory effects of sage extract on the growth of enteric bacteria. Pak J Bio Sci 2008;11:487–489.
17. Pizzorno J, Murray M. Textbook of Natural Medicine. Churchill Livingston, London, 1999.
18. Felter H. The Eclectic Materia Medica, Pharmacology and Therapeutics. Eclectic Medical Publications. Sandy, OR, 1994.
19. Yarnell E, Abascal K, Rountree R. Clinical Botanical Medicine. Mary Ann Liebert, Inc., New Rochelle, NY. 2009.
20. Mulyaningsih S, Sporer F, Reichling J, Wink M. Antibacterial activity of essential oils from *Eucalyptus* and of selected components against multidrug-resistant bacterial pathogens. Pharm Biol 2011;49:893–899.
21. Takahashi T, Kokubo R, Sakaino M. Antimicrobial activities of *Eucalyptus* leaf extracts and flavonoids from *Eucalyptus maculata*. Lett Appl Microbiol 2004;39:60–64.

22. Senthilkumar CS, Kumar MS, Pandian MR. In vitro antibacterial activity of crude leaf extracts from tecoma stans (l) juss. Et kunth, *Coleus forskohlii* and *Pogostemon patchouli* against human pathogenic bacteria. Int J Pharm Tech Res 2010;2:438–442.
23. Saklani S, Gahlot M, Kumar A, et al. Antimicrobial activity of extracts of the medicinal plant *Coleus forskohlii.* Int J Drug Res Tech 2011;1:52–59.
24. Nilani P, Duraisamu B, Dhamodaran P, Ravichandran S, Elango K. Effect of selected antiasthmatic plant constituents against microorganism causing upper respiratory tract infection. Ancient Sci Life 2009;29:30–32.
25. Bishop BL, Duncan MJ, Song J, Li G, Zaas D, Abraham SN. Cyclic AMP-regulated exocytosis of *Escherichia coli* from infected bladder epithelial cells. Nat Med 2007;13:625–630.
26. Shimizu M, Shiola S, Mizushima T, et al. Marked potentiation of activity of beta-lactams against methicillin-resistant *Staphylococcus aureus* by corilagin. Antimicrob Agents Chemother 2001;45:3198–3201.
27. Yu HH, Kim, KJ, Cha JD, et al. Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. J Med Food 2005;8:454–461.
28. Favela-Hernandez JM, Garcia A, Garza-Gonzalez E, et al. Antibacterial and antimycobacterial lignans and flavonoids from *Larrea tridentata*. Phytother Res 2012;12:1957–1960.
29. Coutinho HD, Costa JG, Lima EO, et al. Herbal therapy associated with antibiotic therapy: Potentiation of the antibiotic activity against methicillin-resistant *Staphylococcus aureus* by *Turnera ulmifolia.* BMC Complement Altern Med 2009;9:13.
30. Medina AL, Lucero ME, Holguin FO, et al. Composition and antimicrobial activity of *Anemopsis californica* leaf oil. J Agric Food Chem 2005;53:8694–8698.
31. Grieve M. Botanical.com: A Modern Herbal. Online document at: http://botanical.com Accessed June 16, 2013.
32. Kyung KH. Antimicrobial properties of *Allium* species. Curr Opin Biotechnol 2012;23:142–147.
33. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes Infect 1999;1:125–129.
34. Cutler RR, Wilson P. Antibacterial activity of a new, stable aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*. Br J Biomed Sci 2004;61:71–74.
35. Palaksha MN, Ahmed M, Das S. Antibacterial activity of garlic extract on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin. J Nat Sci Biol Med 2010;1:12–15.
36. Shokrzadeh M, Ebadi AG. Antibacterial effect of garlic (*Allium sativum* L.) on *Staphylococcus aureus.* Pak J Biol Sci 2006;9:1577–1579.
37. Wei LS, Musa N. Inhibition of *Edwardsiella tarda* and other fish pathogens by *Allium sativum* L. (*Alliaceae*) extract. Am Euras J Agric Environ Sci 2008;3:692–696.
38. Kapai NA, Anisimova NY, Kiselevskii MN, et al. Selective cytokine-inducing effects of low dose *Echinacea*. Bull Exp Biol Med 2011;150:711–713.
39. Hudson, JB. Applications of the phytomedicine *Echinacea purpurea* (purple coneflower) in infectious diseases. J Biomed Biotechnol 2012;2012:769896.

Address correspondence to:
Jeffrey Langland, PhD
Biodesign Institute
Arizona State University
Tempe, AZ 85287

E-mail: Jeffrey.Langland@asu.edu