Antimicrobial activity of extracts from exotic fruits and vegetables

Kendrick A. Kirby-Lee[1]

[1]Weston High School, Weston, MA 02493, United States

Corresponding Author:
Kendrick A. Kirby-Lee

Email address: kirby-lee@comcast.net

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Abstract

Novel sources of antimicrobial agents need to be identified to address the looming crisis of antimicrobial resistance. The overall goal of the described experiments was to determine whether extracts of exotic vegetables, mushrooms, and herbs have antimicrobial properties. Vegetable and herbal extracts were purchased from online supplier. Fresh vegetable roots and mushrooms were run through a commercially available juicer to obtain aqueous extracts. Extracts were spotted onto filter paper disks and activity assessed by measuring the zone of inhibition for both *E. coli* K-12 and *B. subtilis* as representative Gram-negative and Gram-positive organisms, respectively. Synergy testing was also performed by placing disks spotted with extracts in close proximity to one another. Amongst commercial extracts, prominent Gram-positive activity was found for, *Echinacea*, grapefruit seed, olive leaf, golden seal extracts; and prominent mixed Gram-negative and Gram-positive activity was found for clove extract. Amongst fresh vegetable and mushroom juicing extracts, Gram-positive activity was detected for celery root.

Introduction

The emergence of drug resistant bacterial infections had led to the need for new therapies. Finding agents that inhibit kill bacteria but are not harmful to humans is a formidable task. Plants live in a sea of microbes and arguably must have a plethora of mechanisms to prevent injury by these neighbors, some of which include elaboration of antimicrobial compounds. This notion is supported by the general observation that vegetables and mushrooms take an extremely long time to undergo spoilage. As such, it was my hypothesis that exotic edible vegetables should be a potential source of antimicrobial activity, and by definition should have low toxicity to human hosts. Identification of vegetables and or extracts with antimicrobial activity would be an ideal start for future exploration. Therefore, the overall goal of the described experiments was to identify crude extracts of exotic vegetables and herbs with antimicrobial activity.

Materials and Methods

B. subtilis, *E. coli* K-12 and 10 ug penicillin and 30 μg chloramphenicol disks were obtained from Carolina Biological Supply (Burlington, NC). Prior to use, bacteria were streak on LB plates incubated at 37 C in ambient air for 20 hours for isolation of individual colonies and to confirm purity and expected growth characteristics. For disk diffusion experiments, isolated colonies were suspended in sterile water to an approximate density of 0.5 McFarland (~5 colonies) and then spread on an LB plate using a disposable sterile cotton tip applicator swab (Medline, Northfield, IL) to evenly distributed bacteria across an entire plate. Sterile 6 millimeter paper disks (Carolina Biological Supply) were placed on plates with sterile forceps or a disk dispenser. Penicillin and chloramphenicol disc controls were performed in parallel during each experiment.

Fresh vegetables (horseradish, jalapeno pepper, shallots, carrots, celery root, sunchoke, jicama, purple top turnip, and parsnip) and mushrooms (portabella, shitake) (Whole Foods Market, Austin, TX) were extracted with a 400-watt Fruit and Vegetable Juice Extractor (Black and Decker, Model JE2200B) according to the manufacturer's instructions. Extracts were purchased from iHerb.com. The following were listed as alcohol-based extracts either directly on label or suggested by alcohol content of at least 19%: *Usnea* spp, garlic, and licorice (Herb Pharm, Williams, OR); thyme (*Thymus* vulgaris) (Nature's Answer, Hauppauge, NY); and *Reishi,* (California Xtracts, Moreno Valley, CA). The following were steam distilled: ginger (*Zingiber officinale*) and clove (*Eugenia caryophillata*) (Now Foods, Bloomington, IL). The extraction method for the following were not indicated: grapefruit seed extract (NutriBiotic, Lakeport, CA); shitake (*Lentinus edodes*, Nature's Answer), *Echinacea purpurea* (Nature's Way, Greenbay, WI), olive leaf (*Olea europaea*, Now Foods), and goldenseal (*Hydrastis canadensis*, Herb Pharm). For initial screening of extracts, 10μl of extract was pipetted onto each disk. Plates were then incubated at 37° C for 20 hours and inhibition zone diameters measured with a ruler using reflected light per consensus standards (Clinical and Laboratory Standards Institute 2017).

Dose response curves were generated by adding to disks 20μl (2X), 10μl (1X) or two-fold serial dilutions of extracts and dispensing onto disks as described above. For synergy testing, discs were placed at a distance that was equal to the sum of the previously measured inhibition radii for the two extracts tested separately. This distance was considered optimal to uncover both synergism and antagonism.

Results shown are representative of mean values from two separate experiments. Growth up to the disc (6 mm) was considered indicative of complete resistance to the extract being tested.

Results

Activity of commercially available herbal extracts was tested against *E. coli* and *B. subtilis*. Results are summarized in Table 1. All herbal extracts except for licorice demonstrated at least some activity against *B. subtilis*. Especially prominent activity was noted for *Echinacea*, grapefruit seed, olive leaf, and shitake mushroom extracts. Gram-negative activity (against *E. coli*) was observed less frequently and was always associated with Gram-positive activity (*B. subtilis*) as well. Activity of garlic, ginger, olive leaf, thyme and *Usnea* was greater for *B. subtilis* than for *E. coli*. In contrast, clove activity was greater for *E. coli* than *B. subtilis*.

Activity of fresh vegetable and mushroom juicing extracts is summarized in Table 2. Celery root alone demonstrated weak Gram-positive activity.

A dose-response (correlation of inhibition zone with amount of extract applied, data not shown) was demonstrated for all active herbal extracts supporting reproducibility and specificity of observed findings. Dilutional testing was not performed for vegetable and mushroom juicing extracts because of minimal activity of undiluted active extract (i.e., celery root) with a zone size that already approached the disk margin. Interestingly, several extracts demonstrated multiple zones of inhibition, suggesting the potential for multiple antimicrobial activities in the same extract (Fig. 1A).

Pairwise synergy combination testing was performed by placing disks for the most active alcohol-based herbal extracts in close to proximity to one another and screening for either unidirectional or bidirectional accentuation of zone of inhibition between the two disks. The following combinations were tested: clove and golden seal; shitake and *Echinacea*; *Echinacea* and olive leaf; olive leaf and grapefruit seed; shitake and grapefruit seed; *Echinacea* and grapefruit seed; clove and *Echinaceae*; and *Echinacea* and golden seal. Amongst these tested extract combinations, only clove extract was noted to unidirectionally potentiated activity of golden seal against *B. subtilis* (Fig. 1B).

Discussion

A large number of commercial herbal extracts demonstrated antimicrobial activity against a representative *B. subtilis and E. coli*. Notably, 65% of extracts demonstrated a detectable zone size against the former, while only 35% demonstrated activity against the latter. This likely relates at least in part to the higher permeability barrier and selective exclusion of antimicrobials from double membrane-based Gram-negative organisms (Zgurskaya et al. 2015). Nevertheless, the potent activity with very large zone sizes for some extracts (> 30 mm for *Echinacea*, grapefruit seed, olive leaf, and shitake extracts) was notable. Interestingly, one extract (clove) demonstrated greater activity for *E. coli* than *B. subtilis*.

Fresh juicing extracts were inactive with the exception of celery root. Notably, while shitake mushroom juicing extract was inactive, the commercial shitake extract (listed on bottle as alcohol free "Bio-Chelated proprietary extraction process") in glycerin and water was extremely potent with the largest inhibition zone size observed amongst extracts tested (37.5 mm for *B. subtilis*). This observation suggests that the activity component was not sufficiently solubilized and concentrated in the aqueous-based juicing extract. This conclusion is consistent with prior observations of Ishikawa et al. who observed the preferential partition of Shitake mushroom

(*Lentinula edodes)* antimicrobial activity in the organic rather than aqueous component (Ishikawa et al. 1991).

This study supports widespread antimicrobial activity in herb and root-based vegetables consistent with the need for protection against adverse effects of environmental microbes. Investigation of antimicrobials found in plant and herb substances are an active area of investigation based on potential for medicinal use. In particular, there is active exploration in the dental literature for use of herbal extracts directly as additives to toothpaste and oral emoluments to treat periodontal disease (Chandra Shekar et al. 2015). Antimicrobial activity of several extracts examined in this study have been described previously including *Echinacea* against Gram-positive, *Propionbacterium acnes* (Sharma et al. 2011); clove against *Streptococcus mutans* and *salivarius* (Mirpour et al.); and golden seal (*Hydrastis canadensis*) against *Staphylococcus aureus* (Ettefagh et al. 2011). Prior studies suggest antimicrobial contribution of several common phytochemical constituents such as tannins, eugonol (Nascimento et al. 2000), and alkaloids (Ettefagh et al. 2011). However, the full identify of substituents underlying antimicrobial activity observed in this study will need further investigation. It is anticipated that several unique natural products will be thereby elucidated with properties worthy of further study.

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Table 1. Average Zone of Inhibition for Commercial Herbal Extracts

Extract	*E. coli* (mm)	*B. subtilis* (mm)
clove	28.5	18.75
Echinacea	0	35.5
garlic	9	8.5
ginger	8.5	15.75
grapefruit seed	0	34
golden seal	0	31
licorice	0	0
olive leaf	9	34
reishi	0	8
shitake	0	37.5
thyme	10	13
Usnea	10	11.75
carrots	0	0
jalapeno peppers	0	0
horseradish	0	0
portabella mushroom	0	0
shallot	0	0
penicillin	0	32
chloramphenicol	33	28.3

Table 2. Average Zone of Inhibition for Juicing Extracts

Extract	*E. coli* (mm)	*B. subtilis* (mm)
celery root	0	8.75
shitake mushroom	0	0
sunchoke	0	0
jicama	0	0
purple top turnip	0	0
parsnip	0	0
chloramphenicol	32.5	28.5

Figure

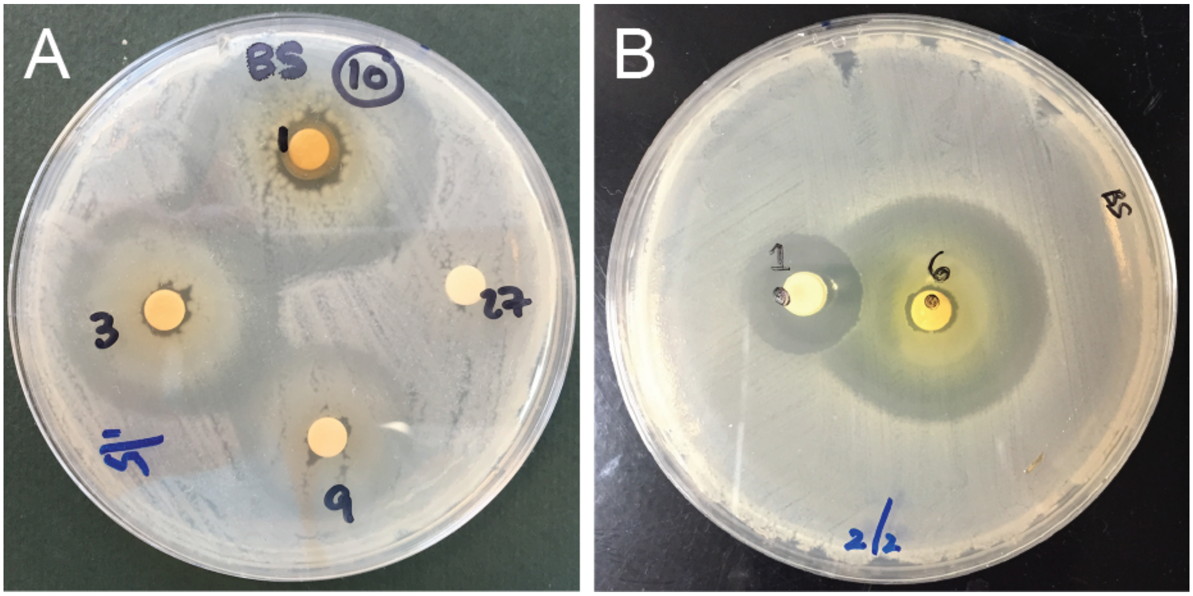

Figure 1. Examples of extract activity against *B. subtilis*. (A) Activity of individual extracts: 1-clove, 3-garlic, 9-*Reishi*, and 27-negative control. Note several zones of inhibition, for example, around clove. **(B)** 1-clove potentiates 6-goldenseal activity as indicated by bowing out of the goldenseal zone of inhibition next to the clove disk.

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