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Antimicrobial activity of crude epicarp and seed extracts from mature avocado fruit (*Persea americana*) of three cultivars

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Abstract

The epicarp and seed of *Persea Americana* Mill. var. Hass (Lauraceae), *Persea Americana* Mill. var. Shepard, and *Persea americana* Mill. var Fuerte cultivars of mature avocados ($n = 3$) were ground separately and extracted with both absolute ethanol and distilled water. Extracts were analyzed for antimicrobial activity using the microtiter broth microdilution assay against four Gram-positive bacteria, six Gram-negative bacteria, and one yeast. Antimicrobial activity against two molds was determined by the hole plate method. The ethanol extracts showed antimicrobial activity (104.2–416.7 µg/mL) toward both Gram-positive and Gram-negative bacteria (except *Escherichia coli*), while inhibition of the water extracts was only observed for *Listeria monocytogenes* (93.8–375.0 µg/mL) and *Staphylococcus epidermidis* (354.2 µg/mL). The minimum concentration required to inhibit *Zygosaccharomyces bailii* was 500 µg/mL for the ethanol extracts, while no inhibition was observed for the water extracts. No inhibition by either ethanol or water extracts was observed against *Penicillium* spp. and *Aspergillus flavus*.

Keywords: *Antimicrobial; avocado cultivar; epicarp; extracts; seed*

Introduction

As the world population grows the demand for food increases (Food and Agriculture Organization of the United Nations, 2002), and there is therefore a need not only to produce more food, but to assure that what is produced is safe for human consumption. One of the most common factors contributing to unsafe food is bacterial contamination (Conte et al., 2007). Synthetic additives have been widely used in the food industry to control microbial pathogens and inhibit microbial spoilage, although in recent years there is an increasing demand for natural food additives such as plant extracts (Conte et al., 2007).

Plant materials such as *Citrus* spp. peel (Johann et al., 2007) and grape (*Vitis vinifera* L.) seeds (Baydar et al., 2006) are some natural products that display antimicrobial activity that has been applied in foods. The avocado fruit [*Persea Americana* Mill. (Lauraceae)], a native of tropical America (Jacob et al., 1971), is commonly grown in many developing countries for the flesh of its fruit (Chanderbali et al., 2008). Three botanical varieties of avocado adapted to different climate conditions have traditionally been recognized: Mexican [*P. americana* var. *drymifolia* (Schlecht. & Cham.) Blake], Guatemalan (*P. americana* var. *guatemalensis* L. Wms.), and West Indian (*P. americana* var. *americana* Mill.). Most commercial avocado cultivars are interracial hybrids developed from chance seedlings. Thus, the most important cultivars in subtropical climates, such as "Hass," "Bacon," and "Fuerte," are Guatemalan–Mexican hybrids with different degrees of hybridization (Newett et al., 2002).

Extracts from the epicarp of the immature avocado fruit have been demonstrated to have both antifungal and antibacterial properties (Jacob et al., 1971; Sivanathan & Adikaram, 1989). The seed of the immature fruit was also

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found to have antibacterial properties (Jacob et al., 1971). The antifungal properties of the immature avocado were established to be due to the idioblast oil cells, which are made up of alkaloids, sesquiterpene hydroperoxides, other terpenes (Platt & Thomson, 1992), persin, and a group of 2-alkylfurans (Rodriguez-Saona et al., 1998).

Tannins, catechin flavones, and polyphenolic compounds are often found in the tissues and seed of the avocado fruit. These chemicals are all antimicrobial in nature and could have contributed to the antibacterial activity of the immature fruit (Jacob et al. 1971; Young & Biale, 1967). However, few data are available on the antimicrobials of mature fruit, which are expected to be different, as protection from plant pathogenic microorganisms is no longer required and microbial degradation may even be desirable to release the seed.

Mature avocado flesh is often used to produce guacamole or oil, leaving the epicarp and seed as a byproduct. The potential use of the discarded epicarp and seed could add value to fruit production in developing countries, and also potentially reduce environmental problems associated with disposal of the epicarp and seed. This study was therefore undertaken to determine the presence and level of antimicrobial activity (if any) associated with simple crude extracts of the epicarp and seed of different varieties of mature avocados against 10 foodborne pathogens and three fungi of food significance.

Materials and methods

Preparation of avocado extracts

Hass (Guatemalan race), Shepard (Guatemalan race), and Fuerte (Guatemalan × Mexican race) cultivars of avocado, selected due to their genetic differences (Newett et al., 2002), were obtained from local suppliers between February and May 2004 and kept matured until ready-to-eat. The epicarp and seed of each individual avocado fruit were retained and ground separately. Voucher specimens were not deposited anywhere, as the fruit is freely available commercially.

Ethanol and water extracts were each prepared from three avocados of each variety. Ethanol extracts of the ground epicarp and seed were prepared by stirring 10 g of the homogenates in 50 mL of absolute ethanol at 4°C for 24 h (Emeruwa, 1982; Ulate-Rodriguez et al., 1997). Extracts were recovered by filtration and dried in a rotary evaporator at 70°C for 15 min. After drying, extracts were weighed, reconstituted in 5 mL of 50% ethanol, and stored at room temperature until use.

Water extracts were prepared as above, but after filtration, the slurries were centrifuged at 10,000 *g* for 15 min after which the supernatants were decanted. The resulting liquid was filtered and the filtered supernatants were freeze-dried. The dried extracts were weighed, reconstituted in 5 mL of distilled water, and then autoclaved. After sterilization, the extracts were stored at room temperature until use (Richter & Vore, 1989).

Microorganisms used

Ten bacteria, *Listeria monocytogenes* (ATCC 7644), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Salmonella* Enteritidis (ATCC 13076), *Citrobacter freundii* (ATCC 8090), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhimurium (ATCC 13311), and *Enterobacter aerogenes* (ATCC 13048), and three fungi, *Aspergillus flavus*, *Penicillium* spp., and *Zygosaccharomyces bailii*, from the Food Microbiology Laboratory collection at the University of Queensland were used.

Antibacterial activity determinations

The antibacterial activity of the extracts was determined using the microtiter broth microdilution assay (CLSI, 2006, 2008). Briefly, 100 µL of tryptic yeast soy glucose broth was dispensed into all wells of a 96-well microtiter plate. A diluted 1000 µg/mL solution of each extract (100 µL) was added to a separate well in the first column of the microtiter plate. Serial two-fold dilutions were made with a multichannel pipette, beginning with the first column, and proceeding until the following concentrations of each antimicrobial agent were obtained: 500, 250, 125, 62.5, 31.25 and 15.625 µg/mL. One hundred microliters of the contents of the sixth column were discarded, and columns seven and eight were left free of antimicrobials. Five microliters of an overnight culture grown in nutrient broth at 37°C containing ~10^8–10^9 cfu/mL was added to each well of the first seven columns. The wells in column eight were not inoculated and served as a negative control. Streptomycin was used as a positive control against these bacteria as described by Amarowicz et al. (2008). Plates were incubated at 37°C for 48 h. Plates were inspected visually for growth at 24 and 48 h. In this study, the minimum inhibitory concentration (MIC) was taken as the lowest concentration of antimicrobial agent at which there was no perceptible growth of the organism. Extracts with MICs equal to or greater than 500 µg/mL are described as having no antibacterial activity. All antibacterial activity studies were performed using three individual avocados of each variety.

Antifungal activity determinations

The antifungal activity of the extracts against the yeast *Zygosaccharomyces bailii* was determined as for the

antibacterial activity determinations described above. The antifungal activity of the extracts against the two molds was determined by the hole plate method using potato dextrose agar (PDA) as the growth medium (Qamar et al., 1996). Briefly, sterilized PDA inoculated with each of the fungi was vortexed and aseptically poured into sterile 90 mm Petri plates and allowed to congeal. Five holes of 0.6 cm diameter were aseptically punched into the Petri plates with a stainless steel borer of uniform edge and size. The holes were filled with 100 µL of epicarp or seed extract from the three different cultivars of avocado at a concentration of 500 µg/mL. There were two negative controls for each extract, one containing ethanol and the other containing sterile distilled water. Petri plates were incubated at 30°C for up to 5 days. Clear zones of inhibition around the holes were recorded as having antifungal activity. All antifungal activities were performed in triplicate as above.

Statistical analysis

Mann–Whitney, Kruskal–Wallis, and *t* tests were performed on all data sets using MINITAB software (MINITAB 15; Minitab Inc., Minneapolis, MN, USA) at a 95% confidence level.

Results and discussion

The results of the determination of antimicrobial activity for all ethanol extracts of avocado against both bacterial and fungal species are presented as MICs in Tables 1 and 2, respectively. The values presented for the bacteria and yeast are those obtained after 24 h (as these did not differ from those obtained at 48 h). Gram-positive bacteria that were susceptible to water extracts of avocado were *Listeria monocytogenes* and *Staphylococcus epidermidis* (data not shown). Antimicrobial activity against *Listeria monocytogenes* for water extracts ranged from 93.8 µg/mL for the Shepard variety epicarp to 375.0 µg/mL for the seed of Fuerte variety. The only water extract that displayed any activity against *Staphylococcus epidermidis* was the epicarp of the Hass variety (354.2 µg/mL). No antibacterial activities were observed against the other seven bacteria tested (Table 1).

In general the ethanol extracts displayed a wider range and higher level of activity than the water extracts, even though the results were not significantly different ($p > 0.05$). Ethanol extracts of avocado (from individual epicarp, seed, and cultivar combinations) displayed activity against most Gram-positive (except *Staphylococcus epidermidis*) and Gram-negative (except *Escherichia coli*) bacteria tested (Table 1). Minimum inhibitory concentration values ranging from 104.2 µg/mL (*Salmonella* Enteritidis) to 416.7 µg/mL (*Listeria monocytogenes* and *Staphylococcus aureus*) were observed.

Water extracts of avocado (from specific epicarp, seed, and cultivar combinations) displayed no activity against any of the fungi tested, while MICs as low as 166.7 µg/mL from the corresponding ethanol extracts were observed against *Zygosaccharomyces bailii* only (Table 2). It has been speculated that as avocados mature, the degree of

Table 1. Antibacterial activity (minimum inhibitory concentration, µg/mL) of ethanol extracts of three varieties of avocado against 10 bacteria[a].

	Shepard		Hass		Fuerte	
	Epicarp	Seed	Epicarp	Seed	Epicarp	Seed
Listeria monocytogenes	416.7 ± 144.3	166.7 ± 72.2	>500	>500	416.7 ± 144.3	125.0 ± 0.0
Staphylococcus epidermidis	>500	>500	>500	>500	>500	>500
Staphylococcus aureus	416.7 ± 144.3	416.7 ± 144.3	291.7 ± 190.9	>500	416.7 ± 144.3	208.3 ± 72.2
Enterococcus faecalis	>500	250.0 ± 0.0	>500	>500	500.0 ± 0.0	500.0 ± 0.0
Escherichia coli	>500	>500	>500	>500	>500	>500
Salmonella Enteritidis	208.3 ± 252.6	208.3 ± 252.6	104.2 ± 36.1	125.0 ± 0.0	125.0 ± 108.3	145.8 ± 95.5
Citrobacter freundii	166.7 ± 72.2	145.8 ± 95.5	208.3 ± 72.2	166.7 ± 72.2	250.0 ± 216.5	250.0 ± 216.5
Pseudomonas aeruginosa	166.7 ± 72.2	166.7 ± 72.2	208.3 ± 72.2	250.0 ± 216.5	250.0 ± 216.5	291.7 ± 190.9
Salmonella Typhimurium	375.0 ± 216.5	250.0 ± 216.5	>500	>500	>500	>500
Enterobacter aerogenes	250.0 ± 216.5	125.0 ± 0.0	250.0 ± 216.5	125.0 ± 0.0	>500	>500

[a]Data are means ± standard deviations of three individual avocados for each variety.

Table 2. Antifungal activity (minimum inhibitory concentration, µg/mL) of ethanol extracts of three cultivars of avocado against one yeast and two molds[a].

	Shepard		Hass		Fuerte	
	Epicarp	Seed	Epicarp	Seed	Epicarp	Seed
Zygosaccharomyces bailii	416.7 ± 144.3	416.7 ± 144.3	375.0 ± 216.5	104.2 ± 36.1	166.7 ± 72.2	375.0 ± 216.5
Penicillium spp.	>500	>500	>500	>500	>500	>500
Aspergillus flavus	>500	>500	>500	>500	>500	>500

[a]Data are means ± standard deviations of three individual avocados for each variety.

antifungal activity decreases due to the breakdown of the active ingredients (Karni et al., 1988).

The evidence presented in this article indicates that crude extracts of the epicarp and seed of mature avocados do have antimicrobials, and possess the potential to be used as a food additive. Furthermore, this may represent an alternative source of income from avocado waste. In order to realize this potential, however, further studies need to be performed to identify the active compounds in the mature fruit and optimize their extraction on a larger scale.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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