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SHORT COMMUNICATION

Antidiarrheal Activity of *Campomanesia xanthocarpa* Fruit

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ABSTRACT The growing list of drug-resistant microorganisms and the persistence of deaths due to diarrhea are compelling reasons to study plants in search of new therapeutic agents. The chemical constitution and popular use of the edible fruits of *Campomanesia xanthocarpa* O. Berg motivated this study to assess the antimicrobial and antidiarrheal properties of the fruits. An extract in 70% ethanol was prepared, and its antimicrobial activity was tested against several strains of bacteria by the agar diffusion and microdilution methods. Antidiarrheal activity was analyzed by testing intestinal motility in an animal model. Preliminary phytochemical study indicated the presence of flavonoids, saponins, and tannins in the hydroalcoholic extract. Antimicrobial activity was significant, but the minimum inhibitory concentration proved to be higher than the maximum extract concentration tested. The extract did not show significant activity for intestinal motility. Although this fruit extract did not show great results as an antimicrobial or antidiarrheal agent, the study contributes to the search for new plant agents and could be referred to as a research protocol by investigators in this area.

KEY WORDS: • *antidiarrheal* • *antimicrobial* • **Campomanesia xanthocarpa** • *diarrhea* • *fruits* • *tannins*

INTRODUCTION

THERE IS SUPPORT FROM the World Health Organization[1] for the study of plants in search of new antimicrobials, and the constant emergence of resistant microorganisms lends an urgency to these studies.[2,3] Many *in vitro* experiments have been done to select plants with potential antimicrobial activity.[4,5] One result that can be cited is the antimicrobial activity of a hydroalcoholic extract of *Piper regnelli* Miq. leaves against *Staphylococcus aureus* and *Bacillus subtilis*.[6]

The development of a medicine or product from a plant should respect regional and world needs both for new safe and efficient agents and for the preservation of biodiversity.[7] Species of the myrtle family Myrtaceae are widely used to treat, mainly, gastrointestinal disorders, hemorrhages, and infectious diseases, probably because of their astringency. Leaves and bark are the parts used most commonly, and the fruits (guava, clove, allspice, etc.) are generally edible.[8] Thus, the myrtaceous shrub *Campomanesia xanthocarpa* O. Berg, known in Brazil as *gabiroba*, occurs in the savannah-like Cerrado of south and southeast Brazil, Argentina, Uruguay, and Paraguay. Beside the widespread consumption of the fruits, its leaves are used as an antidiarrheal, depurative, and anti-inflammatory, and phytochemical analysis has indicated the presence of flavonoids, saponins, and tannins.[7,9–11]

Motivated by these considerations, the aim of the present study was to test the fruit of *C. xanthocarpa* for antimicrobial activity against a range of bacteria and for antidiarrheal activity in mice, by the method of intestinal motility. This fruit was chosen because, besides its promising chemical composition, it is edible and easily found.

MATERIALS AND METHODS

Ripe fruits of *C. xanthocarpa* were collected at Araraquara (SP, Brazil), on the campus of the São Paulo State University. They were dried for a week at 40°C, powdered, and extracted by cold turbo extraction with 70% ethanol. The extract was filtered, concentrated under reduced pressure, frozen and lyophilized, yielding 2% (wt/wt) dried fruit extract.

Bacterial test strains were *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC), *Escherichia coli* (ATCC 25922), *Salmonella setubal* (ATCC 19196), *Shigella sonnei* (clinical sample), *Staphylococcus aureus* (ATCC 25923), and *Staphylococcus epidermidis* (ATCC 27853). These cultures were maintained on slants of brain–heart infusion (BHI) agar at 4°C and were activated in BHI broth at 37°C for 24 hours.

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A preliminary phytochemical screening was carried out on an extract obtained by percolation of the dried and powdered fruit with 70% ethanol. The following chemical reactions were used to characterize secondary metabolites:[12] Shinoda and aluminum chloride reactions for flavonoids; gelatin, iron, and copper salts for tannins; Dragendorff, Bouchardat, Mayer, and Bertrand precipitation reactions for alkaloids; Borntraeger reaction for anthraquinones; Kedde and Liberman–Buchard for steroids; and foaming index for saponins.

The extract was analyzed by thin-layer chromatography on plates of Merck (Darmstadt, Germany) silica gel 60 F254 (0.2 mm thick), eluted in the solvent system chloroform/methanol/*n*-propanol/water (5:6:1:4 by volume) (organic phase). The thin-layer chromatography plate was sprayed with anisaldehyde–sulfuric acid, and spots were revealed by heating or under ultraviolet light.[13]

Agar diffusion was used to determine the activity of extracts against the test strains of bacteria.[14] For these assays, the extracts were dissolved in dimethyl sulfoxide (DMSO) to 200 mg/mL. An inoculum of bacterial cells was prepared at a density matching 0.5 on the McFarland scale (1.5×10^8 colony-forming units/mL), diluted 1:100 in Mueller-Hinton agar (to about 10^6 colony-forming units/mL), and poured into horizontal Petri dishes. Steel templates with six wells of 6 mm in diameter were placed on the solid medium, and 100 μL of the extract (50 mg/mL diluted in DMSO:BHI broth, 1:2 vol/vol), 100 μL of DMSO:BHI broth (1:2 vol/vol) (negative control), and 50 μL of ampicillin solution (50 μg/mL) or 50 μL of chloramphenicol solution (50 μg/mL) were separately added to each well. After 2 hours at 4°C, the plates were incubated at 37°C for 24 hours. Bacterial growth inhibition was determined by the diameter of the inhibition zone around each well, measured with digital calipers. The experiments were performed in triplicate.

Minimum inhibitory concentration (MIC) values were determined by the microdilution method.[15] The extract solution was diluted with BHI (1:5 vol/vol), with the final test concentration ranging from 10.000 mg/mL to 0.078 mg/mL. The wells of 96-well microplates were filled with 100 μL of BHI, and 100 μL of extract solution, diluted with BHI to 40 mg/mL, was added to the first well of a microplate line, starting a 1:1 (vol/vol) serial dilution along each column. Next, 100 μL bacterial suspensions were added separately, to give a final cell density of 2.5×10^5 colony-forming units/mL and a starting concentration of 10 mg/mL extract. The microplates were incubated aerobically at 37°C for 24 hours. Ampicillin and chloramphenicol (for *Salmonella* sp.) were used as positive controls, ranging in concentration from 12.500 μg/mL to 0.013 μg/mL. Bacterial growth was detected by adding 0.01% resazurin aqueous solution, and MIC values were identified as the lowest extract or reference drug concentration at which no growth was indicated by a change in color of resazurin from blue (absence of growth) to pink (growth).[16] Minimum bactericidal concentration (MBC) values were determined by inoculation on Mueller-Hinton agar plates with a sample from each well of the microplates that had been incubated at 37°C for 24 hours and were defined as the lowest concentration of the extract or reference drug at which microorganisms were completely killed. The experiments were performed in triplicate.

Gastrointestinal motility was assayed as described previously.[17–19] The extract was dissolved in sterile water to a concentration of 60 mg/mL. Thirty adult female albino Swiss mice (*Mus domesticus domesticus*), weighing 24–30 g, were selected and housed in polypropylene cages ($30 \times 20 \times 13$ cm) under standard conditions (21 ± 1°C with a 12:12-hour reversed light–dark cycle and relative humidity 50–60%) for 10 days before the experiment. Mice had free access to water and normal commercial laboratory diet (Purina, São Paulo, Brazil). The animal experiments complied with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, Ribeirão Preto, Brazil) and were approved by the Research Ethics Committee of the School of Pharmaceutical Sciences, São Paulo State University, in Resolution 24/2004. On the day of the test, the animals were divided into three groups of 10 mice each. They were weighed and deprived of food, with free access to water. Three hours after food deprivation, animals in the first group were treated by gavage with fruit extract of *C. xanthocarpa* (60 mg/mL) at 1,000 mg/kg body weight, whereas the negative control group received 0.9% NaCl sterile solution, and the positive control group received 5 mg/kg loperamide hydrochloride. Ninety minutes after the treatment with extracts, 0.4 mL of 10% aqueous suspension of charcoal powder in 5% gum acacia was administered to each animal orally. The animals were sacrificed 45 minutes later in a CO_2 chamber, and the abdomen was opened. The percentage of the length of the small intestine (from the pylorus to the cecum) traveled by the charcoal plug was determined.

The results are expressed as mean ± SD values. Statistical significance was defined as $P < .05$, and the significance of differences between groups was determined by one-way analysis of variance with Tukey's *post hoc* test.

RESULTS AND DISCUSSION

The phytochemical test reactions indicated the presence of flavonoids, saponins, and tannins in the fruit extract, all of which were confirmed by thin-layer chromatography.

In the agar diffusion assay, fruit extract showed activity at 50 mg/mL against all the bacteria tested, except *B. subtilis*, as presented in Table 1, and *E. faecalis* showed the highest susceptibility (largest clear zone). However, the MIC and MBC values indicated in the microdilution assay were higher than the maximum concentration tested (10 mg/mL), with one exception: *S. setubal* showed an MIC of 5 mg/mL, but its MBC was again higher than 10 mg/mL (Table 1).

In the gastrointestinal motility test, the percentage distance traveled by the charcoal plug in each group is shown in Table 2. The presence of extract did not decrease the motility significantly at the concentration tested, in contrast to the control (loperamide).

People use medicinal plants from the several different biomes found in Brazil, such as the Cerrado, Atlantic Forest,

Table 1. Antimicrobial Activity of Fruit Extract of *C. xanthocarpa*

	Agar diffusion assay (inhibition zone diameter)[a]		*Microdilution assay*			
			Extract[b]		*Control*[c]	
Microorganism	*Extract*	*Control*	*MIC*	*MBC*	*MIC*	*MBC*
B. subtilis	0.0	13.0 ± 1.4	>10	>10	1.56	1.56
E. faecalis	18.5 ± 1.4	17.7 ± 0.8	>10	>10	0.39	0.39
E. coli	8.3 ± 0.6	16.7 ± 0.6	>10	>10	0.39	0.78
S. Setubal	8.5 ± 0.7	12.5 ± 1.4	5	>10	625	1250
S. sonnei	8.5 ± 0.5	12.0 ± 1.7	>10	>10	3.13	3.13
S. aureus	12.0 ± 0.7	11.6 ± 1.4	>10	>10	3.13	3.13
S. epidermidis	9.0 ± 0.7	14.3 ± 0.7	>10	>10	1.56	1.56

[a]Diameter expressed in mm as mean ± SD of three determinations.
[b]Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values expressed in mg/mL.
[c]MIC and MBC values expressed in μg/mL.

and Amazon Forest, to treat tropical diseases as leishmaniasis, malaria, schistosomiasis, and bacterial and fungal infections.[20] These plants are used mainly in form of crude extracts, infusions, and medicated dressings.[21]

In order to identify new therapeutic agents and to make the use of medicinal plants safer, many groups have been investigating the therapeutic and toxic properties of native and exotic plants.[21,22] Many phytochemical, toxicological, and therapeutic studies of plants have been carried out or are in progress, but much more could be done, given the vast range of plant biodiversity. It has been estimated that the majority of the 250,000 higher plant species are still unscreened with regard to their pharmacological potential or have been studied only for one specific property.[7,23] Another reason to study plants is the multiple drug resistance that many human pathogens have acquired, owing to selection induced by indiscriminate use of antimicrobials, the emergence of uncommon infections, immunosuppressed patients, and the adverse side effects of some medicines. Among the plants studied, several have proved potential sources of antimicrobial agents.[24]

Plants with flavonoids, tannins, and saponins in their composition are potential sources of antimicrobial agents.[25] These metabolites are described as constituents of the fruit of *C. xanthocarpa*,[9,11] and this has been confirmed in this study by standard test reactions and thin-layer chromatography. A recent article describes the fruits of *C. xanthocarpa* as rich in nutritional compounds and monoterpenes constituting their essential oil.[26]

Table 2. Effect of Plant Extract on Gastrointestinal Motility

Treatment	*Distance traveled by charcoal plug (% length of small intestine)*
Saline	47.98 ± 10.31
Extract	48.18 ± 7.05
Loperamide hydrochloride	38.54 ± 10.21*

Data are mean ± SD values ($n = 10$ per group).
*$P < 0.05$ versus negative control and extract sample.

Antimicrobial activity against *S. aureus*, *Salmonella choleraesuis*, and *Candida albicans* has already been described in *gabiroba* leaf extract.[27] The fruit extract formed inhibition zones against all bacteria tested, except *B. subtilis*, but the MICs were higher than the maximum concentration tested. Thus, although the chemical constitution and antimicrobial activity of the leaves had measurable antimicrobial evaluation, it was found that the activity in whole fruit extract was weak and not a potentially usable property.

There are reports of antidiarrheal activity in the leaves of *C. xanthocarpa*,[8,9] and an objective of this research was to test for this activity in the fruit extract, because the tannins in its composition are edible and readily accessible. However, under the conditions used here, in a dose of 1,000 mg/kg of body weight, the extract showed no significant difference from the saline control, indicating that it did not have this property. The absence of any strong activity of the fruit extract against some etiological agents of diarrhea, such as *E. coli*, *Shigella* sp., and *Salmonella* sp., reinforced the indicated absence of therapeutic activity against diarrhea.

Research on chemical composition and popular uses is a non-random way to drive the biological study of plants.[7] In light of such information, as well as the easy access to fruits of *C. xanthocarpa* in Brazil, the antimicrobial and antidiarrheal activities of fruit extract of this plant were analyzed. However, both results were negative, in opposition to what was expected, and this report can be of value in putting these results on record, in order to prevent the use, for these ends, of similar methods of extraction of these fruits by the population.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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