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 Organization1 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, deparative, 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.
C. XANTHOCARPA FRUIT ANTIDIARRHEAL ACTIVITY

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 × 20 × 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 CO2 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*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Agar diffusion assay (inhibition zone diameter)</th>
<th>Microdilution assay (Extract)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>0.0</td>
<td>13.0 ± 1.4</td>
<td>&gt;10</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>18.5 ± 1.4</td>
<td>17.7 ± 0.8</td>
<td>&gt;10</td>
</tr>
<tr>
<td>E. coli</td>
<td>8.3 ± 0.6</td>
<td>16.7 ± 0.6</td>
<td>&gt;10</td>
</tr>
<tr>
<td>S. Setubal</td>
<td>8.5 ± 0.7</td>
<td>12.5 ± 1.4</td>
<td>5</td>
</tr>
<tr>
<td>S. sonnet</td>
<td>8.5 ± 0.5</td>
<td>12.0 ± 1.7</td>
<td>&gt;10</td>
</tr>
<tr>
<td>S. aureus</td>
<td>12.0 ± 0.7</td>
<td>11.6 ± 1.4</td>
<td>&gt;10</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>9.0 ± 0.7</td>
<td>14.3 ± 0.7</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

* MIC and MBC values expressed in μg/mL.

Data are mean ± SD of three determinations.

Microdilution assay (inhibition zone diameter) and Agar diffusion assay (inhibition zone diameter).

MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration.


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