In Vitro Evaluation of the Antimicrobial and Antimycobacterial Activities of Solanum guaraniticum A. St.-Hil. Leaves

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INTRODUCTION

Before the advent of modern medicine, people depended essentially on the plants for the treatment of various diseases, and they still have been an important source for the development of new drugs (Kuete et al., 2009). Plants contain large amounts of chemical compounds which can be explored for preventing microbial infections, through different mechanisms (Cowan, 1999; Savoia, 2012).

Therefore, natural products can be new promising alternatives for the treatment of infectious diseases, taking into account the emergence of antibiotic resistance, and undesirable side effects due to use of synthetic drugs (Cushnie and Lamb, 2011). Tuberculosis (TB) is considered a serious public health problem, taking place among the main infectious diseases (Arruda et al., 2012). Mycobacterium tuberculosis is responsible for more human mortality than any other single microbial species (Tekwu et al., 2012).

Currently, the treatment of TB involve along course of combination of antibiotics, leading to poor patient compliance, moreover, the multidrug resistance is also a problem. Thus, the discovery of new antituberculosis agents is urgent and, in this sense, various extracts and isolated compounds from plants have been tested with great results (Gautam et al., 2007; Tawde et al., 2012; Macabeo et al., 2012).

Solanum guaraniticum A. St.-Hil. (syn. Solanum fastigiatum var. acicularium Dunal) is a shrub popularly known as jurubeba or false jurubeba, occurring in Paraguay, Argentina and Brazil (Soares et al., 2008). According to the Brazilian Pharmacopoeia, the species Solanum paniculatum L. is recognized as the true jurubeba, and used in folk medicine as a tonic for fevers, anemia, erysipelas, cholagogue, bitter, and eupetect to treat gastric and liver dysfunctions (Mesia-Vela et al., 2002, Sabir and Rocha, 2008). Due to the similarities, these two species are used interchangeably by the population. S. guaraniticum has hepatoprotective (Sabir and Rocha, 2008) and antioxidant properties, being detected in the extract of its leaves the presence of...
caffeic, chlorogenic and rosmarinic acids, very active phenolic compounds (Zdra et al., 2012). Several Solanum species are cited in the literature to possess antimicrobial activity against fungi, bacteria and mycobacteria (Bontempo et al., 2013; Balachandran et al., 2012; Das et al., 2010; Lozoya et al., 1992). S. paniculatum had activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa (Lôbo et al., 2010) but, to the best of our knowledge, no studies concerning the antimicrobial activity of S. guaranitum have been reported. Thus, the aim of this study was to evaluate the in vitro antimicrobial and antymycobacterial activities of the crude extract (CE), chloroform (CHCl₃), ethyl acetate (AcOEt) and butanol (BuOH) fractions of the S. guaranitum leaves, against Gram-positive, Gram-negative bacteria, fungi and mycobacteria, by broth microdilution method to achieve the minimum inhibitory concentration (MIC), in order to verify their possible potential in the treatment of infectious diseases.

MATERIALS AND METHODS

Plant material and extractions

S. guaranitum leaves were collected in Guaporé (Rio Grande do Sul, Brazil), on December 2011. A voucher specimen was identified and archived in the herbarium of Department of Biology at Federal University of Santa Maria, under the registration number SMDB 13158. The plant material was dried in a stove with controlled temperature, powdered in a knife mill and extracted with ethanol (70%) for 7 days with daily agitation. After filtration, the material was concentrated on rotary evaporator to remove the ethanol and obtaining the aqueous extract, which was successively fractionated with solvents of increasing polarity, chloroform, ethyl acetate and n-butanol. The solvent was evaporated to obtain the dried fractions (CHCl₃, AcOEt and BuOH, respectively). Part of the aqueous extract was separated and taken to dryness, yielding the crude extract (CE).

Microorganisms tested

CE and fractions were individually tested against Staphylococcus aureus ATCC 25923, Staphylococcus intermedius (clinical isolate), Streptococcus agalactiae (clinical isolate), Enterococcus faecalis ATCC 51299, Micrococcus luteus ATCC 7468, Listeria monocytogenes (clinical isolate), Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae ATCC 700603, Escherichia coli ATCC 25922, Candida albicans ATCC 44373, Cryptococcus neoformans (clinical isolate) and Aspergillus fumigatus (clinical isolate). For the antymycobacterial assay, was used standard strains of Mycobacterium avium LRS41CDC, Mycobacterium tuberculosis H37Rv ATCC 25618 and Mycobacterium smegmatis mc² 155 ATCC 700084.

Antimycobacterial assay

The strains of mycobacteria were grown onto Löwenstein-Jensen medium and incubated for 3-5 days. Suspensions of these cultures were standardized using the scale 0.5 to Mac Farland, diluted in Middlebrook 7H9 broth supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase) (Difco Laboratories, Detroid, Mich) and 0.2% glycerol (MD7H9) until the concentration of 10⁷ CFU/mL. The CE and fractions tested were diluted in dimethylsulfoxide (DMSO) at a concentration of 50 mg/mL and diluted in MD7H9 until the desired concentrations, beginning the series with 2500 µg/mL. Susceptibility tests were performed by the broth microdilution method, according to CLSI M7-A7 (2006). Mycobacterial inoculums (100 µL) were placed in each well of a microdilution plate, as well as the extracts at corresponding concentrations. The test was performed in triplicate. The plates of M. smegmatis were incubated for 2 days, M. avium for 5 days and M. tuberculosis for 7 days at 37°C. In order to verify the growth of microorganisms, the dye 3-(4,5 dimethyl thiazole-2-yl) -2,5 diphenyl tetrazolium bromide (MTT- Sigma, USA) was added to each plate well. Then, the lowest extract concentration can produce inhibition of visible growth of microorganisms was considered as MIC.
RESULTS AND DISCUSSION

The activities of the CE and fractions from the S. guaraniticum leaves against bacteria and fungi are shown in Table 1. It was considered that if the extracts displayed a MIC less than 100 µg/mL, the antimicrobial activity was good; from 100 to 500 µg/mL the antimicrobial activity was moderate; from 500 to 1000 µg/mL the antimicrobial activity was weak; over 1000 µg/mL the extract was considered inactive. Many groups utilize faster growing and/or non-motile bacteria, especially the CHCl₃ fraction, as test organisms. In view of these criteria, it was possible to observe that the extracts were active against Gram-positive bacteria, with variables MICs from 32 to 1024 µg/mL. The AcOEt fraction showed good activity against Staphylococcus intermedius and Listeria monocytogenes. Staphylococcus intermedius is a member of the normal flora of dogs and is also a major opportunistic pathogen responsible for the common canine skin condition pyoderma, and can occasionally cause severe infections of humans (Bannoe et al., 2007). The facultative intracellular bacteria Listeria monocytogenes is associated with serious human and animal infections, including abortion and septicaemia. It is considered a pathogen of major concern due to high occurrence in foods and high mortality rate associated with listeriosis (Wang et al., 2013). Probably, these activities are due to highest content of polyphenols found in this fraction, as these metabolites have antimicrobial activity recognized (Daglia, 2012), and also by good antioxidant capacity by the DPPH method described previously (Zadra et al., 2013). Xiong et al. (2013) when performing a screening and identification of the antibacterial bioactive compounds from Lonicera japonica leaves found that the phenolic compounds present in the plant extract were responsible for the antibacterial activity shown.

Table 1. Minimum inhibitory concentrations (MIC) for CE and fractions of S. guaraniticum against bacteria and fungi.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (µg/mL)</th>
<th>CE</th>
<th>CHCl₃</th>
<th>AcOEt</th>
<th>BuOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>256</td>
<td>256</td>
<td>1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>Staphylococcus intermedius</td>
<td>256</td>
<td>128</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>256</td>
<td>128</td>
<td>256</td>
<td>256</td>
<td>256</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>256</td>
<td>256</td>
<td>128</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>32</td>
<td>128</td>
<td>512</td>
<td>512</td>
<td>512</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>128</td>
<td>128</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&gt;2500</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
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<tr>
<td>Escherichia coli</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
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<tr>
<td>Yeasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>Filamentous fungi</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
</tbody>
</table>

The best activity was found for the CE against Micrococcus luteus (MIC = 32 µg/mL), bacteria that causes minor infections especially in patients with suppressed immune system (Bonjar, 2004), and can be attributed to an interaction between the different components of the extract, such as polyphenols, tannins and alkaloids presented (Zadra et al., 2012), whose nature is very complex. In general, the CE and fractions showed moderate activity against other Gram-positive strains, including Streptococcus agalactiae and Enterococcus faecalis, pathogens associated with important diseases of clinical importance.

Concerning the Gram-negative bacteria, only the CE showed a weak activity against Pseudomonas aeruginosa, the other extracts were inactive against Klebsiella pneumoniae and Escherichia coli. These results can be explained, at least in part, because beside the efflux pumps, Gram-negative bacteria present some other characteristic particularities in their outer membrane like the polysaccharides that contributes to cell surface properties, such as membrane permeability and antibiotic susceptibility (Mahlke et al., 2009). All the extracts were inactive against fungi, both as yeasts Candida albicans and Cryptococcus neoformans and for the filamentous fungi Aspergillus fumigatus.

Mycobacteria are Gram-positive, non-motile and obligate aerobic bacteria. Due to the slow growth rate and pathogenicity of M. tuberculosis, many groups utilize faster growing and/or non-pathogenic mycobacteria as the test organism, including M. smegmatis and M. avium (Copp, 2003; Kuate et al., 2012; Boligon et al., 2012). In this study, the antimicrobial activity of the CE and fractions of S. guaraniticum was evaluated (Table 2), observing moderate activity for the CE, CHCl₃ and BuOH fractions against M. smegmatis, especially the CHCl₃ fraction, which showed the lowest MIC value (156 µg/mL). This activity is probably due to the highest contents of tannins and flavonoids present in the fraction, which also showed the best antioxidant activity assessed by inhibition of lipid and protein oxidation, demonstrating radical scavenging properties (Zadra et al., 2012).

It is well established that the antioxidant activity and phenolic compounds of plant extracts is related to its antimicrobial activity (Katalinic et al., 2013; Koysonboon et al., 2006; Alves-Silva et al., 2013). No significant activity was observed against M. tuberculosis and M. avium. Similar results were described by Cruz et al. (2012) regarding the activity of Ficus luschnathiana, whose butanolic fraction showed MIC value of 156.25 µg/mL for M. smegmatis, and was considered a promising antimycobacterial activity.

Table 2. Minimum inhibitory concentrations (MIC) for CE and fractions of S. guaraniticum against yeasts.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (µg/mL)</th>
<th>CE</th>
<th>CHCl₃</th>
<th>AcOEt</th>
<th>BuOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>1250</td>
<td>1250</td>
<td>2500</td>
<td>2500</td>
<td>2500</td>
</tr>
<tr>
<td>Mycobacterium smegmatis</td>
<td>312</td>
<td>312</td>
<td>2500</td>
<td>2500</td>
<td>2500</td>
</tr>
</tbody>
</table>

The solubility property of plant metabolites extracted with solvents of different polarity appears to contribute to the outcome of the antimicrobial assays employed (Othman et al., 2011). Higuchi et al. (2011) carried a bioassay-guided fractionation of the chloroform fraction from Byrsonima fagifolia leaves, resulting in the isolation of terpenoids with promising...
activity against *M. tuberculosis*. In another similar study, the compound methyl caffeate, isolated from the methanolic extract of *Solanum torvum* fruits, presented prominent antimycobacterial activity (Balachandran et al., 2012).

**CONCLUSIONS**

The results show that the CE and fractions from *S. guaranitireum* leaves possess good activity against *Staphylococcus intermedius*, *Listeria monocytogenes* (AcOEt fraction) and *Micrococcus luteus* (CE), and in most cases, moderate activity against Gram-positive bacteria. Although the CE has shown weak activity against *Pseudomonas aeruginosa*, it can be considered that the extracts were practically inactive against Gram-negative bacteria and inactive for fungi. Moderate activity against *Mycobacterium smegmatis* were observed for CE, BuOH, and especially for the CHCl3 fraction, which can be attributed to its good antioxidant activity and the highest content of flavonoids, tannins and alkaloids previously described. These findings suggest that *S. guarantitireum* leaves have antimicrobial and antimycobacterial potential and may come to be used primarily to treat diseases associated with Gram-positive bacteria, requiring further studies regarding the isolation of compounds in order to better understand which substances are responsible for these activities.

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