Comparative study of the use of jacketed reactor, ultrasound and maceration in the preparation of standardized extracts of "Alternanthera paronychioides" and their antioxidant activity

Fernando Henrique Bosso*, Marcos José Salvador.

Abstract
The objective of this study is to perform the comparative study of the use of jacketed reactor, ultrasound and maceration as extraction methods for the preparation of standardized extracts of "Alternanthera paronychioides" (Amaranthaceae), a Brazilian plant with potential antioxidant activity and antidiabetics. Therefore, ethanol was used as extractive liquid, and different extraction times for the same amount of vegetable powder and extractive liquid. The antioxidant activity in vitro (ORAC-FL assay) of the extracts was evaluated and the chemical characterization of active extracts was analyzed by way ESI(-)-MS and HPLC-UV-DAD/MS. The results indicate that etanol extracts of "A. paronychioides" has a remarkable antioxidant activity and among the extraction methods tested, the jacketed reactor and especially the ultrasound had the highest yields.

Key words:
Alternanthera paronychioides, Flavonoids, Extraction methods.

Introduction
The Amaranthaceae family has promising plants for biological activity and is therefore potential matrices for the search for natural antioxidants, suggesting a relationship between the occurrence of polyphenols, pharmacological properties and the ability to sequester free radicals. However, among many of the known species, little has been studied about Alternanthera paronychioides, with little information regarding its chemical preparation, whose secondary metabolites have apparently not been satisfactorily identified so far. The standardization of the extraction method is also crucial for the correct evaluation of the chemical potential of the species in question, since the method of extraction directly influences the quantities and types of compounds obtained. So, the objective of this project is to perform the comparative study of the use of jacketed reactor, ultrasound and maceration as extraction methods for the preparation of standardized extracts of Alternanthera paronychioides and chemical characterization of extracts by way ESI(-)-MS and HPLC-UV-DAD/MS.

Results and Discussion
The experiments indicate that the ultrasound and the jacketed reactor show higher yields of antioxidant activity, being 919 μmol of TE/g for the jacketed reactor 60 min and 1021 μmol of TE/g for the ultrasound 60 min. Ethanolic extract obtained by maceration showed antioxidant activity of 886 μmol of TE/g and corroborate the literature data1. In initial analyzes by mass spectrometry (ESI (-) - MS and MS/MS), negative ion mode, is suggestive of the presence of O- and C- glycosylated flavonoids, including m/z [M-H]-: 431, indicative of vitexin and m/z [M-H]-: 609 indicative of rutin.

Chart 1. Antioxidant capacity by ORAC-FL assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant capacity in μmol of TE/g (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH extract Maceration (24h)</td>
<td>886 (5.19)a</td>
</tr>
<tr>
<td>EtOH extract Ultrasound (60min)</td>
<td>1021 (4.96)a</td>
</tr>
<tr>
<td>EtOH extract Jacketed reator (60min)</td>
<td>919 (7.39)a</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5.62 (0.89)b</td>
</tr>
</tbody>
</table>

*aORAC data expressed as relative micromole of trolox equivalent per gram of extract, mean (%RSD, relative standard deviation of triplicate assays) of triplicate assays.
*bORAC data for experimental positive control expressed as relative trolox equivalent, mean (%RSD, relative standard deviation) of triplicate assays.

Conclusions
The results indicate that A. paronychioides has a remarkable antioxidant activity which is probably due to O- and C- glycosylated flavonoids, including rutin and vitexin, and among the extraction methods tested, the jacketed reactor and especially the ultrasound had the highest yields.

Acknowledgement
Thanks to the doctoral student Jane V. N. Marinho for all the teachings and great support. Thanks to FAPESP, CAPES, CNPq and FAEPEX-UNICAMP for the financial support.

References