EVALUATION OF EFFICACY OF PRESERVATIVES ASSOCIATED WITH ACHILLEA MILLEFOLIUM L. EXTRACT AGAINST BACILLUS SUBTILIS

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Submitted: August 12, 2004; Approved: February 08, 2006

SHORT COMMUNICATION

ABSTRACT

The antimicrobial efficacy of three preservatives used in cosmetic formulations was evaluated. Phenova® and imidazolidinyl urea inhibited the growth of *Bacillus subtilis* when added to leaf extract of *Achillea millefolium* L., whereas 0.2% Nipagin®/Nipasol® in propylene glycol did not.

Key words: *Achillea millefolium* L., preservatives, antibacterial activity, *Bacillus subtilis*

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(7:3, v/v) to prepare 100 mL of ethanolic extract. A glycolic extract was prepared by diluting 8% of the ethanolic leaf extract in propylene glycol and it was shown in microbiological tests to be free of microorganisms. Half of the glycolic extract was divided into three aliquots, to which were added, respectively: Phenova® (phenoxyethanol plus parabens), imidazolidinyl urea, and Nipagin®/Nipasol® (methyl and propylparabens) (13) in propylene glycol solution, to achieve 0.2% of each one as the final concentration in the growth test (4).

The inoculum prepared to contaminate the extract was adjusted to the 0.5 Mc Farland scale (1-3 x 10^8 CFU/mL) in brain and heart infusion broth (BHI) with *Bacillus subtilis* ATCC 9372 previously grown at 37°C for 24h. Part of the three test solutions and the *A. millefolium* leaf extract, was immediately contaminated with 10^7 CFU/mL of *B. subtilis*. The *A. millefolium* glycolic extracts (100 µL), contaminated or not, were diluted in 1 mL of BHI broth to perform the antibacterial tests. In parallel, the following were incubated at 37°C for 24h: microorganism-free *A. millefolium* L. glycolic and *B. subtilis* contaminated *A. millefolium* L. glycolic extract, with or without the preservatives being tested, propylene glycol, which was the solvent for the preservative, and BHI as a control medium. Bacterial growth was observed by the presence of turbidity, its absence showing a bacteriostatic effect. Next, a sample of each tube was removed and plated on Müller-Hinton agar, which was incubated at 37°C for 24h to observe bacterial growth as a test for bactericidal properties.

In Table 1 we can see that the previously contaminated extracts showed no microbial growth when Phenova® and imidazolidinyl urea were used as preservatives. The preservative imidazolidinyl urea has good bacteriostatic activity with at a concentration of 0.2%, a bactericidal effect with a concentration of 0.5%. The *B. subtilis* was present as a contaminant in plant extracts, in which it does not reveal itself by any modification in physicochemical and organoleptic properties.

### Table 1. Evaluation of preservatives associated with *Achillea millefolium* L. extract against *Bacillus subtilis*.

<table>
<thead>
<tr>
<th>Preservatives 0.2%</th>
<th>Without contamination</th>
<th>With contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>-/ -</td>
<td>+</td>
</tr>
<tr>
<td>Control Preservative 1</td>
<td>-/ -</td>
<td>-/ -</td>
</tr>
<tr>
<td>Control Preservative 2</td>
<td>-/ -</td>
<td>-/ -</td>
</tr>
<tr>
<td>Control Preservative 3</td>
<td>-/ -</td>
<td>+</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>-/ -</td>
<td>+</td>
</tr>
<tr>
<td>Medium Control</td>
<td>-/ -</td>
<td>+</td>
</tr>
</tbody>
</table>

a) (+) Bacterial growth on plate; b) / - / No bacterial growth; c) Preservative 1: Phenova®; d) Preservative 2: Imidazolidinyl urea; e) Preservative 3: Nipagin®/Nipasol®.

Under the conditions used in this analysis, we conclude that 0.2% Nipagin®/Nipasol® mixture was not effective, during the 24h contact against this bacterium. However, under different assay conditions, these preservatives might show better activity against *B. subtilis*. These results are rather significant since *B. subtilis* may be present as a contaminant in plant extracts, in which it does not reveal itself by any modification in physicochemical and organoleptic properties.

### RESUMO

**Avaliação da eficácia de conservantes associados a extrato de *Achillea millefolium* L. contra *Bacillus subtilis***

A eficácia antimicrobiana de conservantes empregados em formulações cosméticas foi avaliada usando Phenova® e Imidazolidinil uréia que inibiram o crescimento de *Bacillus subtilis* no extrato de *Achillea millefolium* L. e Nipagin®/Nipasol® 0.2% em propilenoglicol não apresentaram efeito microbicida.

**Palavras-chave:** *Achillea millefolium* L., conservantes, atividade antibacteriana, *Bacillus subtilis*

### REFERENCES