MICROBIOLOGICAL EVALUATION AND PRESERVATIVE EFFICIENCY OF NEW MANDELC ACID DERIVATIVES IN OINTMENTS

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Abstract
The aim of this study was to investigate the antibacterial and antifungal activity of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid and also to evaluate the preservative efficiency of these two new mandelic acid derivatives in ointments. The antimicrobial activity was determined by the agar disk diffusion method and by microdilution broth technique, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The results show that these two mandelic acid esters were found to be effective against the tested microorganisms and exert a good antibacterial and antifungal activity at low concentrations. The oxi-acetyl mandelic acid and oxi-propionyl mandelic acid were included in 0.0125 g % and respectively 0.05 g % concentrations in ointments samples, and it was evaluated the efficiency of the preservative action for 28 days, in the presence and in the absence of the test microorganisms - Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 90028. The tested mandelic acid esters proved to have a very good preservative action and could be used to preserve ointments in 0.0125 g % and respectively 0.05 g % concentrations.

Resumat
Scopul acestui studiu a fost investigarea acțiunii antimicrobiene și antifungice a acidului oxi-acetil mandelic și a acidului oxi-propionil mandelic în unguente. Acțiunea antimicrobiană a fost determinată prin metoda difuzimetrică și metoda microdiluțiilor în agar Mueller-Hinton, conform recomandărilor Clinical and Laboratory Standards Institute (CLSI). Rezultatele obținute arată că cei doi esteri ai acidului mandelic s-au dovedit eficaci asupra microorganismelor testate și exercită o activitate antibacteriană și antifungică bună, fiind activi în concentrații mici. Acidul oxi-acetil mandelic și acidul oxi-propionil mandelic au fost introdus în probe de unguente în concentrații de 0,0125% și respectiv 0,05% g, evaluându-se eficiența acțiunii conservante timp de 28 de zile în prezența și în absența microorganismelor testate: Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 90028. Cei doi esteri ai acidului mandelic testați au demonstrat o foarte bună acțiune conservantă, putând fi utilizați pentru conservarea unguentelor farmaceutice în concentrație de 0,0125% g și respectiv 0,05% g.

Keywords: mandelic acid, preservatives, antibacterial action, antifungal action

Introduction
Solutions, gels, ointments and other pharmaceutical forms can be invaded by microorganisms that determine changes in their organoleptic or physico-chemical properties [1]. Thus, the use of the antimicrobial preservatives is required in order to impede the development of the microorganisms in the pharmaceutical forms. Preservatives have a major importance in fighting microbial contaminations of pharmaceutical forms. However, over the past few decades, many preservatives have become less effective against certain microorganisms due to the emergence of drug-resistant bacteria. Thus, it is essential to investigate new substances with preservative action with less resistance. Recent trends show that the discovery rate of active new molecular entities is declining [2, 3]. In the current investigation, a screening of new mandelic acid esters was performed in order to identify new preservatives. Mandelic acid is hydroxy-acid active against some bacteria (Staphylococcus aureus, Proteus spp., Escherichia coli, Aerobacter aerogenes etc.) in the range of 350-500 mg/L [4, 5]. It is a nontoxic substance which has a long history of use as an
Antimicrobial activity. Antibacterial and antifungal potential of mandelic acid esters were assessed in terms of zone of inhibition of microbial growth (Figure 1 and Figure 2). Statistical analysis of the results included the calculation of standard deviation. (Table I and Table II).
Figure 1. Antibacterial effects of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid against *Staphylococcus aureus* ATCC 25923

Figure 2. Antifungal effects of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid against *Candida glabrata* ATCC MYA 2590

### Table I
Antibacterial effects of mandelic acid derivatives

<table>
<thead>
<tr>
<th>Compounds/antibiotics</th>
<th>S. aureus ATCC 25923</th>
<th>S. lutea ATCC 9341</th>
<th>E. coli ATCC 25922</th>
<th>Pseudomonas aeruginosa ATCC 27853</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxi-acetyl mandelic acid</td>
<td>36.06 ± 0.05</td>
<td>37.03 ± 0.15</td>
<td>33.96 ± 0.05</td>
<td>30.06 ± 0.05</td>
</tr>
<tr>
<td>Oxi-propionyl mandelic acid</td>
<td>37.1 ± 0.10</td>
<td>37.03 ± 0.15</td>
<td>29.03 ± 0.05</td>
<td>25.1 ± 0.10</td>
</tr>
<tr>
<td>Ampicillin (25 µg/disc)</td>
<td>25.1 ± 0.10</td>
<td>25.1 ± 0.10</td>
<td>15.06 ± 0.05</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol (30 µg/disc)</td>
<td>25.1 ± 0.10</td>
<td>25.1 ± 0.10</td>
<td>32.13 ± 0.11</td>
<td>21.06 ± 0.11</td>
</tr>
</tbody>
</table>

### Table II
Antifungal effects of mandelic acid derivatives

<table>
<thead>
<tr>
<th>Compounds/antibiotics</th>
<th>Candida albicans ATCC 90028</th>
<th>Candida glabrata ATCC MYA 2590</th>
<th>Candida parapsilosis ATCC 22019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxi-acetyl mandelic acid</td>
<td>50.00 ± 0.00</td>
<td>48.03 ± 0.05</td>
<td>48.93 ± 0.05</td>
</tr>
<tr>
<td>Oxi-propionyl mandelic acid</td>
<td>48.03 ± 0.05</td>
<td>47.03 ± 0.05</td>
<td>46.97 ± 0.05</td>
</tr>
<tr>
<td>Nystatin (100 µg/disc)</td>
<td>17.90 ± 0.10</td>
<td>19.00 ± 0.00</td>
<td>20.1 ± 0.10</td>
</tr>
</tbody>
</table>

The data obtained in the quantitative antimicrobial activity are presented in Table III.

### Table III
MIC values (µg/mL) of tested derivatives

<table>
<thead>
<tr>
<th>Sample</th>
<th>S. aureus ATCC 25923</th>
<th>Candida albicans ATCC 90028</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxi-acetyl mandelic acid</td>
<td>125</td>
<td>150</td>
</tr>
<tr>
<td>Oxi-propionyl mandelic acid</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

The results showed that these two mandelic acid esters were found to be effective against the tested microorganisms and exerted a good antibacterial and antifungal activity at lower concentrations. Oxy-acetyl mandelic acid showed a better antimicrobial activity than oxi-propionyl mandelic acid. The efficiency of preservative action in ointments.

The preservative properties are considered adequate if, in the condition of the test, there is a marked reduction or no increase in the number of microorganisms in the inoculated preparation after the test period. The criteria for the evaluation of antimicrobial activity are expressed in terms of the logarithmic reduction in the number of viable microorganisms against the value obtained from the inoculum.

The obtained results demonstrated the antimicrobial and antifungal activity of those two tested preservatives in ointments. These data are presented in Figure 3.

The obtained results showed that the bacteria were killed two days after inoculation. The activity against *Candida* strains was lower, although the two preservatives were able to reduce the viable number of fungus colonies.
In the case of the samples which didn’t contain preservatives but were contaminated with the same test microorganism there was noticed the presence of the microorganisms in a variable number. Thus, in the case of the samples without preservatives there were present a growth of the number of microorganisms, the microorganisms contaminating the ointment samples. The control samples which contained these preservatives but were not contaminated with test microorganisms did not present contaminants 14 days after the inoculation. There wasn’t registered any bacterial growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* or other contaminants during the entire test period.

**Conclusions**

Two mandelic acid derivatives have shown a very effective antimicrobial action. The two compounds, the oxi-acetyl mandelic acid and the oxi-propionyl mandelic acid, totally inhibited the Gram positive and Gram negative bacteria and diminished the number of fungal colonies two days after the inoculation of the ointment samples. The oxi-acetyl mandelic acid and the oxi-propionyl mandelic acid proved to have a very good preservative action and could be used as antimicrobial preservatives in 0.0125 g % and respectively 0.05 g % concentrations in order to prevent proliferation or to limit microbial contamination of ointments.

**References**