ORIGINAL ARTICLE

MICROBIOLOGICAL EVALUATION AND PRESERVATIVE EFFICIENCY OF NEW MANDELIC 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.

Rezumat

Scopul acestui studiu a fost investigarea acţiunii antimicrobiene şi antifungice a acidului oxi-acetil mandelic şi a acidului oxi-propionil mandelic precum şi evaluarea eficienţei acţiunii conservante a acestora în unguente. Acţiunea antimicrobiană a fost determinată prin metoda difuzimetrică şi metoda microdiluţiilor în agar Mueller-Hinton, conform recomandărilor *Clinical and Laboratory Standards Institute* (CLSI). Rezultatele obţinute arată că cei doi esteri ai acidului mandelic s-au dovedit eficienţi asupra microorganismelor testate şi exercită o activitate antibacteriană şi antifungică bună, fiind activi în concentraţii mici. Acidul oxi-acetil mandelic şi acidul oxi-propionil mandelic au fost introduşi în probe de unguente în concentraţii de 0,0125 g % şi respectiv 0,05 g %, evaluându-se eficienţa acţiunii conservante timp de 28 de zile în prezenţa şi în absenţa microorganismelor test: *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028. Cei doi esteri ai acidului mandelic testaţi au demonstrat o foarte bună acţiune conservantă, putând fi utilizaţi pentru conservarea unguentelor farmaceutice în concentraţie de 0,0125 g % şi 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

antibacterial agent in the treatment of urinary tract infections. Nowadays it is also used in the treatment of skin problems, such as acne [6, 7].

Taking into consideration the antimicrobial action of DL-mandelic acid, there were synthesized two mandelic acid esters that could also have an antimicrobial action – oxi-acetyl mandelic acid and oxi-propionyl mandelic acid. These two substances were synthesized in our laboratory, their physico-chemical properties and structure were validated and the results were previously published [8].

Materials and Methods

Microorganisms. The antimicrobial activity was studied using Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and pathogenic yeasts (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC MYA 2950, *Candida parapsilosis* ATCC 22019). All these strains were obtained from the Culture Collection of the Department of Microbiology, Faculty of Pharmacy, "Gr. T. Popa" University of Medicine and Pharmacy, Iaşi, Romania.

Antimicrobial activity. The antimicrobial activity was evaluated by the agar disc diffusion method [9]. A small amount of each microbial culture was diluted in sterile 0.9% NaCl until the turbidity was equivalent to McFarland standard no. 0.5 (10^6 colony forming units (CFU)/mL). The suspensions were further diluted 1:10 in Mueller Hinton agar for bacteria and Sabouraud agar for yeasts and then spread on sterile Petri plates (25 mL/Petri plate). According to the guidelines of Clinical and Laboratory and Standards Institute (CLSI) [9], the antimicrobial activity was determined by the agar disk diffusion method, with an *inoculum* of overnight culture, in final concentrations of about 10^5 CFU/mL. The microorganism's suspensions were further diluted 1:10 in Mueller Hinton agar for bacteria and Sabouraud agar for yeasts and then spread on sterile Petri plates (25 mL/Petri plate). Sterile stainless steel cylinders (5 mm internal diameter; 10 mm height) were applied on the agar surface in Petri plates. Then, 0.1 mL of each compounds were added into cylinders. Commercial available discs containing Ampicillin (25 µg/disc), Chloramphenicol (30 µg/disc) and Nystatin (100 µg /disc) were also placed on the agar surface. The plates were incubated at 37°C for 24 h (bacteria) and at 24°C for 48 h (yeasts). After incubation the diameters of inhibition zones were read in triplicate and the average was taken as final reading.

Broth microdilution method. The antimicrobial activity of the tested mandelic acid esters was quantified by the broth microdilution method [9]. Stock solutions of the test compounds were prepared over a range of concentrations from 2000 to 1 µg/mL. The microorganisms used in this assay were *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 90028. The minimum inhibitory concentrations (MIC) (µg/mL) were recorded after 24 h of incubation at 37°C for *Staphylococcus aureus* or at 24°C for *Candida albicans* as the concentration of the compound which inhibited the visible growth of the tested microorganism.

The efficiency of preservative action in ointments. The preservative properties were determined according to European Pharmacopoeia standards [10]. The ointment samples were prepared according to the following formulas: Rp1/ cetyl alcohol 5 g, sunflower seed oil 20 g, lanoline 5 g, vaseline 20 g, oxi acetyl-mandelic acid 0.0125 g, distilled water q.s. ad 100 g; Rp2/ cetyl alcohol 5 g, sunflower seed oil 20 g, lanoline 5 g, vaseline 20 g, oxi-propionyl mandelic acid 0.05 g, distilled water q.s. ad 100 g; Rp3/ cetyl alcohol 5 g, sunflower seed oil 20 g, lanoline 5 g, vaseline 20 g, distilled water q.s. ad 100 g.

There were used four samples of 50 g ointment (one is the control sample) in which were added as preservative oxi acetyl-mandelic acid (preservative I) in 0.0125% concentration. Another four samples of 50 g ointment were used (one is the control sample) in which were added as preservative oxi-propionyl mandelic acid (preservative II) in 0.05% concentration. There were also used three samples of 50 g ointment without preservatives. The samples were contaminated with the test microorganims *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028.

The ointment samples were tested over a period of 28 days in the presence and in the absence of the test microorganisms. Suspensions of each test microorganisms were used to inoculate the ointment samples in their final containers in order to give a final inoculum of 10^5-10^6 CFU/mL and mixed thoroughly to ensure a homogenous distribution. The inoculated containers were stored at 20-25^{0}C in the absence of light. There were gathered samples at different time intervals after the inoculation (0 h, 6 h, and 48 h, 7 days, 14 days, and 28 days) and the viable microorganisms were counted by plate count.

Results and Discussion

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).

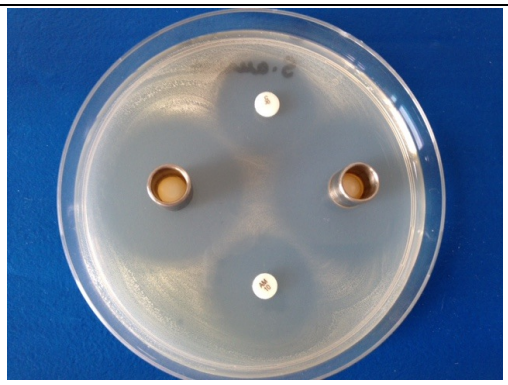

Figure 1.
Antibacterial effects of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid against *Staphylococcus aureus* ATCC 25923

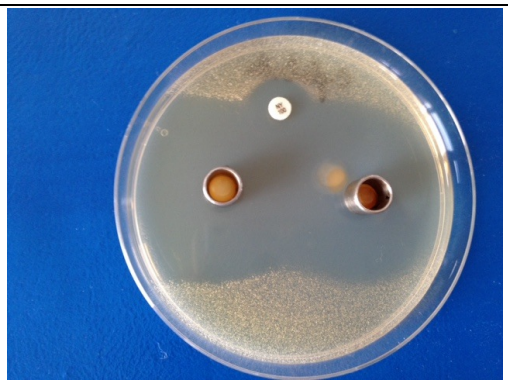

Figure 2.
Antifungal effects of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid against *Candida glabrata* ATCC MYA 2590

Table I
Antibacterial effects of mandelic acid derivatives

Compounds/ antibiotics	Diameter of inhibition zone (mm) ± Standard Deviation (SD)			
	S. aureus ATCC 25923	*S. lutea* ATCC 9341	*E. coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853
Oxi-acetyl mandelic acid	36.06 ± 0.05	37.03 ± 0.15	33.96 ± 0.05	30.06 ± 0.05
Oxi-propionyl mandelic acid	37.1 ± 0.10	37.03 ± 0.15	29.03 ± 0.05	25.1 ± 0.10
Ampicillin (25 µg/disc)	25.1 ± 0.10	25.1 ± 0.10	15.06 ± 0.05	0
Chloramphenicol (30 µg/disc)	25.1 ± 0.10	25.1 ± 0.10	32.13 ± 0.11	21.06 ± 0.11

Table II
Antifungal effects of mandelic acid derivatives

Compounds/ antibiotics	Diameter of inhibition zone (mm) ± Standard Deviation (SD)		
	Candida albicans ATCC 90028	*Candida glabrata* ATCC MYA 2590	*Candida parapsilosis* ATCC 22019
Oxi-acetyl mandelic acid	50.00 ± 0.00	48.03 ± 0.05	48.93 ± 0.05
Oxi-propionyl mandelic acid	48.03 ± 0.05	47.03 ± 0.05	46.97 ± 0.05
Nystatin (100 µg/disc)	17.90 ± 0.10	19.00 ± 0.00	20.1 ± 0.10

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

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

Sample	Microorganism test/ MIC values (µg/mL)	
	S. aureus ATCC 25923	*Candida albicans* ATCC 90028
Oxi-acetyl mandelic acid	125	150
Oxi-propionyl mandelic acid	500	500

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 efficiency of the preservative system is considered achieved if the number of viable microorganisms registers a reduction of no more than 0.1% of the initial *inoculum* (10^5-10^6 CFU/mL) and the number of the viable fungus colonies remains the same or diminishes in the first 4 days of the testing period [11].

The obtained results demonstrate 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.

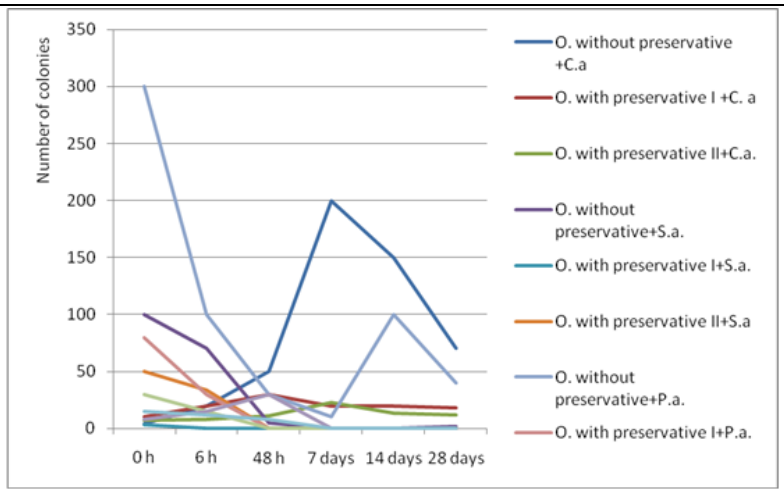

Figure 3.

The preservative action evaluation of some mandelic acid esters in ointments

O = ointment; preservative I = oxi-acetyl mandelic acid; preservative II = oxi-propionyl mandelic acid; C.a. = *Candida albicans*; S.a. = *Staphylococcus aureus*; P.a. = *Pseudomonas aeruginosa*; C = control

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.

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