

# Development and sequential analysis of a new multi-agent, anti-acne formulation based on plant-derived antimicrobial and anti-inflammatory compounds

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## Background

The antibacterial and anti-inflammatory potential of plant-derived compounds have been reported in many studies. Emerging evidence indicates that plant-derived essential oils and/or their major compounds may represent a plausible alternative treatment for acne, a prevalent skin disorder in both adolescent and adult population. Therefore, the purpose of this study was developing and sequential analysis of the antimicrobial activity of a new multi-agent, synergic formulation, based on plant-derived antimicrobial compounds (i.e., eugenol, beta-pinene, eucalyptol, limonene) and anti-inflammatory agents (salicylic acid) for potential use in the topic treatment of acne and other skin infections. The synergism between the natural compounds and conventional preserving system, 2-phenoxyethanol : potassium sorbate was also assessed.

## Material/methods

The antimicrobial qualitative screening was carried out against standardized microbial strains recommended by the European Pharmacopoeia (8th ed.) for the microbiological quality assessment of topical drug formulations, i.e.: *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231. Quantitative analysis was performed by the serial microdilution technique. The fractional inhibitory concentrations (FIC) and index (FICI), were calculated based on the MIC results, using specific formula for determining the possible synergism of action between the tested compounds.

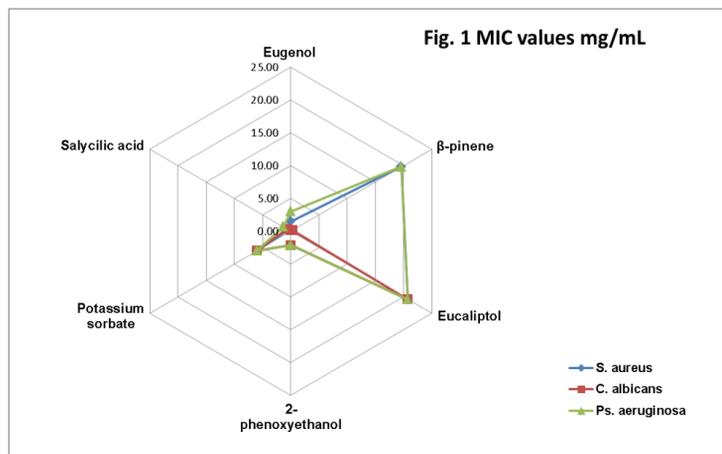
## References:

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The flow cytometry assay was carried out in order to evaluate the possible mechanism of action of the tested compounds, as well as of their mixtures with the conventional preserving system 2-phenoxyethanol : potassium sorbate – mixture 1, or with salicylic acid – mixture 2. The tested concentrations for the flow cytometry assay were MIC and MIC/ 2. The antimicrobial combinations were included in a cream base formulation

## Results

In the present study the MIC (mg/ mL) values of eugenol, eucalyptol,  $\beta$ -pinene, conventional preservatives, i.e. 2-phenoxyethanol and potassium sorbate and salicylic acid, an antiacne active component were under 5 mg/ mL for all the tested strains (Fig.1)



The optimal antimicrobial combinations selected in this study were: eugenol:  $\beta$ -pinene: salicylic acid and eugenol:  $\beta$ -pinene: 2-phenoxyethanol: potassium sorbate (Table 1).

Table 1. Percentage concentration for the components

Tested mixtures	Percentage concentration for the components (%)				
	eugenol	$\beta$ -pinene	salicylic acid	2-phenoxyethanol	potassium sorbate
Mix 1	0,7	3,17	0,17	-	-
Mix 2	0,7	3,17	-	0,37	0,094

Mun et al., 2014 proposed an interpretation model for the calculated FICI, i.e.:  $\leq 0.5$ , synergism of action; 0.5 - 0.75, partial synergism;  $> 0.75 - 1$  additive effect;  $> 1$ , without effect, and  $> 4$  - antagonistic effect. The tested mixtures revealed FICI values in the range of 0.5-0.75, demonstrating a partial synergism, excepting FICI calculated for mix2 against *C. albicans* strain, with a value of 1.23, suggesting the lack of interaction between the mix' components.

The possible mechanisms of action revealed by flow cytometry were cellular coatings permeabilization and inhibition of efflux pumps activity.

Concerning the flow cytometry analysis of the antimicrobial mixtures, a significant cellular coatings permeabilisation of *P. aeruginosa* and *S. aureus* cells was induced by both mix1 and mix2 at concentrations MIC/8, while in case of *C. albicans* only mix 1 exhibited this effect at MIC/8 (Fig. 2).

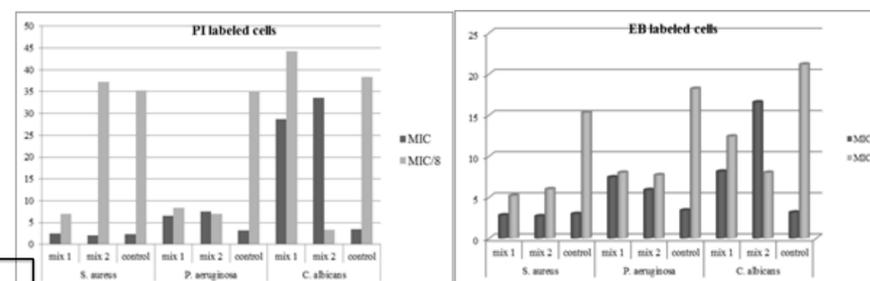


Fig 2. PI and EB labeled cells – median of fluorescence intensity

Similar effects were obtained for the individual tested components which exhibited a general efflux pumps activating effect at MIC/8 in case of all tested strains .

Formal methods used to assess the effectiveness of various conservation systems and the similarities and differences between them were analyzed by several authors. For this study, the challenge test has been used. The topical formula conservation system meets the A criteria specified in the European Pharmacopoeia edition in use. The cream base formulation demonstrated thermodynamic stability and optimum microbiological characteristics.

## Conclusions

The complex algorithm proposed in this study allowed the *in vitro* selection of natural active compounds for optimized combinations with antimicrobial activity, with applications in cosmetic and pharmaceutical industries. The possible mechanisms of action revealed by flow cytometry were cellular permeabilization and inhibition of efflux pumps activity induced by concentrations corresponding to sub-MIC values. The most active antimicrobial combination represented by eugenol: $\beta$ -pinene:2-phenoxyethanol: potassium sorbate was included in a cream base formulation alongside salicylic acid as a well known antiacne compound.

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