

# Synergic complex of two plant flavonoids as a novel tyrosinase inhibitor

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## INTRODUCTION

Tyrosinase is a key enzyme in melanin formation. Its inhibition is a crucial step for hyperpigmentation reduction. Glabradin is a isoflavonoid derived from licorice (*Glycyrrhiza glabra*). Its main activity is inhibition of melanin synthesis. It can also act as an antioxidant and anti-inflammatory agent. Ellagic acid is known polyphenolic compound derived from pomegranate fruit. It is strong antioxidant and whitening agent. However its role in melanin synthesis is unclear<sup>1,2,3</sup>. Thus the aim of this study was to evaluate the efficacy of two plant flavonoids: ellagic acid and glabradin, on tyrosinase inhibition as well as their whitening properties *in vivo*.

## MATERIALS AND METHODS

### In vitro tests

The IC<sub>50</sub> for ellagic acid, glabradin and their mixture in tyrosinase inhibition reaction was estimated. Kojic acid served as a reference sample. L-DOPA was used as tyrosinase substrate during enzymatic reaction. Measurement of absorbance increase was conducted for 5-6 min in 475 nm wavelength. For each sample we measured the rate of change in absorbance per min ( $\Delta A/\text{min}$ ). Level of inhibition was shown in % by division of  $\Delta A/\text{min}$  value obtained for each sample containing different concentrations of inhibitor (reference, tested substances and its mixture), by  $\Delta A/\text{min}$  value obtained for sample without inhibitor. GraFit programme was used to calculate IC<sub>50</sub>.

### In vivo test

The *in vivo* efficacy of an emulsion with ellagic acid and glabradin on 14 patients with hyperpigmentation was performed. Mexameter and Visia were used to assess skin parameters before and after 4 weeks of cream usage. Self-evaluation of the skin condition was also performed.

## RESULTS

### Results IN VITRO

IC<sub>50</sub> count according to 4-parameter equation:

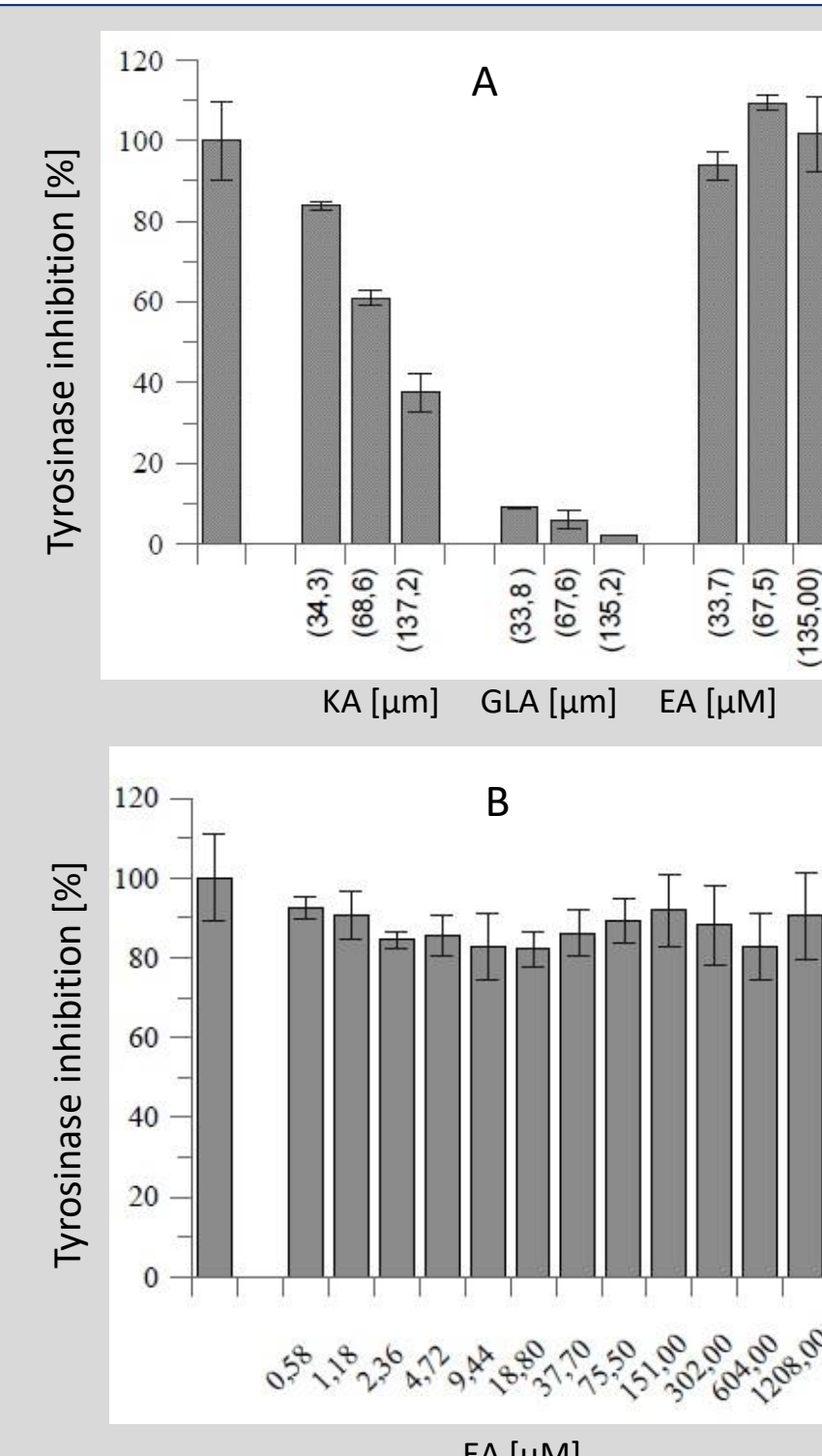
$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^s} + \text{background}$$

IC <sub>50</sub> [μM] value					
	Measurement 1	Measurement 2	Measurement 3	Measurement 4	Mean
Kojic acid	100,60±9,28	92,77±9,87	127,2±15,2	-	<b>106,8±11,4</b>
Glabradin	1,045±0,226	1,218±0,375	5,254±1,260	-	<b>2,506±0,620</b>
Mixture <sup>A</sup>	0,165±0,046	0,316±0,012	0,223±0,043	0,245±0,037	<b>0,246±0,034</b>
IC <sub>50</sub> [mg] value					
Glabradin	9,83±2,13×10 <sup>-5</sup>	1,15±0,35×10 <sup>-4</sup>	4,94±1,18×10 <sup>-4</sup>	-	<b>2,35±0,58×10<sup>-4</sup></b>
Mixture <sup>B</sup>	0,0155±0,0043	0,0296±0,0109	0,0210±0,0041	0,0231±0,0035	<b>0,0223±0,0057<sup>C</sup></b>
Ellagic acid	Lack of inhibitory activity				

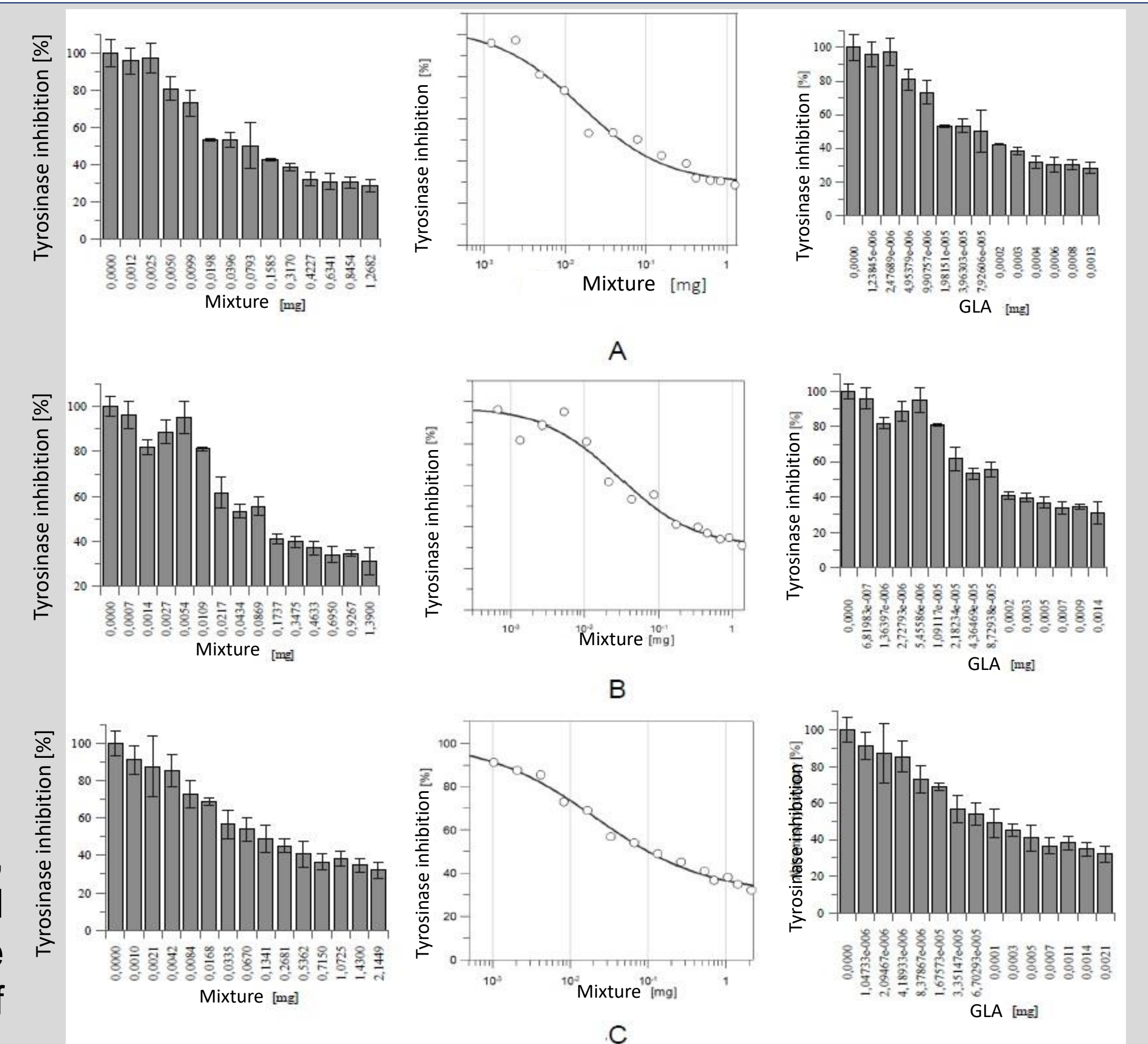
A – in case of mixture, IC<sub>50</sub> value express concentration [μM] of glabradin  
B – quantity in mg of mixture  
C – concentration in 0,0223 mg of mixture – glabradin (0,1%)=2,23×10<sup>-5</sup> mg, ellagic acid (1%)=2,23×10<sup>-4</sup> mg

**Table 1.** IC<sub>50</sub> of glabradin, ellagic acid, its mixture, and reference (kojic acid). Kojic acid is weaker tyrosinase inhibitor than glabradin. Kojic acid IC<sub>50</sub> is by 43-times higher than IC<sub>50</sub> for glabradin. Ellagic acid is not tyrosinase inhibitor.

**The mixture containing glabradin and ellagic acid has a highest inhibitory effect on tyrosinase. Its 10-times higher than pure glabradin.**



**Figure 1.** A. IC<sub>50</sub> of glabradin (GLA), ellagic acid [EA] and reference kojic acid [KA]. **Glabradin is stronger tyrosinase inhibitor than kojic acid.** B. IC<sub>50</sub> of ellagic acid [EA]. EA is not tyrosinase inhibitor.



**Figure 2.** IC<sub>50</sub> of mixture containing glabradin (GLA) and ellagic acid. Quantity of glabradin was calculated in mixture. A, B, C - triplicates.

### Results IN VIVO

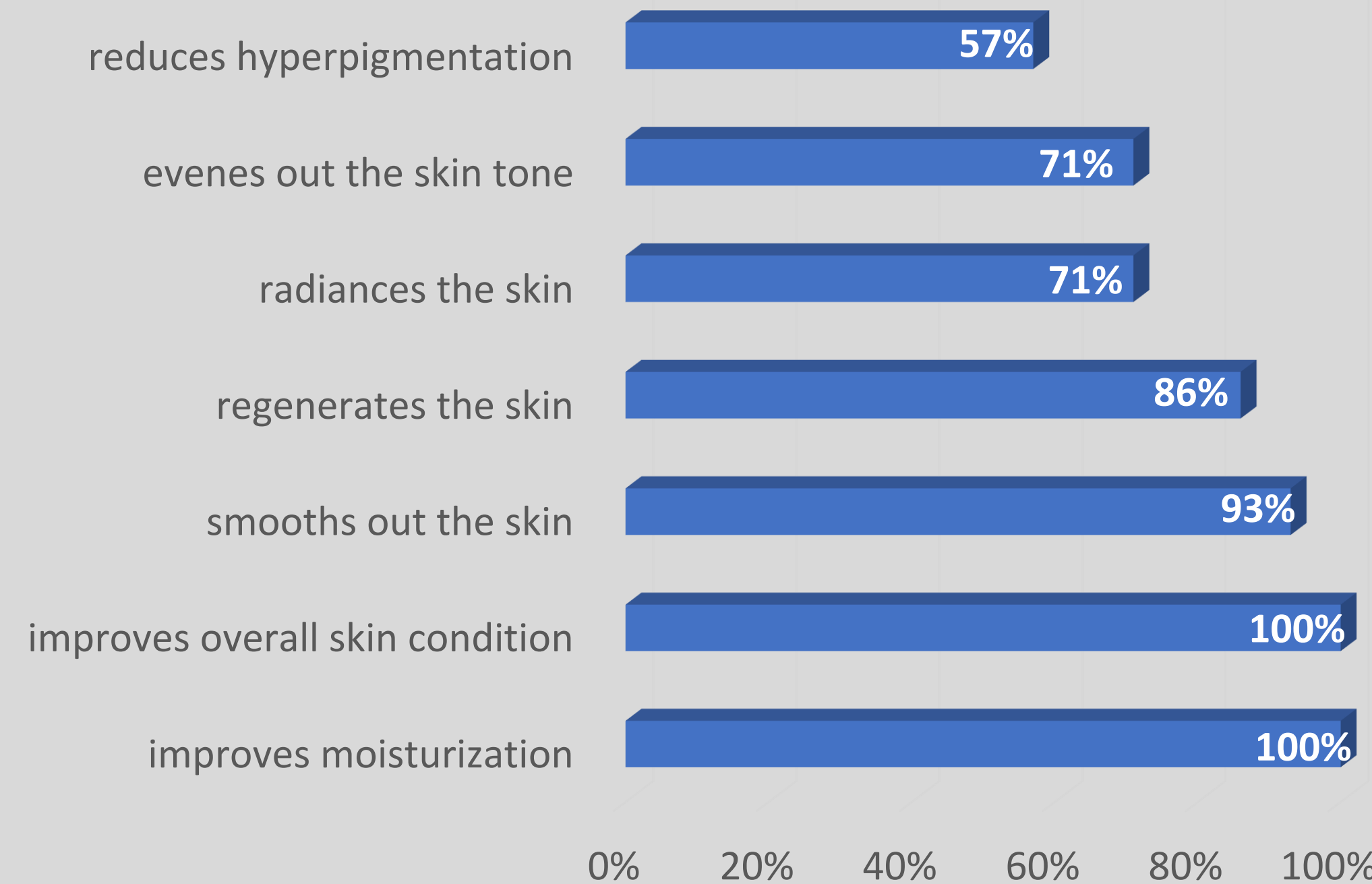
Skin parameter (n=14)	Baseline	Average improvement after 4 weeks	Actual improvement after 4 weeks
Spots		- 9%	Reduction in spots by 9% in 79% of the study subjects
Melanin	100%	- 15%	Skin lightening by 17% in 86% of the study subjects
Erythema		- 6%	Decrease of erythema by 13% in 64% of the study subjects

**Table 2.** Change in selected skin parameters (Mexameter, Visia) after 4 weeks of treatment with cream containing tested complex. The reductions in spots, melanin and erythema were observed.

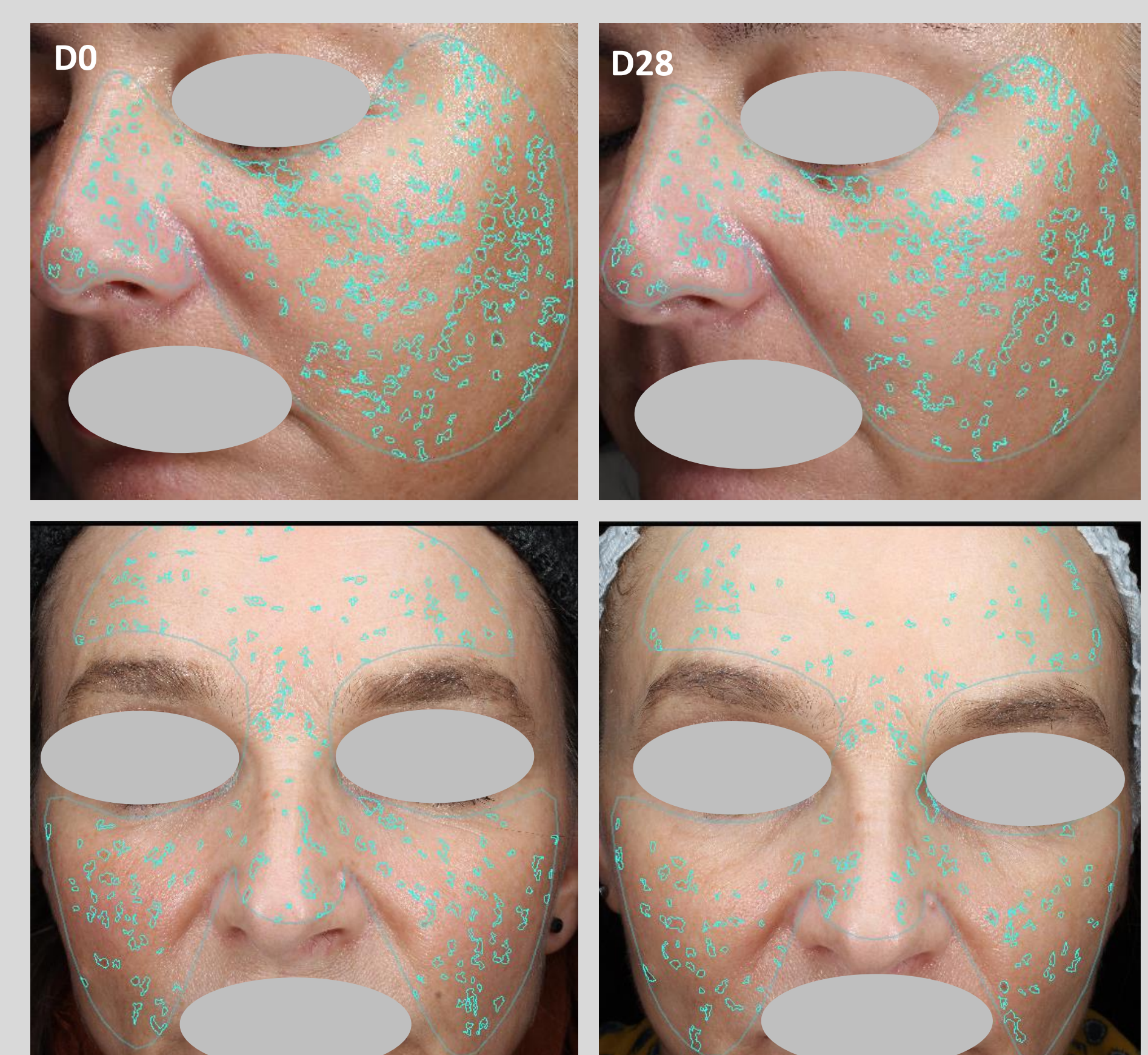
VISIBILITY OF SKIN HYPERPIGMENTATION IN 5-POINT ANALOGUE SCALE						
	NONE	MILD	MODERATE	INTENSE	VERY INTENSE	MEAN
	1	2	3	4	5	
D0	0%	7%	57%	29%	7%	3,36
D21	0%	57%	29%	14%	0%	2,57
DECREASE OF HYPERPIGMENTATION VISIBILITY BY...						24%

**Table 3.** Self-evaluation of skin hyperpigmentation visibility. The overall reduction of hyperpigmentation by 24% was observed.

### Self evaluation of cream containing glabradin and ellagic acid



**Figure 3.** Self-evaluation of skin condition after 4 weeks of cream with glabradin and ellagic acid usage. Reduction of hyperpigmentation was noticed by 57% of volunteers.



**Figure 4.** Visia photography of volunteer AM (upper photo) before and after 4 weeks of cream containing glabradin and ellagic acid usage. The reduction of spots number by 20% and its size by 14%. Volunteer BF (lower photo), also the reduction of spots number by 17% were measured.

## CONCLUSION

It was observed that ellagic acid is not a typical tyrosinase inhibitor, however the mixture containing glabradin and ellagic acid displayed greater tyrosinase inhibition than glabradin alone. Moreover the complex showed much higher inhibitory activity than kojic acid. Mexameter measurements showed reduction of melanin by 15% after 4 weeks of emulsion usage. Also patients estimated that the emulsion evens the skin tone and reducing skin hyperpigmentation visibility by 24% (in 5-point analogue scale). Both tested compounds are well known from their whitening properties. However ellagic acid is not a typical tyrosinase inhibitor, but rather an alternative substrate according to literature<sup>3</sup>. Nevertheless it's boosting inhibitory activity of glabradin. In conclusion, for the first time the synergy of glabradin and ellagic acid in tyrosinase inhibition was demonstrated. Also emulsion containing both flavonoids exhibited efficient whitening properties.

## LITERATURE

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