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Psoralea corylifolia

New cosmetic formulations based on N-Prolyl Palmitoyl Tripeptide-56 Acetate and Bakuchiol Complex with anti-aging properties

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INTRODUCTION

- The objective of this study was to evaluate the biological potential of a pharmaceutical O/W emulsions containing a synergistic biologically active complex based on a ***plant-derived meroterpene phenol, bakuchiol (BAK)*** [1- (4-hydroxyphenyl) -3,7- dimethyl-3-vinyl-1,6-octadiene] and ***a peptide as a n-prolyl palmitoyl tripeptide-56 acetate (TPA***), in order to highlight the optimal concentration and combination in the formulation, in terms of antioxidant property.

- The objective of this study was to evaluate the biological potential of a pharmaceutical O/W emulsions containing a synergistic biologically active complex based on a ***plant-derived meroterpene phenol, bakuchiol (BAK)*** [1- (4-hydroxyphenyl) -3,7- dimethyl-3-vinyl-1,6-octadiene] and ***a peptide as a n-prolyl palmitoyl tripeptide-56 acetate (TPA***), in order to highlight the optimal concentration and combination in the formulation, in terms of antioxidant property. **Bakuchiol** is a active substance found in the species ***Psoralea corylifolia*** which exhibits antioxidant and antibacterial activity and it is an alternative to the retinoids. **The n-prolyl palmitoyl tripeptide-56 acetate** is a small peptide which has been reported to stimulate the production of elastin, fibronectin, glucosaminoglycan and collagens.
- O/W emulsions were prepared using a non-ionic, non-ethoxylated emulsification system that is composed of hydrolyzed wheat protein olivoyl, cetearyl alcohol, glyceryl oleate, glyceryl stearate, able to build-up anisotropic lamellar phases O/W from vegetal oils, by means of totally natural ingredients. As as a dispersed phase, we use a mixt of vegetable oil like *Plukenetia volubilis, Ribes nigrum* and *Triticum vulgare* and as a as a continuous phase *Lavandula latifolia* hydrosol.
- There were formulated three emulsions with different concentrations in active complex **(0.5% BAK + 0.5% TPA, 1% BAK + 1%TPA, 1% BAK + 2% TPA).**

METHODS

Determination of total phenolic content

The total phenolic content was determined following previously described methods (Grochowski et al., 2017; Trifan et al., 2021). Briefly, 50 µL of sample were mixed with 100 µL Folin–Ciocalteu reagent and vigorously mixed. After 3 min, 75 µL of 1% Na_2CO_3 solution were added and the mixture was incubated for 2 h at room temperature in the dark. Then, the absorbance was read at 760 nm and the total phenolic content was expressed as micrograms of gallic acid equivalents (µg GAE/mL).

Determination of antioxidant activity

DPPH and ABTS assays were used to evaluate the antioxidant activity of the the active complex (BAK /TPA)

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The assay was performed following a previously described method (Grochowski et al., 2017), with slight modifications. Thus, 50 µL of sample was added to 150 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.004% methanol solution. After 30 min incubation at room temperature in the dark, the absorbance was read at 517 nm. DPPH radical scavenging activity was expressed as milligrams of Trolox equivalents (mg TE/mL).

2,2′-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid radical scavenging assay

The assay was performed following a previously described method (Grochowski et al., 2017), with minor changes. $ABTS^{\bullet+}$ was generated by mixing 7 mM 2,2′-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) solution with 2.45 mM potassium persulfate (1:1, *v/v*). The mixture was allowed to stand for 12–16 min in the dark at room temperature. In the beginning of the assay, ABTS solution was diluted with methanol to reach an absorbance of 0.700 ± 0.02 at 734 nm. Then, 30 µL sample was added to 200 µL ABTS solution and vigorously mixed. After 30 min incubation at room temperature, the absorbance was read at 734 nm. The ABTS radical scavenging activity was expressed as milligrams of Trolox equivalents (mg TE/mL).

RESULTS

Sample	TPC (µg GAE/mL)	DPPH (mg TE/mL)	ABTS (mg TE/mL)
0.5% Bak + 0.5% TPA	0.035 ± 0.002	1.06 ± 0.01	3.61 ± 0.02
1% Bak + 1% TPA	0.043 ± 0.004	1.28 ± 0.01	3.62 ± 0.01
1% Bak + 2% TPA	0.046 ± 0.007	1.30 ± 0.01	3.61 ± 0.02
1% Bak	0.069 ± 0.005	1.35 ± 0.01	3.61 ± 0.01
2% TPA	0.017 ± 0.003	0.03 ± 0.00	0.10 ± 0.02

Data are presented as mean ± standard deviation (SD) of three determinations.
Abbreviations: ABTS - 2,2′-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid;
Bak – bakuchiol [1- (4-hydroxyphenyl) -3,7- dimethyl-3-vinyl-1,6-octadiene];
DPPH - 1,1-diphenyl-2-picrylhydrazyl; GAE, gallic acid equivalents;
TE - trolox equivalents; TPC - total phenolic content;
TPA - n-prolyl palmitoyl tripeptide-56 acetate

Evaluation of microbial contamination after 24 hours incubation

The microbiological assay has been realized by following an adapted protocol from ISO 18415:2007, for the total aerobic mesophilic microorganisms (total aerobic microbial count, total yeast and mold count), to analyze pathogenic strains: *Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa* or *Salmonella*.

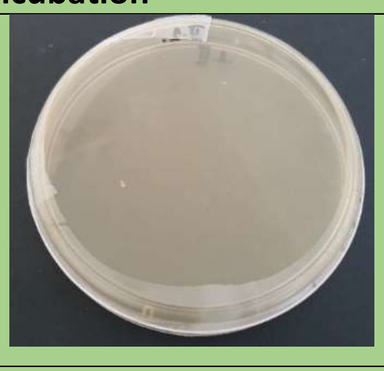
Optical microscopy images of O/W emulsions

Optical microscopy, through the direct observation of the sample, allows the quality of the product. This method allows to detect the homogeneity of the formulations, the morphology, the size and the interaction of the drops of the internal phase of the emulsions and, finally, the organization and the structure of the crystal lattices formed by the various rheological modifiers included in the formula.

The morphology of the designed emulsions was carried out using a Binocular Optika B-159 optical microscope model S8AP0, with increase power of 1000x

Evaluation of microbial contamination after 24 hours incubation

Emulsion O/W II
without active substances

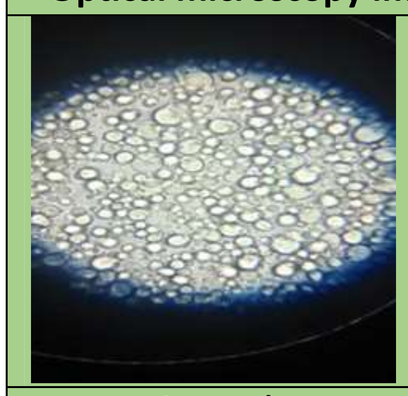


Emulsion O/W II.1
0.5% Bak + 0.5% TPA

Emulsion O/W II.2
1% Bak + 1% TPA

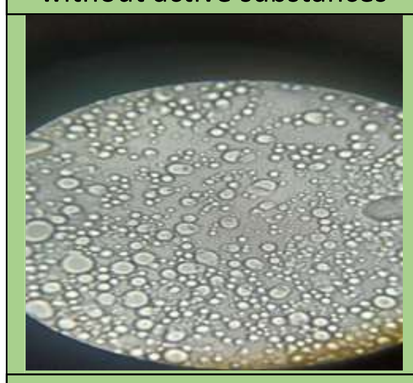
Emulsion O/W II.3
1% Bak + 2% TPA

Optical microscopy images of O/W emulsions



Emulsion O/W II
without active substances

Emulsion O/W II.1
0.5% Bak + 0.5% TPA



Emulsion O/W II.2
1% Bak + 1% TPA

Emulsion O/W II.3
1% Bak + 2% TPA

CONCLUSION

- The results suggested that the biologically active complex showed good antioxidant activity. This research confirmed that the proportions used for preparing emulsions with BAK and TPA are suitable for topical use due to their antioxidant effect and to the potential utilization in antiaging therapy.
- Microbiological evaluation indicates that the total number of viable aerobic mesophilic microorganisms does not exceed 103 cfu/g or 103 cfu/ml of product. *Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans* were not detected in the emulsions.
- Optical microscopy indicate that the samples are stable, no creaming, flocculation or sedimentation phenomena are observed. The addition of cosmetic actives after emulsion production does not change the emulsion structure.
- It can be concluded that the designed emulsions presented physico-chemical properties adequate for cosmetic skin care product formulations.