In vitro release of antioxidant compounds from *C. sativa* leaf extract incorporated in a surfactant-free semisolid formulation

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INTRODUCTION

Topical natural antioxidants are a useful strategy for the prevention of photoaging and oxidative stress mediated skin diseases (1). In view of this underlying principle, the screening of natural plant extracts with scavenging activity for pro-oxidant reactive species is a primary requirement for the development of new topical antioxidant formulations. A *C. sativa* leaf extract has previously shown antioxidant activity against several reactive species that have been detected in the skin after UV exposure (2). This extract was successfully incorporated into a semisolid surfactant-free emulsified formulation (Table I).

MATERIALS AND METHODS

In this study, *in vitro* release of the antioxidant compounds was assessed using the DPPH assay. DPPH is a stable free radical that can accept an electron or hydrogen radical converting it into a stable, diamagnetic molecule. Reaction mixtures contained DPPH (190 µM) dissolved in ethanol 96%, and extract solutions at different concentrations or samples withdrawn during the release test, in a final volume of 200 µL. After 20 min, the absorbance was measured at 515 nm in a microplate reader (ELX 808 IU, BIO-TEK, USA). The effects were expressed as the percentage inhibition of the DPPH reduction The methodology was validated regarding linearity, intra and inter-day precision. The test was conducted on amber Franz diffusion cells provided with a water jacket, at 32 ºC during 6 h. Receptor phase was a pH 5 acetate buffer solution. Samples (0.5 ml) were withdrawn at 0.5, 1, 2, 3, 4 and 6 h and this volume was replaced with acetate buffer. Membrane selection was carried out based on recovery (measured with DPPH antioxidant activity assay) after filtration of *C. sativa* extract solutions (10 and 20 µg/ml) with polyethersulfone and cellulose nitrate. membranes. A formulation without the extract (base) was used as control.

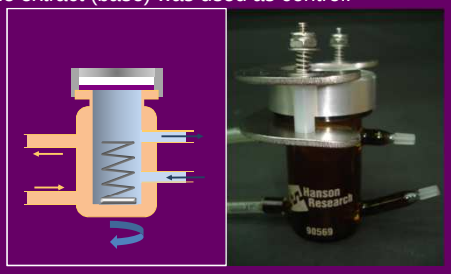

Figure 1. Franz diffusion cell used in this work.

RESULTS AND DISCUSSION

Validation parameters for DPPH assay are presented in table II. Precision was lower than 9 % for all the *C. sativa* extract concentrations evaluated. The method was also found to be specific to the antioxidant formulation because the base did not present measurable antioxidant activity. Nitrate cellulose membrane presented the lowest retention of the antioxidant compounds (Table III) and thus it was selected to perform the test.

Table I. Composition of the antioxidant formulation

Compound	wt %
Carbopol 940®	1.0
Glycerin 85 wt%	27
Liquid paraffin	13
Titanium dioxide	0.5
Methylparaben	0.15
Triethanolamine	0.26
C. sativa extract	0.5
Deionized water	e.q.100

Table II. Validation parameters

Linearity	Intra-day precision Concentration, µg/mL (CV, %) n=5	Inter-day precision Concentration, µg/mL (CV, %) n=4	Range µg/mL
y = 3.4367x + 3.7359 R^2 = 0.9969	2.5 (2.3)	2.5 (8.3)	2.5-20.0
	5.0 (5.1)	5.0 (5.1)	
	10.0 (4.2)	10.0 (7.3)	
	14.0 (2.3)	14.0 (5.7)	
	17.5 (4.5)	17.5 (5.4)	
	20.0 (5.2)	20.0 (4.8)	

The formulation released the antioxidant compounds in the first 4 h according to the Higuchi model (R^2=0.977), being the release rate (K) of 610 70 µg.$h^{-0.5}$. Initially it was observed a faster release rate that slowed down with time (Fig. 2) which is characteristic of a diffusion mechanism of release. Hence, when applied to the skin, the formulation (Fig. 3) will deliver the antioxidant compounds for a long period of time.

Table III. Recovery after filtration with different membranes

Concentration µg/mL	Recovery %	
	Polyethersulfone	Cellulose nitrate
10	85.9	101.8
20	97.3	102.1

Figure 3. Appearance of the formulation containing *C. sativa* extract.

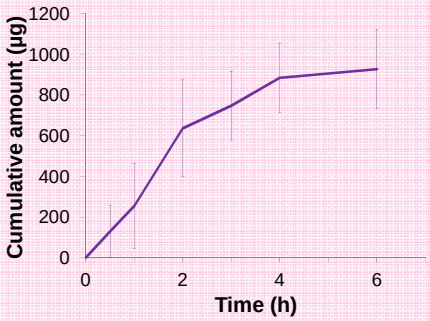

Figure 2. *In vitro* release of antioxidant compounds from the surfactant-free semisolid formulation containing *C. sativa* extract.

CONCLUSIONS

DPPH method proved to be a suitable methodology to quantify the release of the antioxidant compounds from the semisolid formulation. This methodology measures the antioxidant activity of the whole extract taking therefore into account possible synergisms, which is an advantage over analytical methods that quantify only isolated compounds whose relation to the activity of the extract may be uncertain. The Franz diffusion cell conveniently allowed to describe the release profile of the antioxidant compounds from the surfactant-free formulation. This antioxidant topical formulation finds application in the prevention or treatment of oxidative stress-mediated dysfunctions.

REFERENCES

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2) Almeida IF, Fernandes E, Lima JLFC, Costa PC, Bahia MF. Protective effect of *Castanea sativa* and *Quercus robur* leaf extracts against oxygen and nitrogen reactive species. J. Photochem Photobio B. 91(2008) 87-95.