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multidimensional dos alimentos

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Valorization of *Calluna vulgaris* (L.) Hull as potential cosmetic ingredient: Characterization, antioxidant activity and cell viability study

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ABSTRACT

The ecosystems sustainability is an increasing challenge around the world. Considering the rational use of plants, extracts with therapeutic effects can be an option for different industries such as cosmetic and pharmaceutical. An example is the *Calluna vulgaris* (L.) Hull, a native Portuguese plant with a long history of medicinal properties. In the present study *C. vulgaris* was chemical characterized by the total protein content, ash, dietary fiber, and moisture. The protein content represents 6,80%, ash 2,31%, dietary fiber 38,96% and moisture 12,03 %. Three extracts (aqueous, ethanolic and hydro-alcoholic) were screened for antioxidant activity (DPPH and FRAP assays) and cell viability in keratinocytes. The hydro-alcoholic extract displayed the highest antioxidant activity (51.83 ± 3.90 $\mu\text{mol Trolox eq./g}$ for DPPH; 2138.30 ± 15.46 $\mu\text{mol/g}$ for FRAP). The total flavonoids content (TFC) and total phenolic content (TPC) varied, respectively, from 53.96 mg to 121.92 mg GAE/g and 14.80 mg to 118.26 CEQ/ g dry basis. The cell viability suggested that this extract has no adverse effect on keratinocytes until a concentration of 1000 $\mu\text{g/ml}$. This study showed the interesting potential of *C. vulgaris* as new promising ingredient for pharmaceutical and cosmetic industries.

1. INTRODUCTION

Actually there is an increasing interest of cosmetic industry on natural extracts, frequently based on questions of sustainability and the search of new cosmetic active ingredients. *C. vulgaris* has been used in traditional folk medicine as an antiseptic, antibacterian, cholagogue, diuretic, expectorant, antirheumatic and anti-inflammatory agent [1]. Different phytochemical studies reported the presence of large amounts of phenolic compounds, which could be the main responsible for the antioxidant activity [2-5]. Furthermore, according to some authors, *C. vulgaris* can reduce the skin level of proinflammatory cytokines, protecting HaCat cells against UVB and inhibiting UVB-induced lipid peroxidation [6, 7].

The aim of this work was to evaluate the macronutritional composition of *C. vulgaris* and study the antioxidant activity, TFC, TPC and cell viability (in keratinocytes) of three extracts (aqueous, hydro-alcoholic and alcoholic).

2. MATERIALS AND METHODS

2.1 Plant materials and extracts preparation

Flowers of *C. vulgaris* were collected in Moreira de Cónegos, Portugal, in October 2015, and milled at particle size of approximately 0.1 mm. Powdered samples (1 g) were submitted to solvent extraction by maceration with 20 mL of ethanol; ethanol: water (1:1) or distilled water, at 40 °C during 30 minutes. Extracts were filtered through Whatman No. 1 filter paper, concentrated under vacuum or freeze-dried, and kept under refrigeration (4°C) prior to use.

2.2 Nutritional composition

Moisture, protein, ash and fiber contents were determined according to the AOAC (2012) methods [8, 9].

2.3 Determination of TPC, TFC and antioxidant activity

TPC was determined spectrophotometrically according to the Folin-Ciocalteu [10] procedure with minor modifications [11]. TFC was determined by a colorimetric assay based on the formation of flavonoid–aluminum compound according to Rodrigues *et al* [12]. The antioxidant activity of the samples was evaluated by DPPH (2,2- diphenyl-2-picrylhydrazyl) radical-scavenging activity and ferric reducing antioxidant power (FRAP) [13].

2.4 Cell viability assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was used to assess keratinocytes viability after exposure to extracts [12]. Triplicate wells were incubated with fresh medium in the absence or presence of the extract dissolved in cell culture (HaCaT cells) medium containing 0.1, 1.0, 10, 100 and 1000 µg/ml of extracts.

2.5 Statistical analysis of data

Data were reported as mean ± standard deviation of at least triplicate experiments. Statistical analyses of the results were performed with GraphPad Prism 6.01. $p < 0.05$ was accepted as denoting significance.

3. RESULTS AND DISCUSSION

3.1 Nutritional composition

The macronutritional composition of *C. vulgaris* flowers revealed a high content of fiber (38.96 ± 1.64 %), followed by moisture (12.03 ± 0.15 %), protein (6.80 ± 0.27 %) and ash (2.31 ± 0.09 %).

3.2 Determination of total phenolic, total flavonoid contents and antioxidant activity

The TPC, TFC and antioxidant activity results of *C. vulgaris* flowers are summarized in Table 1.

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant potential assay (FRAP), and 2,2- diphenyl-2-picrylhydrazyl (DPPH[•]) scavenging assay of *C. vulgaris* extracts (CS) (mean value \pm standard deviation).

Extract	TPC (mg GAE/g)	TFC (mg CEQ/g)	DPPH (μ mol eq. Trolox/g)	FRAP (μ mol/g)
Aqueous	75.43 ± 7.345	88.72 ± 1.945	23.66 ± 0.649	1354.5 ± 102.14
Hydro-alcoholic	121.92 ± 0.815	118.26 ± 3.598	51.83 ± 3.903	2138.3 ± 15.46
Alcoholic	53.96 ± 5.389	14.80 ± 1.136	15.02 ± 1.574	634.9 ± 99.00

*GAE- gallic acid equivalent; CEQ- catequin equivalents

Results showed that *C. vulgaris* extract had a significant antioxidant activity when compared to other plant extracts [14]. This high activity could be related to the phenolic content of the plant [6, 14]. Also, *C. vulgaris* extracts showed a high TPC, mainly in the hydro-alcoholic extract. According to some authors, the addition of water to polar organic solvents such as acetone, methanol or ethanol, creates a polar medium that improves the extraction of phenolic compounds [15]. The TFC content ranged between 14.80 and 118.26, respectively, for the alcoholic and hydroalcoholic extract.

3.3 Cell viability assay

The extracts effect on keratinocyte (HaCaT) growth were investigated by an MTT assay. As it is possible to observe in Figure 1, none of the extracts leads to a decrease on cell viability at all concentrations up to 1000 μ g/mL.

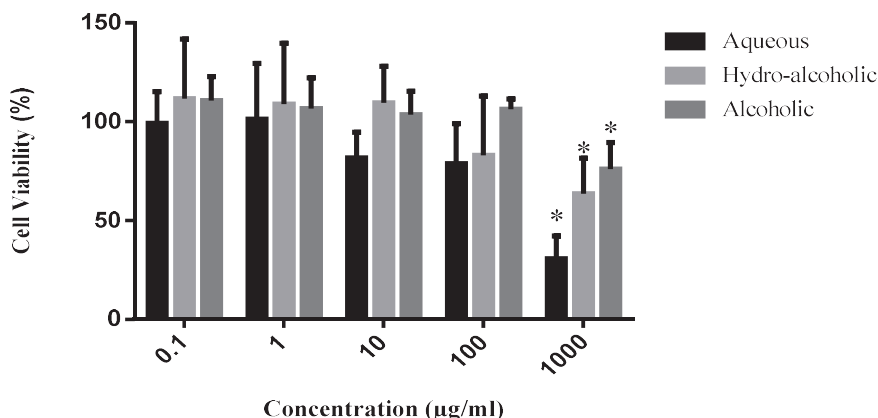


Figure 1. Effect on the metabolic activity of HaCaT cells after the exposure to *C. vulgaris* extracts (aqueous, hydro-alcoholic and alcoholic) at different concentrations, measured by the MTT assay. Values are expressed as means \pm SD (n=4). * $p < 0,05$.

4. CONCLUSION

The results obtained in the present study suggest that *C. vulgaris* could be considered as a promising ingredient for different industries, such as cosmetic or even pharmaceutical.

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