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Production and characterization of nanoparticles containing methanol extracts of Portuguese Lavenders

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ABSTRACT

Lavenders (*Lavandula* species) are aromatic plants of great economic value for fragrance, pharmaceutical and cosmetic industries. Their biological activities and application on therapy can be compromised due to bioactive compounds physicochemical instability. Therefore, nanotechnology can be used as a way of achieving this stabilization.

The antioxidant profile of different extracts from *Lavandula stoechas* ssp. *luisieri* and *L. pedunculata* were established by lipid peroxidation inhibition and the antioxidant activity confirmed by the free radical scavenging method. Methanol extracts of *L. stoechas* ssp. *luisieri* and *L. pedunculata*, due to their high antioxidant activity, phenolic content (1387.21 and 1044.19 mg gallic acid equivalents g_{sample}^{-1}, respectively) and flavonoid amount (482.4 and 360.0 mg rutin equivalents g_{sample}^{-1}) were selected for encapsulation. The produced polymeric poly (lactic-co-glycolic) acid (PLGA) nanoparticles observed by scanning electron microscopy (SEM) showed a well-defined spherical shape and a high encapsulation efficiency (>96%) when the concentration of the extracts main component, rosmarinic acid, was used as indicator of encapsulation efficiency.

The epidermal permeation of both extracts through human epidermis and their *in vitro* cytotoxicity in human keratinocytes studies were suggestive of low risks of toxicity. In conclusion, the current study provides data for promising new cosmetic or dermatological formulations for the pharmaceutical industry, as anti-aging and antioxidant agents.

1. Introduction

Lavenders are aromatic evergreen shrubs from *Lamiaceae* family that have been traditionally used as culinary herbs and medicine for headaches, digestive troubles, burns, skin sores and insect bites. Nowadays, these plants are extensively cultivated as ornamental plants for garden, landscape use, potpourris and essential oil production to fragrance, cosmetic, pharmaceutical, food and flavor industries [26]. *Lavandula stoechas* ssp. *luisieri* (Rozeira) Rozeira (Syn: *L. luisieri*; *L. stoechas* var. *luisieri*) and *Lavandula pedunculata* (Mill.) Cav. (Syn: *L. pedunculata* ssp. *sampaiana*; *L. stoechas* ssp. *pedunculata*) are two common *Lavandula* species growing wild in Portugal [13].

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In the last decade, the essential oils from *L. stoechas* ssp. *luisieri* were analyzed and their antifungal, anti-inflammatory and antioxidant activities studied [11,6,28]. The essential oils from *L. pedunculata* were also analyzed and their antifungal activity demonstrated [27]. In addition, several extracts of both species showed antibacterial activity [20], anticholinesterase inhibition and antioxidant capacity [5]. Taking into account these reports, we found reasonable to foresee, in the present study, an application for these lavender extracts as skin anti-aging and antioxidant agents, for topical and cutaneous treatment.

Naturally, the skin acts as an efficient barrier to external environment factors, against toxic substances, pathogens and other organisms [2]. Some of these aggressive conditions, such as ultraviolet or ionizing radiation, are related with the formation and accumulation of free radicals in cells, damaging or modifying the proteins and nucleotides structure and, as a consequence, resulting in cancer or other pathologies [12]. The interaction of antioxidant and anti-inflammatory compounds with radical species results in a decrease of cellular damage and oxidative stress [25], playing and important function against several pathologies. However, many active compounds have the disadvantage of showing toxicity, which is one of the main challenge in skin diseases treatment [7,15].

It is now well known that nanotechnology provided new approaches and alternatives for diseases treatment [19]. The encapsulation into nanoparticles of a wide range of drugs resulted in significant advances in the treatment efficiency for topical acne and anti-aging, anti-inflammatory, antimicrobial or antifungal drugs [16]. An example of higher stability and antioxidant activity of encapsulated plant extracts was achieved after encapsulation process by our group [24]. Poly (lactic-co-glycolic) acid (PLGA) nanoparticles were commonly used to encapsulate selected extracts, in order to enhance their antioxidant activity by protecting them from instability. In addition, PLGA has been extensively employed in drug delivery applications, since it decomposes through hydrolysable ester bonds in the body, it is excreted as CO_2 [18,21] and it is biodegradable and biocompatible [10]. As an example, our team successfully developed nanoparticles of PLGA containing azelaic acid for acne treatment and this nanosystem demonstrated similar antimicrobial activity after encapsulation when compared to the free drug [21].

This study aimed to encapsulate into PLGA nanoparticles methanol extracts of *Lavandula stoechas* ssp. *luisieri* and *L. pedunculata* that previously have shown to have a high antioxidant activity. In this way, we would improve the stability of the active compounds and provide a better efficiency for treatment of cutaneous diseases.

2. Materials and methods

2.1. Chemicals

PURASORB PDLG 5002 – Poly-DL-Lactide/Glycolide copolymer (PLGA) Ratio L/G% 50:50 (MW 45,000–75,000 Da) was obtained from PURAC. Pluronic® F68 was obtained from Sigma–Aldrich™ (USA). All other reagents were of analytical grade.

2.2. Plant material

The aerial parts of *Lavandula stoechas* ssp. *luisieri* (Rozeira) Rozeira and *Lavandula pedunculata* (Mill.) Cav. were collected from natural populations occurring throughout the Center and Southwestern regions of Portugal. The plant material was identified and vouchers specimens deposited in the Herbarium of the Lisbon Botanical Garden (LISU 236672) and in the Lisbon Agronomic Institute Herbarium (LISI 164/2011), respectively. The plant material was air-dried at room temperature and powdered. Several extractions (during 24 h at room temperature) with organic solvents of increasing polarity were performed to obtain the essential oils and *n*-hexane, dichloromethane, ethyl acetate, methanol and water extracts.

2.3. Determination of total phenol content

Total phenol content (TPC) was determined using Folin–Ciocalteu reagent according with the method modified describe by Singleton and Rossi [23] and Cheung et al. [3]. In summary, 0.2 mL of Folin–Ciocalteu reagent was added to 0.1 mL of sample (2 mg mL^{-1}), 1 mL of sodium carbonate (15%) and 2 mL of distilled water. After incubation for 2 h, the absorbance was measured at 765 nm. Gallic acid was used as the standard and the results were expressed in mg mL^{-1} of gallic acid equivalents (GAE).

2.4. Determination of total flavonoids content

Total flavonoids content (TFC) was determined according to Dowd method modified by Zhishen et al. (1999). To a 0.5 mL of sample solution (2 mg mL^{-1}) was added 2 mL of distilled water and 150 μL of sodium carbonate (5%). After incubation for 5 min in the dark at room temperature, 150 μL of $AlCl_3$ 10%, 1.0 mL of sodium chloride (1 M) and 2 mL of distilled water was added to solution and incubated for more 10 min in the dark at room temperature. The absorbance was measured at 510 nm against blank sample. Rutin was used as the standard and the results were expressed in mg mL^{-1} of rutin equivalents (RE).

2.5. Antioxidant activity

2.5.1. Lipid peroxidation inhibition

The lipid peroxidation inhibition capacity (LPIC) was realized according to Liegeois et al. (2000). Briefly, 30 μL of linoleic acid (16 mM), 2.81 mL of phosphate buffer (50 mM, pH 7.4) and 20 μL of sample (0.3 mg mL^{-1}) were added to 150 μL of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) solution. The absorbance measurement at 234 nm was carried out after incubation for 20 min in the dark at room temperature. Butylated hydroxytoluene (BHT) was used as positive control.

Free radical scavenging activity (DPPH): The antioxidant activity was evaluated according with Sarikurkcu et al.