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Potential use of *Cytisus scoparius* extracts in topical applications for skin protection against oxidative damage

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ABSTRACT

Cytisus scoparius L. is used in folk medicine for the treatment of several ailments in which the antioxidant and anti-inflammatory effects of its carotenoid and flavonoid content is suggested to play an important role. We postulate that flavonoid- and carotenoid-rich extracts from *C. scoparius* may become useful in the preparation of formulations for topical application to protect the skin against oxidative damage mediated by high energy UV light radiation. The aim of this work was to apply an extraction process to obtain a bioactive extract from *C. scoparius* for the potential use in topical applications. Aqueous and ethanolic extracts from *C. scoparius* were characterized for its reducing capacity, radical scavenging capacity, and on the reactive oxygen and nitrogen species (ROS, RNS). The extracts showed activities comparable to that of synthetic antioxidants, and absence of skin-irritant effects at 1%. Those make them good candidates to be used in topical applications as active ingredients.

1. Introduction

Cytisus scoparius L., also called *Sarothamnus scoparius*, is a perennial, leguminous shrub native to western and central Europe, used in folk medicine for its diuretic, anti-diabetic, hepatoprotective, lithotriptic, hypnotic and sedative properties [1,2]. Traditional uses in relation to cardiotonic, cathartic, emetic, and vasoconstrictor activities have also been reported [3]. Chemical analysis of *C. scoparius* revealed the presence of flavones (6″-*O* acetylscoparin), flavonols (rutin, quercetin, isorhamnetin and kaempferol), isoflavones and its glycosides (genistein, sarothamnoside), carotenoids (chrysanthemaxanthin, xanthophylls and xanthophillepoxide) and alkaloids (spartein, sarothamine and lupamine) [1,4,5]. It is presently well established that flavonoid derivatives including flavones, flavonols, and isoflavones [6] and carotenoids [7] are endowed with antioxidant and anti-inflammatory properties that strongly contribute for the protective and healing effect of plants with this type of chemical constituents.

The *in vitro* antioxidant activity of *C. scoparius* was confirmed by the radical scavenging activity against superoxide and hydroxyl radicals, nitric oxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and by the protection against peroxidation of both β-carotene-linoleic acid in emulsion and lipids in rat liver microsomes [3,4,8]. The *in vivo* antidiabetic, hypnotic, sedative, anxiolytic, antidepressant and antioxidant action have been demonstrated in rats, as it improved the endogenous antioxidant enzymes (superoxide dismutase, catalase), and non-enzymic (ascorbic acid) levels and controlled the lipid peroxidation in brain, kidneys and adrenals tissues of stressed rats [5].

In addition, *C. scoparius* extract was shown to protect in Wistar albino liver from carbon tetrachloride-induced oxidative stress rats, by preventing the rise of serum glutamate oxaloacetate and serum glutamate pyruvate transaminases, lactate dehydrogenase and thiobarbituric acid reactive substances levels. Superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase and glutathione reductase levels were increased by treatment with the plant extract [1].

Natural antioxidants may be proposed for the treatment and prevention of ultraviolet-induced oxidative stress and skin alteration, especially due to their ability to neutralize pro-oxidant reactive species. Carotenoids and flavonoids may provide skin protection against excess light in plants and contribute to the prevention of UV damage in humans. Carotenoids and flavonoids are micronutrients ingested with the diet and are distributed into light-exposed tissues, such as skin or the eye where they may provide systemic photoprotection [9]. On the other hand, little is known about the potential protective potential of plant-extracted

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carotenoids and flavonoids against photo-oxidative damage when topically applied. Noteworthy, incorporation of botanical extracts into formulations for topical application can be proposed to protect the skin against oxidative damage [10,11] and to protect cosmetic products from oxidation. The aim of this work was to develop an active extract from *C. scoparius* for the potential use in topical applications. The antioxidant activity was assessed in chemical and biological systems. The reducing capacity, the radical scavenging activity against the coloured stable free radicals DPPH· and $ABTS^{\cdot+}$, as well as the scavenging effects against reactive oxygen species (ROS) and reactive nitrogen species (RNS) was studied. The ability of the extracts to protect biological systems was preliminary evaluated by the protection that conferred the extract against the β-carotene oxidation in emulsion and the lysis and lipid peroxidation induced in erythrocytes. Finally, to discard possible irritant reactions, the skin irritation potential of the *C. scoparius* extract was assessed by an *in vitro* test with reconstituted human epidermis.

2. Materials and methods

2.1. Chemicals

All the chemicals and reagents were of analytical grade. Ascorbic acid, β-carotene, chloroform, 4,5-diaminofluorescein (DAF-2), diethylenetriaminepentaacetic acid (DTPA), dihydrorhodamine 123 (DHR), 3,5 dinitrosalicylic acid, Folin–Ciocalteau reagent, gallic acid, 30% hydrogen peroxide, sodium hypochlorite solution with 4% available chlorine, linoleic acid, lucigenin, β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium chloride (NBT), phenazine methosulfate (PMS), potassium ferricyanide, rutin, 2-thiobarbituric acid, 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5), trichloroacetic acid, tripyridyl-s-triazine and tween 40 were obtained from Sigma–Aldrich (St. Louis, USA). α,α′-Azodiisobutyramidine dihydrochloride (AAPH), α,α-Diphenyl-β-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonate) (ABTS), histidine and Trolox were obtained from Fluka Chemie GmbH (Steinheim, Germany). Fluorescein sodium salt and quercetin were obtained from Aldrich (Milwaukee, USA). Butylhydroxytoluene (BHT) and butylhydroxyanisol (BHA) were obtained from Roig Farma (Barcelona, Spain). Episkin (batch 09-EKIN-038) supplied by SkinEthic Laboratories (Lyon, France). Chloridric acid, ferric chloride, ethanol and methanol were obtained from Panreac (Barcelona, Spain).

2.2. Preparation of extracts

C. scoparius branches (CsB) were locally collected (Lugo, Spain) in spring 2009, and were dried at room temperature, crumbled, grounded and stored in a dry and dark place at room temperature until utilization. Two particle sizes were selected, between 0.25 and 1 mm (S1) and smaller than 0.25 mm (S2). The grounded CsB were processed according to the flow diagram presented in Fig. 1. The CsB samples were extracted in a shoxlet extractor with hexane at a liquid solid ratio (LSR) of 20 g/g, during 8 h; this protocol was repeated three times to remove the hydrophobic components, which were discarded due to the low observed contribution of this fraction to antioxidant activity. The extracted solids of each particle size were processed with an acetone:water mixture (AW) and its raffinate was then processed with 70% Ethanol acidified (EAA). Extractions were carried out in an orbital shaker at a liquid solid ratio of 10 g/g, during 60 min at 40 and 55 °C (AW and EAA respectively). Extracts were vacuum filtered, vacuum

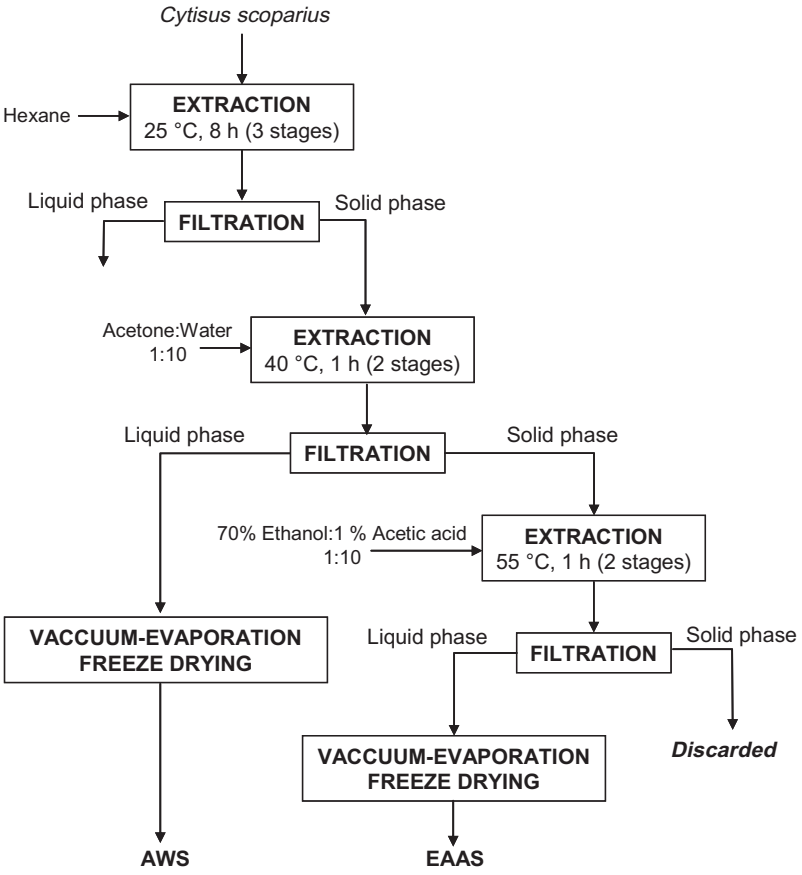

Fig. 1. Flow diagram of the extraction and fractionation process proposed for *C. scoparius* branches.