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Protective effect of *Opuntia ficus-indica* L. cladodes against UVA-induced oxidative stress in normal human keratinocytes

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ABSTRACT

Opuntia ficus-indica L. is known for its beneficial effects on human health, but still little is known on cladodes as a potent source of antioxidants. Here, a direct, economic and safe method was set up to obtain water extracts from *Opuntia ficus-indica* cladodes rich in antioxidant compounds. When human keratinocytes were pre-treated with the extract before being exposed to UVA radiations, a clear protective effect against UVA-induced stress was evidenced, as indicated by the inhibition of stress-induced processes, such as free radicals production, lipid peroxidation and GSH depletion. Moreover, a clear protective effect against apoptosis in pre-treated irradiated cells was evidenced. We found that eucomic and piscidic acids were responsible for the anti-oxidative stress action of cladode extract. In conclusion, a bioactive, safe, low-cost and high value-added extract from *Opuntia* cladodes was obtained to be used for skin health/protection.

Natural products are receiving a great deal of interest by scientists and pharmacologists for their use in the prevention of oxidative stress-related pathologies, which include obesity, atherosclerosis, diabetes, cancer, neurodegenerative diseases, and aging.[1]

Opuntia is widely distributed in Mexico and in all American hemispheres, as well as in Africa and in the Mediterranean basin.[2] Among all the species, *Opuntia ficus-indica* (referred to here on as *Opuntia*) is the most widely distributed. The multiplicity of health-promoting properties of *Opuntia* are well known. Indeed, in traditional medicine it has been recognized as a source of prebiotics and phytochemicals.[3] Most of the studies report the protective effect of fruits and stems; for example, extracts from *Opuntia ficus-indica* var. *saboten* have been reported to protect against neuronal damage produced under oxidant conditions,[4] or against renal and hepatic alterations caused by mycotoxins.[5] On the other side, only few studies have been performed on the antioxidant, anti-inflammatory, wound healing, hypoglycemic and antimicrobial activities of *Opuntia* cladodes.[6,7] Lee and colleagues showed that an ethanol extract of cladodes decreased the oxidation of linoleic acid and DNA.[8] Recently, Avila demonstrated an increased antioxidant activity in plasma and blood in subjects consuming cladodes (300 g/day for 3 days).[9]

Given these premises, and taking into account the high annual productivity of biomass per hectare (10–40 tones dry weight), it is undeniable that *Opuntia* cladodes represent an economic and suitable substrate for isolation of antioxidants. However, it has to be considered that all the above studies have a common drawback, namely the use of organic solvents to extract bioactive compounds. Indeed, it was previously demonstrated that the extraction procedure, as well as the extraction solvent, notably affect the yield of natural products, their content as well as their antioxidant

Abbreviations: ABTS, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); CASP-3, caspase-3; CASP-7, caspase-7; DCF, 2′,7′-dichlorofluorescein; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; HAA, hydrophilic antioxidant activity; H_2-DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ORE, *Opuntia* raw extract; OSMF, *Opuntia* small molecular weight fraction; P-p38, phosphorylated p38 MAP kinase; P-MAPKAPK-2, phosphorylated MAP kinase-activated protein kinase; ROS, reactive oxygen species; SPF, sun protection factor; TEAC, Trolox equivalent antioxidant capacity; TBA, thiobarbituric acid; TBARS, TBA reactive substances; TNB, 5-thio-2-nitrobenzoic acid.

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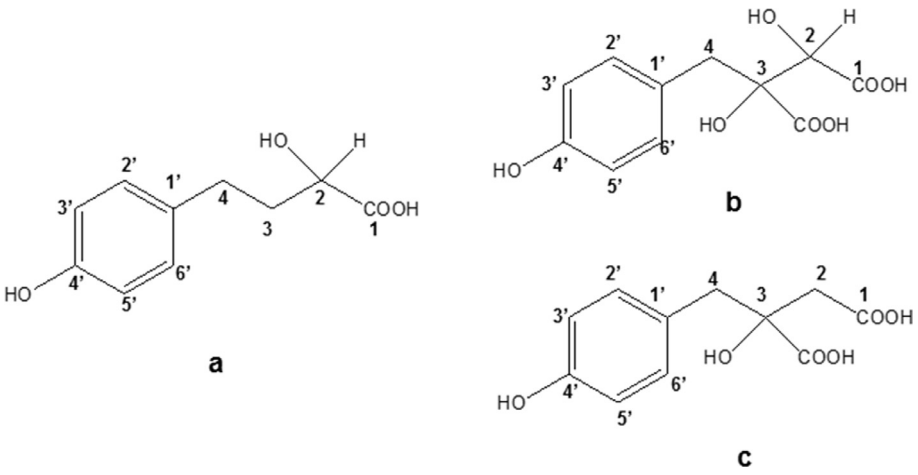

Fig. 1. Structure of 2-hydroxy-4-(4′-hydroxyphenyl)-butanoic acid (a) found in OSMF C, and of piscidic (b) and eucomic (c) acids found in OSMF D.

activity.[10] Furthermore, the use of organic solvents has elevated costs with the risk of contamination of the extract by solvent residues.

We previously reported that *Opuntia* extracts can be obtained by simple mechanical press and that the *Opuntia* raw extract (ORE) was constituted by two main components: i) a high molecular weight constituent consisting in two polysaccharide entities: a linear β-(1→4)-galactose polymer and a highly branched xyloarabinan; ii) a low molecular weight component consisting in lactic acid, D-mannitol and three phenolic derivatives, i.e. piscidic, eucomic and 2-hydroxy-4-(4′-hydroxyphenyl)-butanoic acids (Fig. 1).[6]

Phenolic compounds are used in several applications due to their proved antioxidant and potentially health-promoting properties.[11–13] Importantly, the chemical structure of phenolic compounds, in terms of their reducing properties as electron or hydrogen-donating agents, determines their potential for action as antioxidants.[14]

In this context, we investigated whether ORE is able to protect human keratinocytes against UVA-induced oxidative stress. UVA radiations are known to increase reactive oxygen species (ROS) production, thus causing oxidative damage of proteins, lipids and nucleic acids. These damages result in different detrimental effects on the skin, such as inflammation, premature aging and development of cancer.[15,16] Keratinocytes are essential components of skin and connective tissue, normally present in the outermost layer of the skin.[17] This prompted us to select cultured human keratinocytes (HaCaT cell line) as an excellent experimental model to test the protective role of *Opuntia* extracts against UVA radiations.

We first tested the *in vitro* antioxidant activity of the whole ORE and of each isolated fraction (OSMF A-D), obtained following the procedure described in Di Lorenzo et al.[6] and reported in Supplementary material.

By the ABTS colorimetric assay, we found that ORE is endowed with a significant antioxidant activity, as a low IC_{50} value was obtained (0.52 ± 0.01 mg/mL). Fraction OSMF A, namely the lactic acid component,[6] showed the highest IC_{50} value (1.4 ± 0.01 mg/mL), in agreement with findings obtained in a different experimental system by Lampe, who reported that lactic acid is able to scavenge free radicals.[18] The IC_{50} values of OSMF C (namely 2-hydroxy-4-(4′-hydroxyphenyl)butanoic acid, Fig. 1a) and D (namely piscidic and eucomic acids, Fig. 1b and c, respectively)[6] were found to be much lower (0.09 ± 0.02 and 0.03 ± 0.01, respectively) than those obtained for the whole extract and for OSMF B (D-mannitol,[6]) (IC_{50} = 0.79 ± 0.37 mg/mL). These results were confirmed by the TEAC (Trolox equivalent antioxidant capacity) test, from which much higher TEAC values were obtained for OSMF C and D, with respect to OSMF A and B, indicating a high content in antioxidants in the former two fractions (Table 1).

Table 1
Opuntia raw extract (ORE) and its fractions (OSMF A-D) were tested for their *in vitro* antioxidant properties. The antioxidant activity is expressed as the concentration required to scavenge 50% of free radical $ABTS^{\cdot+}$ (IC_{50}), Trolox equivalent antioxidant capacity (TEAC), and the ability to counteract UV radiations, expressed as sun protecting factor (SPF). Values are normalized to the concentration of each sample.

Sample	IC_{50} (mg/mL)	TEAC (μM/mg)	SPF
ORE	0.52 ± 0.01	43.2 ± 4.53	2.25 ± 0.5
OSMF A	1.4 ± 0.01	10.6 ± 3.39	1.4 ± 0.1
OSMF B	0.79 ± 0.37	24.08 ± 0.34	0.94 ± 0.11
OSMF C	0.09 ± 0.02	225.79 ± 15.85	0.2 ± 0.01
OSMF D	0.03 ± 0.01	749.65 ± 11.81	2.23 ± 0.41

We also tested the UV-protective properties of ORE and its OSMF fractions by measuring their sun protection factor (SPF) *in vitro*, according to a spectrophotometric method.[19] As shown in Table 1, while OSMF B and C did not show any significant protective effect, a value of about 2 was obtained when ORE or OSMF D

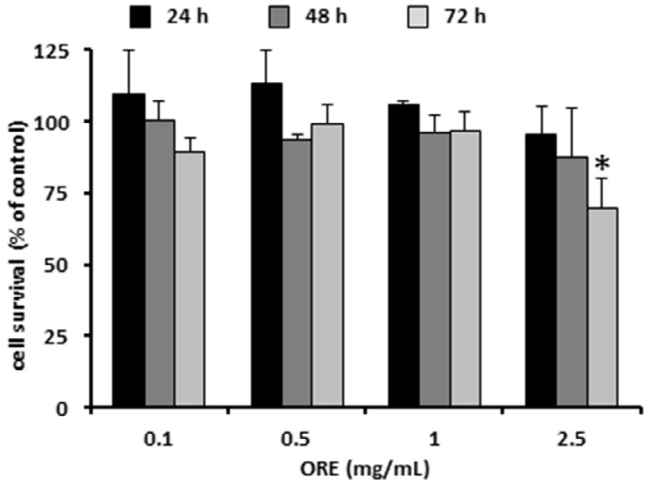

Fig. 2. Effect of ORE on the viability of human keratinocytes. Dose-response curve of HaCaT cells after 24 h (black bars), 48 h (dark grey bars) and 72 h (light grey bars) incubation in the presence of increasing concentrations of ORE. Cell viability was assessed by the MTT assay; the cell survival percentage was defined as described in Supplementary material. Values are given as means ± S.D. ($n \geq 3$). * indicates $p < 0.05$ with respect to untreated cells.