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Phenolic compounds accumulation in wild and domesticated cladodes from *Opuntia* spp. and its benefits in cardiovascular diseases

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Abstract

Opuntia spp. are plants native from Mexico where the largest varieties of wild and semi-domesticated species are found. Nowadays the most domesticated species, *O. ficus-indica* is widely distributed worldwide. Since pre-Hispanic times, young cladodes or nopalitos have been used as a source of food and in folk medicine. Reports indicate that dietary intake of *Opuntia* spp. exhibit antioxidant, anti-inflammatory, and antiatherogenic properties, which emphasizes their nutritional benefit for preventing cardiovascular and most chronic non-infectious diseases. However the *Opuntia* biological properties may fluctuate depending on the cladode phytochemical composition, variations that can be quantitative and qualitative as function of the plant growing

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conditions. For these reasons cell tissue cultures represent an excellent alternative for the control of *Opuntia* metabolite production.

INTRODUCTION

The genus *Opuntia* belongs to the Cactaceae family with high adaptation capacity to grow under conditions such as drought, high temperature, and UV radiation. Mexico is considered as the center of origin for wild and semi-domesticated *Opuntia* spp. species *(1)*. Most of the wild species are still mainly located in their own wild habitats such as backyards, plantations, and agricultural terraces *(1).* Each *Opuntia* species may present several variants and cultivars, resulting in a wide range of morphological characteristics such as colour, shape, flavour, texture, quantity and quality of mucilages, or the number and size of spines. Most morphological characteristics of domesticated *Opuntia* species (e.g. less spines, less amount of mucilages) have been obtained through the domestication process *(1).*
Due to climate changes, the increasing arid zones, and declining water availability, *Opuntia* spp. are gaining importance as food and feed resources. Nowadays, the most propagated *Opuntia* for commercial production is the domesticated *O. ficus-indica* that is widely distributed in arid and semiarid regions worldwide. In Mexico the production of *O. ficus-indica* represents 821 mil tons/year, with the United States as the main country for nopal exportation *(2)*.

Opuntia have been used in traditional medicine since the pre-Hispanic times and recent scientific investigations have confirmed their therapeutical properties *(3).* Several reports indicate that *Opuntia* spp. consumption helps to reduce the blood levels of glucose, cholesterol, and triglycerides *(4,5),* which is mainly attributed to the presence of phenolic and antioxidant compounds *(3)*. Opuntia cladodes are also used in diets for weight loss and fitness, because of their high mineral and natural fiber content *(6)*. However, some changes in the concentration of phytochemicals, mucilages, and fiber have been reported between wild and domesticated species, and the consequences in term of health benefits are almost unknown. In this chapter we reviewed the differences between wild and domesticated species concerning the phenolic compounds composition. The protective effects of *Opuntia* spp. cladodes in atherosclerosis and colon cancer are presented, and cell tissue cultures are proposed as a biotechnological process to improve the yield of the *Opuntia* spp. bioactive compounds.

Phytochemical composition of wild and domesticated *Opuntia* species

Plant domestication is a vital adaptive process to provide food security for the growing population worldwide. Understanding domestication, as an adaptive process, is an interesting way for identifying genes, proteins, and metabolites that contribute to the selection of agronomic important traits *(7)*.

It is well known that secondary metabolite accumulation depends on biotic and abiotic factors as well as the domestication grade. Studies have been carried out with the aim to analyse the molecular composition and properties of various *Opuntia* species as function of their domestication gradient, from the wildest *O. streptacantha* to the most domesticated the *O. ficus-indica* (Figure 1) (8).

The phytochemical composition of those species indicates that *O. streptacantha*, the wildest species, contains the highest phenolic acids concentration (56.8 μmol gallic acid/g). No differences were detected concerning the phenolic acids content among *O. megacantha*, *O. albicarpa*, and *O. ficus-indica,* with values of 44.7, 40.8 and 40.1 μmol gallic acid/g, respectively (Figure 2). The flavonoid content presented less variation among species, with values oscillating between 19.4 and 16.8 μmol of quercetin/g (Figure 2). These values are quite similar to those reported for cladodes cultivated in Egypt *(9)*. The phenolic compounds are related to plant domestication grade, the highest contents being observed in wild species.

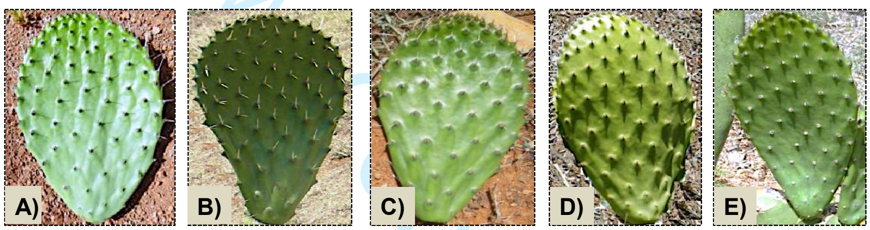

Figure 1*. Changes in size, colour, and number of spines are observed in Opuntia cladodes through domestication. A) O. streptacantha, B) O. hyptiacantha, C) O. megacantha, D) O. albicarpa, D) O. ficus-indica.*

Through LC-MS/MS analysis, it was found that *Opuntia* wild and domesticated species could be classified according to the presence of major and minor compounds present in their cladodes *(8)*. One of the major compounds detected in all the analyzed species was eucomic acid, the highest level was observed in *O. hyptiachanta* and *O. megacantha*. Eucomic acid has been associated as a protective agent against UVA radiations, therefore an extract based on *Opuntia* cladodes could be a safe product for skin protection and health *(10)*.

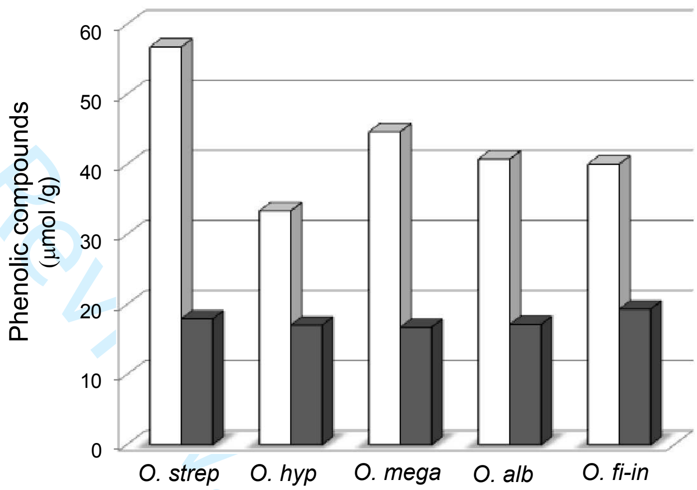

***Figure 2**. Phenolic compounds present in Opuntia spp. White bars, phenolic acids as μmol of Gallic Acid /g sample. Dark bars, flavonoids as μmol of Quercetin/g sample. Opuntia: O. strep= O. streptacantha; O. hyp=O. hyptiacantha; O. mega= O. megacantha; O. alb= O. albicarpa; O. fi-in= O. ficus-indica.*

Chlorogenic acid content could be correlated with domestication, its concentration increased from the wildest *O. streptacantha* to the semi-domesticated *O. albicarpa*. However, the content of this acid present in *O. ficus-indica* was comparable to that of the wild species O. *hyptiacantha (8).* In contrast, the isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside) content seems to decrease along *Opuntia* domestication. Quercetin-3-*O*-xylosyl-rhamnosyl-glucoside is mainly present in *O. albicarpa,* while high concentration of kaempferol 3-*O*-(rhamnosyl-glucoside)-7-*O*-rhamnoside was detected only in *O. ficus-indica*. Kaempferol 3-*O*-arabinofuranoside could be used as a marker of the wild species *O. streptacantha* and *O. hyptiacantha (8).*

These results are very useful for the characterization and authentication of *Opuntia* species and for the selection of species exhibiting the highest potential in compounds with nutritional and therapeutic properties. It is for instance, interesting to note that the compound 3-O-arabinofuranoside, that was detected only in wild species, has been reported to have neuroprotective properties and may delay the cellular senescence of human dermal fibroblast *(11)*.

Opuntia **cladodes and atherosclerosis**

Atherosclerosis and its complications represent the first cause of morbidity and mortality worldwide *(12-14)*. Lipids and lipoproteins play an important role, at least in the development of early atherosclerotic lesions characterized by the formation of foam cells and their accumulation as fatty streaks *(15)*. The formation of early lesions could be due, for a large part, to the oxidative modification of lipoproteins in the vascular wall, and their metabolic deviation towards the scavenger receptor system of macrophages, which accumulate lipid droplets in their cytosol, and are transformed into foam cells *(16-18)*.

Oxidized lipids (oxidized phospholipids, oxysterols, aldehydes issued form lipid peroxidation such as 4-hydroxynonenal or 4-HNE), are proinflammatory and toxic *in vitro* for vascular cells, which suggests a role for these agents in the mechanisms of plaque progression and destabilization *(19-21)*. In animal models for atherosclerosis, diets enriched with antioxidants prevent or slow-down the development of atherosclerosis *(22,23)*.
Opuntia species exhibited cardiovascular properties, possibly resulting from their high antioxidant content *(3,24-26)*. However, the antiatherogenic potential of *Opuntia* species had not been demonstrated neither *in vitro*, nor in animal models for atherosclerosis.

The effect of diets enriched with *Opuntia* cladode powder from wild species *O. streptacantha* and from domesticated *O. ficus-indica*, was investigated on the progression of atherosclerotic lesions in the apoE-knock-out mouse model of atherosclerosis. Results showed that cladode powders from both domesticated and wild Opuntia spp., exhibited a similar efficacy to inhibit *in vivo* the accumulation of 4-HNE-adducts (Figure 3A) and the development of atherosclerotic lesions (Figure 3B) in the intima of apoE-KO mice *(27,28)*.

In vitro, both *Opuntia* cladode powders reduce LDL oxidation by vascular cells, and the inflammatory signaling of oxidized LDL, that is characterized by an increased intracellular and extracellular production of reactive oxygen species, and the expression of adhesion molecules ICAM-1 and VCAM-1 at the endothelium cell surface. *Opuntia* spp. thereby reduced the adhesion of mononuclear cells on activated endothelium, as well as the formation of foam cells. These data enlighten the antiatherogenic properties of wild and domesticated *Opuntia* species, and their nutritional interest as cardiovascular protective agents *(27,28)*.

***Opuntia* cladodes and colorectal cancer**

Several authors reported that Opuntia cladodes exert protective effects toward cancer, based on *in vitro* studies but also on studies conducted on animal models for cancers *(reviewed in 29)*. Foodstuffs may play an important role in colon cancer development. In fact, foodstuffs containing fibers or antioxidants (polyphenols), can be protective, but some foods like red and processed meats may have a promoting effect on the development of colon cancer.

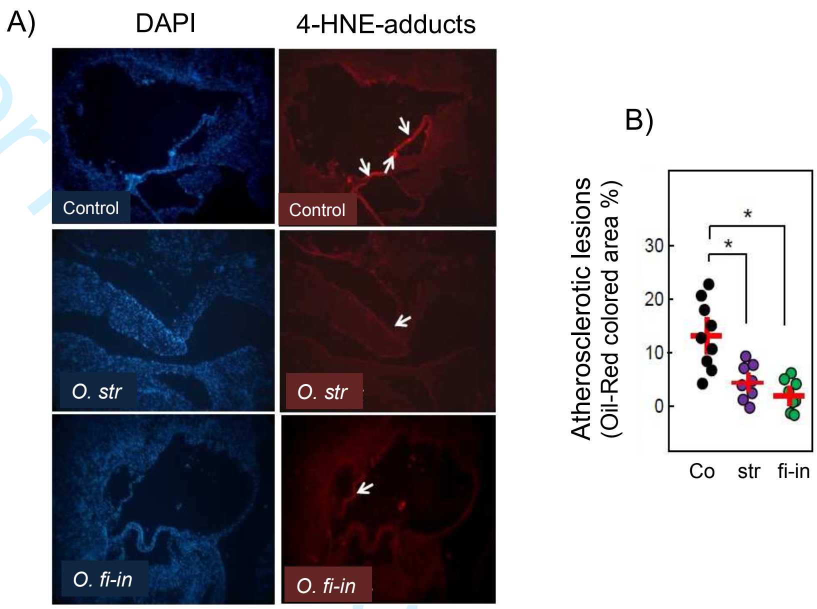

Figure 3. Opuntia cladode powder reduces atherosclerosis lesions in apoE$^{-/-}$ mice. *ApoE$^{-/-}$ male mice aged 6 weeks (8-9 animals/group) were fed a regular powdered mouse chow diet supplemented with O. streptacantha (str) or O. ficus-indica (fi-in) cladode powder (1%). After 15 weeks, the animals were sacrificed. The heart was embedded in OCT (Tissue-Tek), and the aortic sinus were cut and stained. A) shows the expression of 4-HNE-protein adducts in aortic sinus. Slides were labeled by anti-4-HNE Michael adduct antibody (Abcam), revealed by Alexa Fluor 568-conjugated antibody (red, right panels). Nuclei were stained by DAPI (blue, left panels). The white arrows point out the expression of 4-HNE-adducts characteristic of atherosclerotic lesions. B) shows the quantification of the fatty streak area in aortic sinus after staining with Oil Red O, using Image J software. The data are expressed as percent of red-colored atherosclerotic lesion to total arterial wall surface. Each point represents the mean value of 3 determinations in one aortic sinus. The data are expressed as percent of the mean value of the untreated controls and presented as mean ± SEM. Statistical analysis by one-way ANOVA and Holm–Sidak.*P<0.05.(Adapted with permission from Ref. 28, License No. 4297560306070, copyright 2015 Springer).*

The World Cancer Research Fund pointed out a convincing association between red and processed meat intake and the risk of developing colorectal cancer *(30)*. They recommended to eat no more than 500 g/week of red meat and to avoid processed meat consumption. In October 2015, the WHO/IARC classified processed meat as carcinogenic for humans and red meat as probably carcinogenic to humans *(31)*. Among hypothesis linking red and processed meat consumption and colorectal cancer, the high concentration of heme iron (the red pigment of red meat) in those meats has gained attention, particularly because this compound is able to catalyse the peroxidation of dietary lipids and the subsequent formation of toxic lipid oxidation products, such as 4-hydroxynonenal (4-HNE) *(32)*.

Studies were carried out on conditionally immortalized murine colon epithelial cells, bearing or not a mutation on the *Apc* gene, a frequent and early mutation during the development of human colorectal carcinogenesis. In those studies, 4-HNE was found more toxic to normal cells than to preneoplastic ones *(33)*. Due to this differential effect, 4-HNE is suspected to be a promoting agent of colorectal cancer. It was reported that *Opuntia* cladode powders from wild and domesticated species showed a protective effect against the cytotoxic effect of 4-HNE, only in normal cells, but not in preneoplastic cells (Figure 4). Cladode powders could counteract the effect of 4-HNE *in vitro (27)*, with the most domesticated *O. ficus-indica* being less efficient than wild species.

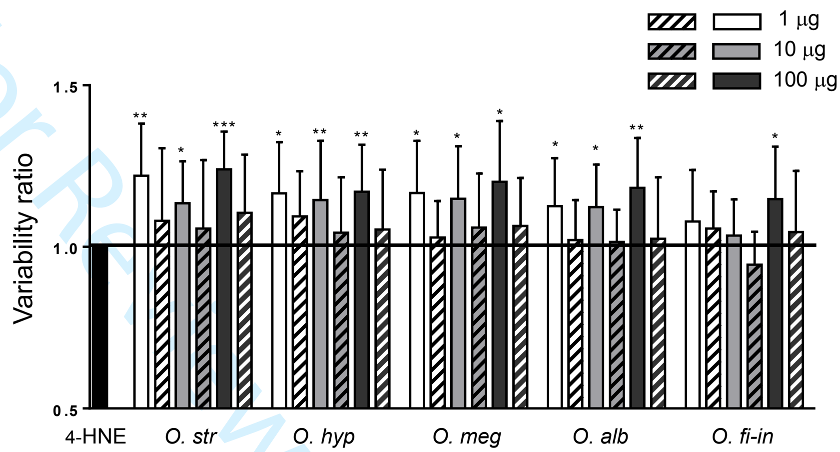

***Figure 4**. Bars represent the viability ratio that means the viability with/without Opuntia cladode powder pretreatment at different dosages (1, 10 and 100 µg/mL) in the culture medium in normal (normal bars) and preneoplastic (hatched bar) cells, upon 4 hydroxynonenal treatment (4-HNE, 40 µM). These results are representative of at least three independent experiments. * means a ratio significantly different from 1, with * : p<0.05; **: p<0.01 and ***: p<0.001. The selected cultivars were: O. str, O. streptacantha; O. hyp, O. hyptiacantha; O. meg, O. megacantha; O. alb, O. albicarpa; and O. fi-in, O. ficus-indica. (Adapted with permission from Ref. 27, License N° 4297711004433 Copyright 2015 Springer*

Production of bioactive compounds by *Opuntia* cultures *in vitro*

Opuntia grown *in vitro* in cell plant culture could be an important tool for increasing the amount of phenolic compounds that are present in *Opuntia* fresh cladodes. Previous studies showed that *O. streptacantha, O. megacantha,* and *O. ficus-indica* exhibited high levels of phenolic compounds, high antioxidant activity *(8)*, and presented important biological effects *(27,28)*. Therefore, these species were selected to establish *in vitro* cultures, as an alternative to improve metabolite production under controlled conditions.

The *in vitro* culture techniques present several advantages for the production of bioactive compounds in relation to whole plant including: (a) a continuous supply of plant material independently from environmental or seasonal changes; (b) a defined culture medium under aseptic conditions assuring that material is free from pathogens; (c) a growth rate of *in vitro* cultures faster than *in vivo* natural conditions, and (d) a possible increase of metabolite production by optimization of culture media, selection of high-producing cell lines, use of bioreactors, or enhancement the metabolic pathways by elicitation *(34,35)*.

Robles-Martínez et al. *(36)* reported the establishment of *in vitro* cultures of *O. streptacantha*, *O. megancantha* and *O. ficus-indica* using embryos as explant. The plant material was cultivated on Murashige and Skoog (MS) media *(37)* supplemented with different concentrations of the auxin 2,4,dichlorophenoxiacetic acid, 2,4-D (1, 2, 3 mg L^{-1}) and the cytokinin benzyladenine, BAP (0.25, 0.5, 1.0 mg L^{-1}). Fast-growing calli were generated on media with 3 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BA *(36)*.

To assess the potential of *Opuntia* calli as a source of metabolites, the content of total phenolic acids (μmol gallic acid/g DW), flavonoids (μmol quercetin/g DW) and antioxidant activity (μmol Trolox/100 g DW) were quantified. The phenolic acid content was higher in *O. streptacantha* and *O. megacantha* calli than those present in *O. ficus-indica* calli. The flavonoid concentration and antioxidant activity (AO) were also higher in *O. streptacantha* (Table I) *(38)*. These results are in accordance with the proteomic profile, which showed that this species accumulates proteins, related to photosynthetic process, and to primary and secondary metabolism *(39)*. Thus, the high enzymatic activity present in *O. streptacantha* cladodes seems to be also present in calli.

Table I. Comparison of metabolite content and antioxidant activity in control and stressed calli from *Opuntia* species[1]

Compound[2]	**Calli**	***Opuntia* *ficus-indica***	***Opuntia* *megacantha***	***Opuntia* *streptacantha***
	Control	24.6	44.1	44.0
Phenolics	Stressed	25-28	25-41	73.6*
(μmol Gallic acid/g)		NS	NS	(UV 240 min)
	control	3.7	5.3	8.9
Flavonoids	Stressed	3.4-5.1	1.8-5.3	15.3*
(μmol Quercetin/g)		NS	NS	(UV 240 min)
Antioxidant activity	Control	699	511	713
(μmol Trolox/100g)	Stressed	986*	816*	945*
		(1.25% PEG)	(25 mM JA)	(50 mM JA)

[1]On dry weight basis. *Statistically significant ($p<0.05$); NS=not significant in relation to control calli. Data in parenthesis indicate the stress condition.

It is well documented that stresses such as UV radiation, drought stress induced with polyethylene glycol (PEG) and jasmonic acid (JA), stimulate the production of compounds such as glycosides, coumarins, saponins, alkaloids, anthocyanins, psoralens, and flavones *(40-42)*. Hence, these stressors or elicitors were used to stimulate the production of such compounds and/or AO activity in *Opuntia* calli *(38).*

Table I summarizes the stress conditions for which a significant increase in metabolite content or AO activity was observed in relation to control calli. The content of phenolic and flavonoid compounds was increased 1.67 to 1.7 times in *O. streptacantha* calli after 240 min UV irradiation. Likewise AO activity was significantly enhanced in *O. ficus-indica* in media treated with 1.25% PEG, *O. megacantha* treated with 25 μM JA, and in *O. streptacantha* treated with 50 μM JA *(38)*. The exposure to JA stimulated both phenolic and flavonoid synthesis, while abiotic stress (UV, PEG) induced mainly the flavonoid route *(38).* These results indicate that the use of elicitors is a successful strategy to improve the metabolite content and AO in *Opuntia* calli.

The results showed that: (a) *O. streptacantha* calli synthesized 3.5-4 times more phenolic acid and flavonoid compounds than *O. megacantha* and *O. ficus-indica* calli under stress conditions; (b) there was no clear correlation between phenolic/flavonoid content and antioxidant activity in *Opuntia* calli suggesting that other undetected antioxidant compounds could be present in those species.

Conclusions

Wild species are a source of bioactive compounds that are not present in domesticated ones. A striking exemple is the kaempferol 3-*O*-arabinofuranoside detected in *Opuntia* wild species. This compound exhibits neuroprotective and inhibitory effects against cellular senescence with high therapeutic potential for the treatment and prevention of diverse age-related diseases and cancer. Nevertheless, *in* *vitro* and *in vivo* studies indicated that wild and domesticated *Opuntia* spp. exhibit antioxidant and anti-inflammatory properties with potential nutritional benefit for preventing diseases such as cardiovascular complications and colon cancer.

The use of cell culture tissue with induction of biotic and abiotic stress is an efficient strategy to improve metabolite production. This is a promising system to obtain compounds with antioxidant properties and to study their protective effect on degenerative diseases.

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