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Phytochemical Content, Nutraceutical Potential and Biotechnological Applications of an Ancient Mexican Plant: Nopal (*Opuntia ficus-indica*)

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Abstract: Nopal is by far the most important cactus worldwide. Since its pre-Hispanic use for food and cochineal dye production, domestication of this plant was one of the most important inheritances left by ancient Mexicans. Nowadays, nopal research is increasing mainly due to the many nutraceutical properties that have been attributed to this plant. Its potential use against type 2 diabetes is gaining importance which has been related to its high antioxidant and dietary fiber contents. Even more, the consumption of nopal cladodes and fruits has been associated with anticancer properties or cancer chemoprevention issues. Other nutraceutical properties also elucidated include antiulcer activity, antiallergenic potential and helping long-term memory. Moreover, new biotechnological tools are now been applied in nopal such as *in vitro* tissue culture techniques and genetic plant transformation. Important industrial enzymes have been isolated, purified and characterized from nopal. Finally, food industry applications for nopal are increasing because of its nutraceutical properties and its potential use as a functional ingredient in the production of bakery products, bars and tortillas, among other interesting new technological uses. Therefore, the aim of the present review is to summarize the information generated in the last years around this important plant.

Keywords: *Opuntia* health benefits, biotechnology, cacti.

1. INTRODUCTION

Since the first human settlements in Mexico nopal constituted an important source of food for indigenous people [1]. Cochineal dye production was one of the main uses associated with nopal plants which contributed with its dispersion around the world. Nopal is the most important cactus species grown worldwide, with the highest economic importance due to its use as food and forage along with its nutraceutical potential. In view of this, nopal research has increased over the last two decades. Many reports indicate its use as an important medicinal or nutraceutical agent. Traditionally, nopal use has been closely linked with its antidiabetic potential; many research groups have associated this effect with its high fiber content, nevertheless, today some other authors suggest that this might be due to the compounds isolated from the fiber free extracts [2]. Similarly, many reports indicate that this ancient plant exerts an anticarcinogenic effect attributed mainly to the plethora of antioxidant compounds present in both cladodes and fruits. Even more, other studies show that due to its high calcium and magnesium content nopal cladodes can aid in bone density increment [3]. In addition, nopal can be related to an anti-ulcer effect after ethanol consumption [4]. Biotechnological applications for nopal are increasing. Some of them include the establishment of *in vitro* culture techniques suitable for massive propagation of endangered nopal species, as well as for commercial and ornamental cultivars [5, 6]. Hence, these techniques can likewise serve for the purpose of genetic transformation in prickly pear cactus. In this sense, genetic transformation techniques are under development, such as the biolistics process and the *Agrobacterium tumefaciens* mediated transformation [7]. On the other hand, nowadays novel applications for nopal are emerging, most of them mainly in the food industry. In this context, nopal has been incorporated as an extra ingredient in new healthier food alternatives. In addition, it has been used for the manufacturing of edible films, as powdered food additive and for the production of colorants. Furthermore, new applications of the cacti species have been developed for water treatment, raw material for Pb^{2+} removal from contaminated water, improvement of lime mortars for restoration, and as bio-insecticide against termites.

2. HISTORY

Opuntia ficus-indica (L.) Mill is the cactus species with the highest economic importance worldwide [8]. It is grown in America, Africa, Asia, Europe and Oceania [9]. Since the arrival of humans to Mesoamerica, some 20,000 years ago, and specifically to the desert and semi-desert regions, nopal plants were an important source of food for indigenous people as well as drinks and medicines; long before the horticultural management of this plant was known, ancient Mexicans consumed it in abundance [1]. Even more, the Codex Mendoza describes the use of nopal in Aztec tribute rolls [10]. Such Codex includes a representation of *Opuntia* cladodes amongst other items such as ocelot and jaguar skins [11]. These plants were distributed from Mesoamerica to Cuba, and other Caribbean islands, at the time they were discovered by European explorers [12].

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It is generally accepted that this specie was domesticated in Mexico [11, 13], where the highest richness of traditional cultivars are found [13]. No wild plants have been found and it has been proposed that it is derived from *O. amyclaea* [14]. The domestication process of *Opuntia* was directed towards the production of plants with cladodes lacking spines and with large sweet fruits [15]; a process which was developed for the south of the meridional highlands of Mexico [16]. Although there is evidence (archaic and botanical) of the various uses of *Opuntia* spp. by several ethnic groups since 8,000 years ago, none is related to the use of *O. ficus indica*. It is worth mentioning that this cactus plant was already an important crop in the XVI century in central Mexico, later on, the Spaniards spread its use in the old World, because of its many properties, specially of the fruits, for the use against scurvy and most importantly for the production of red colorants as a result of *Dactyliopus coccus* infestation in nopal cladodes [14]. In this way, this plant was distributed around the globe from Mexico to the whole world.

3. BIOLOGY AND DISTRIBUTION

The *Opuntia* genus forms part of the Cactaceae family, including different species from North and South America [17]. This genus includes around 160 to 250 species; however, the exact number is unknown [18-20]. The difference is mainly due to nomenclatural problems occurring not only in *Opuntia,* but also within other genera of the *Opuntioideae* sub-family. This can be due to the phenotypic plasticity within many opuntioid taxa, to the recent diversification and to the polyploidy level, among others [21].

Among the *Opuntioideae* subfamily, nopal plants present different ploidy levels that vary from diploid to octoploid [22]; in fact, the majority of the species in this subfamily are polyploid (64% of the total) [23].

The correct taxonomical description of *O. ficus-indica* is difficult, because it is generally described as spineless and different from other *Opuntia* species (*O. megacantha, O. streptacantha* and *O. amyclaea*) [14, 20, 24]. In other cases, spined cultivated genotypes have been classified as *O. ficus-indica* [25]. Nowadays, the use of presence/absence of spines is not an accurate form to differentiate *O. ficus-indica* from other nopal plants [8, 25]. At present, most nopal commercial genotypes are octoploid [22]; however, their ancestor is unknown.

Still more, several authors reported that it is difficult to correctly assign cultivated genotypes to a defined taxon [26, 27]. The species designation in the *Opuntia* genus has long been a problem, since they present a continuous morphological variation and a high level of hybridization with individuals with mixed or intermediate characteristics. Very often the same cultivars are classified as being part of different species. In view of this problem, the use of molecular markers has become an alternative for the correct species assignment in this genus that might unravel the differences which are not obvious by morphological characterization [17].

On the other hand, the overall structure of these plants is rather complex. Even though not matching with morphological criteria, cladodes replace leaves in their photosynthetic task. Cladodes are also known as “cactus pads”, “stems”, “cactus vegetable”, “phyloclades”, “nopales” or “pencas” [28]. The cactus stems are composed of a hard skin that surrounds an outer layer of chlorenchyma and a central core [29]. Likewise, the stems are covered by spines which are modified leaves, and multicellular hairs and trichomes which all together form a structure named “areole”, characteristic of all cacti plants [28]. Randomly disposed on both sides of the cladode we find the stomata; *O. ficus-indica* presents around 15 to 35 stomas per mm^2 [30]. Under proper environmental conditions, new cladodes, flowers or roots will arise from the meristematic tissue underlying in the areoles [31]. Fruits from *O. ficus-indica* typically range from 120 to 200 g with 45 to 60% of the fruit being edible. The fruit color varies from lime green, yellow, orange and red to purple [28]. It presents many small barbed spines hosting a juicy pulp with 150 to 300 non edible seeds. Many of the nopal seeds are abortive, which could be related with problems in the ovule or with a young adventitious embryo [31]. Some structural analyses on the seed pericarp of *O. ficus-indica* [32] have revealed an important amount of lignin (20%), and polysaccharides (62%) including cellulose (35%). Sexual and vegetative propagation are both possible for the *Opuntia* genus [33]. Although the vegetative multiplication is the most extensively used cultivation technique, the adventitious roots developing from the areoles in the portion of the cladode make contact with the soil, allowing rooting as well as water and nutrient absorption. This type of multiplication is more efficient than sexual propagation for plant recruitment. In brief, both systems have accounted to the evolutionary and ecological success of the genus [14].

The overall distribution of this plant is large. With more than 360 species, the *Opuntia* species represent the largest genus of the Cactaceae. We can find this plant from Canada to the Argentinean Patagonia, from sea level or up to 5,100 masl in Peru [24, 34]. Nopal is commercially cultivated for fruit and nopal (cladode) production for human consumption in two main countries: Italy and Mexico. In Italy, fruit production is focused mainly in the island of Sicily with more than 4,000 ha which produces an average of 60,000 t. In Mexico, nopal production is distributed among various states mainly in Distrito Federal, Estado de Mexico, San Luis Potosí, Zacatecas, Tamaulipas, Aguascalientes and Guanajuato [35].

4. CHEMICAL AND PROXIMATE COMPOSITION

4.1. Cladodes

Proximate composition in different cladodes from nopal cultivars has been evaluated by many authors, and it is always variable. This can be due to different environmental conditions prevailing in the site of cultivation as well as to structural differences among these cultivars. The proximate composition of some wild and cultivated *Opuntias* has been described [36]. According to these results, wild nopal Blanco had the highest protein content (19% dw), Cristalino and Tapón II wild materials exhibited the highest lipid concentration (1.5 and 1.8%, respectively). The highest carbohydrate content was observed in Morado (80.9%) and the lowest value in Tapón II (42.4%). The ranges for chemical composition study are described in (Table **1**). In the same way, interesting compounds have been identified in the petroleum ether extract of *O. ficus-indica* var Milpa Alta by GC/MS [37]. Among the 26 compounds identified (representing

Table 1. Chemical composition, dietary fibers, antioxidant compounds and antioxidant capacity of nopal cladodes.

Chemical Composition	**Fresh Nopal (g/100g)[a]**	**Nopal Flour (% dw)[b]**	**Dehydrated Nopal (%)[c]**	**By Products of Nopal Cladodes (g/100 g dw)[d]**
Protein	0.8	6.7-19.0	ND	1.13
Lipids	0.2	0.1-1.8	ND	ND
Total dietary fiber	2	5.5-15.0	45-69	
• Soluble	ND	ND	13.1-25.26	8.92-9.8
• Insoluble	ND	ND	23.76-37.94	53.13-54.45
Ash	2	5.2-19.7	ND	16
Carbohydrate	5	42.4-80.9	ND	ND
Moisture (if reported)	90		6.67-13.33	
Antioxidant Compounds	**Fresh Nopal (g/kg fw)[f]**	**Nopal Flour (dw) [e]**	**Dehydrated Nopal[c]**	**By Products of Nopal Cladodes[d]**
Free phenols	60	562-905 (µg of GAE/g)	0.57-2.3 mg GAE/g	2.7-3.7 (g GAE/100 g dw)
Flavonoids	23.4 (g/kg dw)			
• Kaempferol	2.2	1.8-474.6 (µg/g)	0.17-0.53	ND
• Isorhamnetin	4	58.9-762.2 (µg/g)	0.24-0.72	ND
• Quercetin	2	ND	2.62-3.61	ND
β-Carotene (mg/g dw)	1.6	ND	0.2-0.77	21-22
Lutein (µg/g dw)	ND	ND	10.03-21.10	ND
Antioxidant Capacity (µmol of TE/g)		**Nopal Flour[a]**	**Dehydrated Nopal[c]**	**By Products of Nopal Cladodes[d]**
ORAC		264-738		
DPPH			5.48	
ABTS*			6.11	52-57
FRAP				52-66

[a][39];[b][36]; [c][40]; [d][41]; [e][42] [f][43]. ORAC: oxygen radical absorbancy capacity; DPPH: diphenylpicrylhydrazyl scavenging method; ABTS*: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); FRAP: ferric reducing ability of plasma assay; ND: not determined

95.6% of the total extract), phytosterol (36.03%) was the highest component, and polyunsaturated fatty acids (18.57%) represented the second largest group, followed by palmitic acid (13.54%) and phytol (12.11%).

At our laboratory, Ortiz-Escobar *et al.* [38] assessed the folate content in cladodes of nopal (*O. ficus-indica*) by a microbiological assay, using *Lactobacillus casei* (ATCC 7469) in extracts that were enzymatically treated to release the bound vitamin. In brief, no statistical differences were found between the techniques employed and the folate content was in the range of 5.0 to 5.62 ng/g of fresh tissue. Nopal cladodes are a good source of minerals; according to Feugang *et al.* [39], they contain Ca, Mg and K, among others.

In nopal, by-products are constituted by the outer coating of cladodes and fruits, which are removed before food preparation and contain spines and a large quantity of glochids. In view of this, Bensadón *et al.* [41] evaluated the chemical composition of cladode and prickly pear fruit by-products from two Mexican commercial cladode cultivars, Atlixco and Milpa Alta as well as from two prickly pear fruit crops, Alfajayucan and Pelón Rojo. Wherefore, the amount of by-products obtained from cladodes and fruit was approximately 17 and 53% (fresh weight, respectively). The total dietary fiber was significantly higher in cladodes than in fruits.

The cultivars Atlixco and Milpa Alta, showed no significant difference in the soluble and insoluble dietary fiber (SDF, IDF) from the cladodes. Nevertheless, in fruits IDF was significantly higher in Alfajayucan than in Pelón Rojo (Table **1**). Both cladodes and fruits contained a high concentration of soluble dietary fiber. The two cladode samples exhibited around 8.9 to 9.8 g/100 g dry matter of soluble fibers, while those from Alfajayucan and Pelón Rojo contained between 7.98 and 8.14 g/100 g dry matter of SDF, respectively. Similarly, total carotenoids were detected in the order of 21 to 22 mg/g dry matter for both cladodes and between 16 and 15 mg/g dry matter for both prickly pear fruits (Table **1**). In brief, these authors [41] stated that by-products from cladodes and fruits of *Opuntia* spp. may be utilized as potential functional food ingredients because of their good quality dietary fiber and natural antioxidants.

Nopal cladodes present high calcium content mostly in the form of oxalate crystals. Calcium accumulation in nopal can be related to heat stress, since calcium is able to mitigate the effects of heat stress by improving stomatal function and other cell processes, because this mineral is implicated in many signal transductions that control stomatal aperture [44]. Contreras-Padilla *et al.* [45] evaluated the oxalate and calcium contents of *O. redonda* cladodes at different maturity stages. The results obtained revealed that calcium content in nopal increases as a function of maturity, ranging from 17.4 mg/g dry weight at 40 days to 34.4 mg/g at 135 days, showing an increase of 97%; however, no bioavailability studies were reported.

Another study revealed the calcium bioaccessibility in two *O. ficus-indica* (L.) Mill cultivars namely Milpa Alta and Atlixco. High calcium content was observed in the cladodes, but only 16 and 9% of total calcium was dialyzable in these two crops, respectively. For the Milpa Alta cultivar, only a 14% of total calcium was bioavailable, contrasting with only 3% in the Atlixco cultivar. Ionic dialyzable calcium (IDC) is nutritionally important since calcium has to be soluble and ionized in order to be absorbed in the upper intestinal tract [46]; with this in mind the authors analyzed the levels of IDC in nopal cladodes, finding a 90% for Milpa Alta and a 33% for the Atlixco cultivar. Conversely, the non dialyzed calcium was the major fraction obtained for calcium in both cultivars (84-91% of total calcium). Total oxalate and calcium bioaccessibility had no significant changes after the cooking conditions applied. The ash content contributed to an 18% of dry matter. The most abundant mineral was K for both cultivars, followed by Ca and Mg; in addition, the presence of other minerals was observed but their content was low (Na, Cu, Fe, Mn and Zn). According to these authors, cladodes are a good source of calcium (1701-1966 mg/100 g dry matter) compared to other vegetables [47]. Nopal cladode sugar composition has also been reported. Glucose and galacturonic acid were the main constituents while arabinose, galactose, mannose, xylose and rhamnose were present in lower levels. In the same way, significant amounts of either starch or xyloglucan were present as revealed by the samples treated by I_2/KI. Starch content was 89.5 mg/g for one of the fractions analyzed, and it was consistent with a previous reported value. It is interesting to note a high percentage of calcium oxalate and Klason lignin (16%) in the cladodes [29].

4.2. Fruits

Prickly pear cactus fruits are good sources of minerals such as K, Ca and Mg. The total caloric value of these fruits is 50 kcal/100 g fw. They contain ascorbic acid in the range of 7.6 to 23.3 mg/100 g fw and carotenoids content varies from 2.58 to 4.71 µg/100 g fw (Table **2**). In addition, they contain important amino acids such as lysine and methionine [39]. One main characteristic of prickly pear is the presence of water soluble betalain pigments that are accumulated into the vacuole. Betalainic phytochemicals are nitrogen-containing pigments occurring in the Caryophyllales [28]. The main betacyanin and betaxanthin in this fruit are betanin and indicaxanthin, so modifications in the proportion of these pigments are reflected in pulp color [48]. The betalain pathway of prickly pear cactus fruits has been partially analyzed in order to elucidate the mechanisms underlying pulp color. This was done analyzing the partial genomic sequences of two important genes from this pathway [49]. No differences were found between colored and non-colored cultivars, suggesting that the regulatory mechanisms that generate prickly pear color in the inner core, peel and epidermic tissues function in an independent manner. Core pigmentation takes place first and before the start of fruit maturity, which can be related to maximum soluble solids. Other mechanisms, such as fruit ripening, also control color appearance in these as well as in other fruits [50].

Fruit quality can be affected by environmental conditions; in this sense, a study on two *O. ficus-indica* Italian cultivars, Rossa and Gialla, was conducted [51]. Significant changes were observed among fruit weight, shape and total soluble solid content with the environmental conditions (site, altitude) while flesh percent, pH and total titratable acidity did not change. Fruit weight and seed content were influenced by the type of cultivar, while cladode surface area was poorly related to fruit quality. The role of cultivars in determining fruit quality did not change with site and, moreover, the sensory analysis was unable to discriminate for cultivar and environment. Light interception and cladode dry weight were the main sources of fruit dry weight variability and sugar content [51].

Many mechanisms affect fruit quality in prickly pear, some of them are caused mainly by the fruit ripening process which controls color appearance and flavor. The leading cause for these changes is a tight genetic regulation of the transcription factors of many maturation related genes (up and down-regulation), as well as their regulation type which can be constitutive or variable. All together as a whole, these genetic changes displayed during the ripening process comprehend metabolic control, communication among plant organelles, plant growth regulators and the developmental regulation of gene expression [50]. In addition, variable environmental conditions also play a key role in fruit quality since they mark a trend on fruit yield and fruit ripening time, having an impact on fruit shape, size, flesh percent, sugar content, flavor and taste. Remarkably, fruit size depends on crop yield per plant and cladode [54], as well as on crop management in terms of irrigation and fruit thining [55].

4.3. Seeds

Prickly pear seeds present high levels of protein, lipids, fiber, ash and carbohydrates, according to a study conducted on protein content and nutritional value [56]. Accordingly, all essential amino acids levels for the seed flour and a protein concentrate were above than those reported for the FAO/WHO reference protein [57], except for methionine, threonine and tyrosine. In another study, seeds from *O. ficus-indica* growing in Turkey were evaluated for their nutritive value and chemical composition [58]. Proximate composition for these seeds was crude protein 4.78%, crude lipids 5.0% and fiber 12.47% dw. Significant levels of minerals were observed for them including P 1,628, K 533, Ca 471, Mg 117, Na 71 and Fe 290 12 mg/kg dw. In addition, the seed oil contains linoleic acid as the major fatty acid (61.01%), followed by oleic (25.52%) and palmitic acids

Table 2. Chemical composition, antioxidant compounds and antioxidant capacity of prickly pear fruit.

Chemical Composition	**Fruit Pulp (*O.* spp)**[a]	***O. matudae***[b]	**By-Products**[c*]	
Moisture	84	94.11	5.41-7.52	
Carbohydrate	12	1.7	ND	
Ash	0.3	0.11	13.14-17.07	
Dietary fiber	1.7	1.74	ND	
• Soluble	ND	1.16	7.98-8.14	
• Insoluble	ND	0.58	19.39-34.95	
Protein	1.5	0.56	Traces	
Lipids	0.5	0.04	0.94-1.14	
Antioxidant Compounds	***O. ficus-indica***[d]	***O. stricta***[d]	***O. matudae***[b]	**By-Products**[d*]
Ascorbic acid (mg/100 g fw)	18.5	23.3	31.67	ND
Total phenolics (mg GAE/100 g fw)	218.8	204.4	33.71	0.00154- 0.0027
Total carotenoids (μg β-carotene equivalents/100 g fw)	2.58	4.71	0.02 (mg/mL extract)	0.015-0.016
Betacyanins (mg betanin/100 g fw)	15.2	80.1	ND	ND
Betaxanthins (mg indicaxanthins/ 100 g fw)	25.4	ND	ND	ND
Betalains (mg/100g fw)	40.6	80.1	ND	ND
Taurine (mg/100 g fw)	7.70	6.80	ND	ND
Quercetin (μg/g fw)	90	87.5	ND	ND
Isorhamnetin (μg/g fw)	49.4	50.3	ND	ND
Kaempferol (μg/g fw)	7.8	7.7	ND	ND
Antioxidant Capacity	***O. ficus-indica***[d]	***O. stricta***[d]	***O. matudae***[b]	**By-Products** [c*]
ABTS (μmol TE/g fw)	6.70	5.98	ND	65.76-66.33
DPPH (μmol TE/g fw)	5.22	4.72	16 mg/mi extract (EC_{50} value)	ND
FRAP (μmol TE/g dw)	ND	ND	ND	40.39-47.35

[a] [39]; [b][52][c][41]; [d][53]* glochids, spines, epidermis manually removed from prickly pear fruits, without edible portions to simulate food industry processing by-products; ABTS: 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH: diphenylpicrylhydrazyl scavenging method; FRAP: ferric reducing ability of plasma assay; ND: not determined

(12.23%). Similarly, myristic, stearic and arachidonic acids were detected in *O. ficus-indica* seed oil in low amounts. The fatty acid composition of prickly pear oil was close to those of sunflower and grape seed oils [58].

4.4. Flowers

Opuntia flowers are commonly used in traditional Tunisian medicine for their diuretic activity, their capacity to expulse renal calculus and to cure ulcer [59]. The purified methanol extract of *O. ficus-indica* flowers was analyzed by means of HPLC-PDA-ESI-MS/MS [60]. Many secondary metabolites were detected, some of them belonging to the flavonol glycoside class, as indicated by their absorbances (255-265 and 350-355 nm). Kaempferol, quercetin and isorhamnetin were detected according to the MS/MS spectra of all their precursor ions. The flavonoid content for these flower extracts was 81.75 mg/g of fresh tissue. Isorhamnetin 3-O-robinobioside was found in high levels (52.22%) and was followed by other compounds such as: isorhamnetin 3-O-galactoside (11.98%), isorhamnetin 3-O-glucoside (8.86%), quercetin 3-O-rutinoside (8.67%), quercetin 3-O-glucoside (5.47%), kaempferol 3-O-rutinoside (4.89%), kaempferol 3-O-arabinoside (3.96%) and finally two compounds that had not been previously described. Correspondingly, 18 volatile compounds were identified which represent 84.2% of the whole volatile profile in nopal flowers. The principal compounds detected were: germacrene D (12.6%), 1-hexanol (12.3%), *n*-tetradecane (9.1%) and decanal (8.2%). No monoterpene hydrocarbons were found. Nevertheless, other compounds such as oxygenated monoterpenes were detected (16.5%). On the other hand, sesquiterpene hydrocarbons represented 18.0% with germacrene D being the highest compound. The presence of oxygenated sesquiterpenes was not detected. Nonanal (2.5%) and decanal (8.2%) represented the group of open chain aldehydes with a non terpenic structure (10.7%). Non terpenic open chain hydrocarbons with 13, 14, 15 and 16 carbon atoms accounted for the 20.6% of the total constituents. Three open chain non-terpenic alcohols occurred at 17.2%, and 2-ethyl hexyl acetate was at 1.2%. This is the first description of *O. ficus-indica* volatile composition in flowers [60].

5. PHYTOCHEMICALS AND NOPAL

Many phytochemical compounds are being studied in the search for their capacity to address health problems. These compounds from plant origin can work as substrates for biochemical reactions providing important health benefits. They act mainly as scavengers for toxic chemicals; and in other cases they enhance the absorption and stability of essential nutrients. Some others act exerting beneficial effects on human gut flora (growth factors, fermentation substrates, or inhibitors of harmful intestinal bacteria). Some of these phytochemicals comprehend terpenoids, phenolics, alkaloids and fiber. Research supporting beneficial roles for phytochemicals against cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections and ulcers is based on chemical mechanisms using *in vitro* and cell culture systems, various disease states in animals and epidemiology of humans [61].

These chemicals of plant origin which exert benefits to overall health can be included in De Felice´s definition of a nutraceutical: “a nutraceutical is any non-toxic food extract supplement that has scientifically proven health benefits for both disease treatment and prevention”. In this context, we can clearly distinguish at least three main chemical compounds groups: terpenoids, phenolics and alkaloids [61].

In the case of nopal, it has been recognized as a phytochemically important plant due to the many different compounds it presents and the associated health benefits they provide.

Nopal plants present high levels of phenolic compounds, which are associated with the prevention of cancer metastasis. These naturally occurring compounds are highly present in vegetative foods and nutraceuticals [62] and can be defined as phytochemical compounds presenting at least one aromatic ring with one or more hydroxyl groups, although they are structurally diverse, and are roughly categorized into several classes. First class phenolic compounds include phenolic acids, polyphenols or monophenols classified by the number of hydroxylated aromatic rings and by the type of functional moiety. Second class phenolics are represented by polymerized phenolic compounds such as condensed tannins, proanthocyanidins, lignans and lignin. Finally, the third class of phenolics includes simple phenols, phenylpropanoids, benzoic acid derivatives, flavonoids and stilbene [63].

Many other reports have indicated the important effects exerted by nopal fruit betalains, mainly in the prevention of diseases and some other beneficial effects on health [64]. Betalains are water soluble nitrogenous compounds found in plants and are responsible for some fruit colors. Betaxanthins are a group of betalains that confer the yellow and orange color in fruits while betacyanins are responsible for the red and purple ones [65].

Phytochemicals might prevent the multi-step process of carcinogenesis. Some of these compounds are found in staple crops for human consumption as well as in herbs; nonetheless, all of them are gaining importance as a source of anti-cancer drugs or compounds used in cancer chemoprevention or adjuvants in chemotherapy. These types of chemical compounds found in plants can aid in preventing cancer initiation, promotion and progression by exerting anti-inflammatory and antioxidant effects. Moreover, they have been associated in the induction of cancer cells apoptosis and in the inhibition of tumor growth *in vivo* [66].

Some strategies have been described to fight cancer development, which include: 1) early detection of cancer development by discovering new biomarkers or 2) prevention (by delaying or blocking) of cancer formation or its progression into metastasis by using chemical drugs [67]. Some chemical agents can inactivate carcinogens, helping in the inhibition of each stage of carcinogenesis; such compounds can function as antioxidants or antioxidant enzymes, nonetheless, other agents that suppress tumor growth or stimulate apoptosis can act in later stages [68]. Described as early as 1960s, cancer chemoprevention is a strategy to reverse or suppress the process of carcinogenesis using chemical compounds [69] and it is currently been used to act on all stages of cancer development. Besides being used in cancer onset prevention by means of DNA repair, it also helps in the detoxification, free-radical scavenging and carcinogen metabolism, prevention of tumor, promotion and progression through inhibition of proliferation and angiogenesis, induction of apoptosis and differentiation, reduction of inflammation and immunity increase [70, 71].

Phytochemicals present in different structures of nopal plants can include quercetin, kaempferol, betaxanthin, indicaxanthin, luteolin, isorhamnetin and ascorbic acid among other components [28, 39]. Many of these phytochemical compounds have been associated with nutraceutical properties that will be described in the following section.

6. NUTRACEUTICAL POTENTIAL AND HEALTH BENEFITS

6.1. Antioxidants

In the last decades, antioxidant compounds have gained interest worldwide, mainly due to their capacity to prevent the onset of many serious diseases. In this sense, prickly pear cactus fruits constitute a good source of vitamins and interesting compounds such as polyphenols and betalains [72].

Fruits from *O. joconostle* were analyzed for their phenolic content [73]. According to the results, a total phenolic content of 2.07 mg GAE/g of fresh pericarp was observed. Fresh pericarp contained the largest amount of flavonoids, followed by endocarp and mesocarp. So, the highest betacyanin content was observed in the endocarp followed by mesocarp and pericarp. The whole fruit concentration was 7.57 mg betanin/100 g fw.

In this same way, a group in Spain conducted a study in three different cactus pear fruit species: *O. ficus-indica, O.*

undulata and *O. stricta* [53]. Total phenolics were found around 128-218.8 mg GAE/100 g fresh fruit. In addition, ascorbic acid levels for these varieties were between 18.5 to 23.3 mg /100 g fresh fruit. Two of them*, O. ficus-indica* and *O. stricta* are presented in (Table **2**). Significant amounts of flavonoids were detected among all the *Opuntia* species tested, being quercetin the most abundant compound followed by isorhamnetin, luteolin and kaempferol. Conversely, *O. stricta* fruits displayed the highest total flavonoid content, while *O. undulata* presented the lowest value.

Among total flavonoids, quercetin derivatives were around 7.8-90 μg/g fw, isorhamnetin derivatives between 9.6 and 50.3 μg/g fw and kaempferol derivatives around 5.6-7.8 μg/g fw. Betaxanthin was only present in *O. ficus-indica* (25.4 mg/100 g fresh fruit) and in *O. undulata* (17.8 mg/100 g fresh fruit), being indicaxanthin the main betaxanthin identified. Taurine was found in all three nopal species; this compound works as a neuroinhibitory transmitter and is considered a cell protector amino acid (Table **2**) [53]. Antioxidant capacity for two of these cultivars is described in (Table **2**). Accordingly, the values observed were around 5.98 to 6.7 μmol TE/g fw for the ABTS method, and around 4.72 to 5.22 μmol TE/g fw for the DPPH (diphenylpicrylhydrazyl) scavenging method.

Prickly pear fruits exhibiting different colors were examined for their antioxidant contents in Argentina [65]. Variable results were observed in terms of total soluble values and ascorbic acid content ranging from 0.26 to 0.48 mg/g fw. The total phenolic content was found between 0.54 and 1.2 mg of gallic acid/g fw. Purple *Opuntia* spp., dark purple *O. ficus-indica* and orange *O. megacantha* presented the highest levels amongst the samples studied. The antioxidant activity of the prickly pears analyzed was very variable and presented vitamin C equivalent values (VCEAC) between 0.25 and 0.57 mg/g fw; in this sense, *O. ficus-indica* fruits presented the highest antiradical ability.

Two xoconostle cultivars (*O. joconostle* F.A.C. Weber ex Diguet and *O. matudae* Scheinvar) of high consumption in Mexico were analyzed in terms of their nutritional and antioxidant properties. Such properties were evaluated in nopal pulp and seeds [52]. Palmitic and octanoic acids were found in significant amounts; in the same way two active vitamin C forms were also identified: ascorbic acid and dehydroascorbic acid. Additionally, four isoforms of tocopherol (α, β, γ and δ tocopherols) were identified; interestingly, total tocopherol content was higher in seeds of both cultivars than in the pulp. Seeds were high on γ- tocopherol, while in the pulp α-tocopherol was the main isoform, with relatively low values. In terms of phenolic content, *O. matudae* pulp presented 33.71 mg GAE /g of extract and the EC_{50} for the antioxidant activity of this same cultivar was 16 mg/ml analyzed by the DPPH method (Table **2**).

6.2. Antidiabetics

Nowadays, one of the most important and serious metabolic disorders of mankind is diabetes mellitus, which is among the three leading causes of death worldwide [74]. Enormous societal costs and high implications for all healthcare systems are the two main consequences of type 2 diabetes. It has been estimated that worldwide, diabetes mellitus will increase from 171 million people in 2000 to 366 million people in 2030 [75].

A decrease in insulin-stimulated glucose uptake (insulin resistance) is associated with obesity, ageing and inactivity. The pancreatic islets respond to insulin resistance by enhancing their cell mass and insulin secretory activity. However, when the functional expansion of the islet β-cells fails to compensate for the degree of insulin resistance, insulin deficiency and ultimately type 2 diabetes develop. The onset of type 2 diabetes leads in turn to the development of its long-term consequences: macrovascular complications (including atherosclerosis and amputations) and microvascular complications (including retinopathy, nephropathy and neuropathy). Insulin resistance is typically present throughout the progression from pre-diabetes to type 2 diabetes. By contrast, the onset of this disease and its progression are largely determined by the progressive failure of β-cells to produce sufficient levels of insulin. Interestingly, many insulin-resistant individuals do not become diabetic, because their β-cells are able to compensate for the increased demand for insulin [76].

On the other hand, pharmaceuticals utilized to treat diabetes mellitus are sometimes expensive or might have adverse effects or contraindications. Accordingly, other alternatives to treat and ameliorate the symptoms associated with this illness, have been found in plant derived drugs [77]. In this sense, nopal plants (*Opuntia* spp.) have traditionally been used to treat gastritis, intestinal colic, ulcers and recently, some researchers have highlighted their potential use on type 2 diabetic patients [2]. Many of the properties exerted by nopal plants have been related to the plethora of chemically bioactive compounds they possesses. Some of the health benefits associated with nopal consumption are described in (Table **3**).

This was partially demonstrated in a study where a total of 26 compounds were detected in extracts of *O. ficus-indica* cultivar Milpa Alta [37]. Interestingly, three new compounds were observed: monobutyl 2-(4-hydroxybenzyl) tartrate, ethyl 2-(4-hydroxybenzyl) tartrate and diethyl 2-hydroxy-2-(4-hydroxybenzyl) succinate. Nevertheless, no activity has been associated with them so far. The extract antidiabetic activity was evaluated on streptozotocin (STZ) induced diabetic mice. Blood glucose on *Opuntia* treated groups was significantly reduced *versus* the control group, likewise body weight increased compared to a slight decrease in mice from the control group [37].

In another study, the effect of a complex formed by 65% nopal (*O. ficus-indica*) and other Asian medicinal plants on blood glucose metabolism in *db/db* mice was analyzed [78]. In brief, food intake was reduced after ingestion of this complex and a significant increase in total water intake was observed. In addition, this complex exerted an effect on fasting glucose levels, which decreased after 2 weeks of feeding and even more after 4 weeks of nopal feeding; pancreatic islet integrity of mice was also improved and pancreatic cell proliferation increased significantly mainly in β-cells of the pancreatic islets followed by insulin secretion induction, elevating plasma insulin levels and glucose tolerance. The authors assumed that the hypoglycemic effects of this complex may operate differently from conventional diabetic drugs such as sulfonylureas [78].

Table 3. Diseases and health benefits related to nopal cladodes and fruits consumption.

Disease	Cultivar	Experimental Model	Results	References
Type 2 diabetes	*O. dillenii*	STZ induced diabetic mice	Reduced levels of blood glucose levels, total cholesterol, triglycerides, plasma urea nitrogen and malondialdehyde. Increase of hepatic glycogen levels, high density lipoprotein cholesterol levels and hepatic superoxide dismutase, and glutathione peroxidase activity.	[80]
	O. streptacantha	STZ induced diabetic mice	Reduced plasma glucose levels	[2]
	O. humifusa	STZ induced diabetic mice	Significant lower fasting glucose levels as well as of lower serum total cholesterol and low density lipoprotein (LDL) cholesterol were observed. Higher high density lipoprotein (HDL) cholesterol levels, lower serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations. A significant increase in relative β-cell volume was also detected.	[81]
	O. ficus-indica	Zucker (*fa/fa*) rats	Attenuation of hepatic steatosis by nopal consumption was accompanied by a higher serum concentration of adiponectin and a greater abundance of mRNA for genes involved in lipid oxidation and lipid export and production of carnitine palmitoyltransferase-1. Hepatic reactive oxygen species and lipid peroxidation biomarkers were significantly lower in rats fed with nopal compared with the control.	[89]
Cancer	*O.* spp fruit extract	Ovarian epithelium cells (IOSE), ovarian cancer cell lines OVCA420, SKO3, the HPVEG immortalized cervical epithelium cell line TCL-1; cervical cancer cell lines, HeLa and Me180; and bladder cancer cells UM-UC-6, T24	Prickly pear solution induced apoptosis in all three cancer cell lines tested. In cancer cell lines the strongest effect of apoptosis induction was found in cervical cell.	[98]
	O. humifusa	MCF-7 human breast cancer cells	The nopal extract significantly decreased viable cell numbers, in a concentration dependent manner. A G1 arrest in MCF-7 cells was induced as well.	[101]
	Nine nopal cultivars	Mammary (MCF-7), prostate (PC3), colon (Caco2) and hepatic (HepG2); normal fibroblast (NIH 3 T3) was used as control	Prostate cancer cells proliferation was diminished by the Rastrero cultivar and it was also effective against all four cancer cell lines investigated. Colon cancer cells proliferation was affected by the use of Gavia cultivar juice, and it also affected prostate and hepatic cancer cell growth. *O. robusta* and *O. rastrera* juices were the only samples capable of diminishing the viability of hepatic cancer cells.	[102]
	O. humifusa	U87MG human glioblastoma cells	Hexane soluble fraction obtained from fruits, cladodes and roots was effective on U87MG cells decreasing their proliferation by 49, 55 and 52%, respectively.	[107]
Overweight	Litramine IQP G-002AS natural fiber complex with nopal	125 overweight and obese adults	More IQP G-002AS fed subjects (IQ group) lost at least 5% of their initial body weight compared to placebo. These results suggest that the natural fiber complex Litramine IQP G-002AS is effective in promoting weight loss.	[78]
Peptic ulcer	*O. ficus-indica f. inermis* flowers	Etanol ulcerated rats	Animals pre-treated with nopal flower extract (250, 500 and 1000 mg/kg) and ranitidine exhibited a dose-dependent reduction of ethanol-induced gastric damage with a rate of 50.34, 86.51, 94.43 and 96.59, respectively.	[123]

Another possible mechanism for the antidiabetic effect exerted by nopal plants could be related to their polysaccharide content. Some studies have already revealed that the presence of compounds such as arabinose, xylose, fructose, glucose, galacturonic acid and rhamnose exhibits various functional properties, among them protective effects against H_2O_2 induced damage, free-radical scavenging, anti-inflamatory and antitumor activity, blood lipid lowering effects and wound-healing activity [79, 80]; all of them related in one way or another to the onset of type 2 diabetes. With these previous results in mind, Zhao *et al*. [80] studied the effect of *O. dillenii* polysaccharides (ODP)-la on type 2 diabetes in STZ induced diabetic mice. According to their results, three kinds of polysaccharides were isolated and administered to the mice during 3 weeks. This resulted in a marked decrease in food and water intake, fasting blood glucose levels, total cholesterol, triglycerides, plasma urea nitrogen and malondialdehyde (MDA); a significant increase of mice body weights, hepatic glycogen levels, high density lipoprotein cholesterol levels and hepatic superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activity were also observed, all of them being positive biomarkers of this disease. The proposed mechanism by which polysaccharides might have a positive effect on ameliorating these biomarkers levels is based on the fact that nopal polysaccharides can protect the liver from peroxidation damage and maintain tissue function, improving sensitivity and response of target cells to insulin [80].

Traditionally, in Mexico nopal cladodes are used as a liquefied blend against many diseases but most importantly to ameliorate diabetes effects. In this sense, a study was conducted to elucidate if this liquefied extract and even more a filtered extract from *O. streptacantha* could have any antidiabetic effect on STZ diabetic rats [2]. At the same time, another hypothesis emerges to explain the possible mechanism of action of nopal cladodes against diabetes, suggesting that this effect is independent of mucilage content which was demonstrated by testing the filtrated extract without fibers and pectins, contradicting the accepted theory that overall antidiabetic effect of nopal is caused mainly because of its high fiber and pectin content which may decrease carbohydrate absorption [2].

On the other hand, other researchers still support the idea that the antidiabetic effect of nopal is caused by high fiber and pectin content. This was evaluated using *O. humifusa* stems on STZ induced diabetic mice [81]. According to their results, no effect was observed on daily water intake, food intake or food efficiency. Significant lower fasting glucose levels, serum total cholesterol and low density lipoprotein (LDL) cholesterol levels were observed in nopal treated animals. Similarly, treated animals presented higher high density lipoprotein (HDL) cholesterol values, lower serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations. A significant increase in relative β cell volume was also detected. Thus, this action was attributed to high dietary fiber content and possibly other carbohydrate components in *O. humifusa* which can act on a similar way of other high-fiber containing foods [81].

Obesity and ectopic fat deposition are major risk factors for many diseases ranging from insulin resistance to type 2 diabetes and atherosclerosis [82]. In this sense, increasing numbers of elderly individuals with diabetes look for natural means to manage their disease to reduce diabetes-related complications. The American Diabetes Association recommends a goal of 25 to 35 g dietary fiber daily for a healthy diet. Fiber studies show decreased glucose concentrations and decreasing all-cause mortality. Some studies on *Psyllium* fiber have shown to be associated with lower mean daily glucose concentrations, lower postmeal glucose concentrations, fewer hypoglycemic events, and lower insulin concentrations in people with diabetes mellitus [83].

Nopal is considered among the high-fiber containing foods and one of its traditional uses in some countries is for weight loss or its control, because the benefits derived from nopal cladode consumption results in managing the diabetes related complications as well as helping to avoid the onset of a pre-diabetic state. From this point of view, a recent study claims to provide evidence on the use of nopal fiber as a weight loss agent. A proprietary natural fiber complex (Litramine IQP G-002AS) derived from *O. ficus-indica*, and standardized on lipophilic activity, has shown in preclinical and human studies to reduce dietary fat absorption through gastrointestinal (GI) fat binding [84]. Thereupon, the next phase was to analyze the efficacy and safety of IQP G-002AS in body weight reduction. One hundred twenty-five overweight and obese adults participated in the study. After a 2-week placebo run-in phase, subjects were randomized to receive either 3 g/day of IQP G-002AS (IQ) or a placebo. The primary endpoint was changes in body weight from baseline; and secondary endpoints included additional obesity measures and safety parameters.

Average body weight change from baseline was 3.8 ± 1.8 kg in IQP *vs* 1.4± 2.6 kg in placebo. A minimum of 5% body weight loss was observed in IQP subjects when compared to placebo. In addition, the subjects treated with IQP exhibited a significant reduction in body mass index, body fat composition and waist circumference. No adverse reactions to IQP formulation were found; in brief, the natural fiber complex Litramine IQP G-002AS exerts an effect on weight loss reduction [84]. Therefore, the use of nopal for weight loss could have a positive secondary effect, reducing the initiation of obesity, which is becoming a major health problem around the globe and that derives in other major problems such as diabetes, hypertension, osteoarthritis and heart disease [41, 45, 81].

Oxidative stress is implicated in major health problems such as insulin resistance in type 2 diabetes [85]. In other words, high production of reactive oxygen species (ROS) might trigger the development of type 2 diabetes, which results in accelerated rate of apoptosis in growth arrested cells [86]. Another parameter associated with diabetic patients is the low levels of plasma adiponectin which can also be a result of high ROS generation [87]. ROS production in adipocytes is associated with insulin resistance and adiponectin serum levels alterations and the subsequent inflammatory response [88]. Wherefore, several if not all *Opuntia* species contain high amounts of phenolic compounds with proved antioxidant properties. A research group reported that *O. ficus-indica* attenuates hepatic steatosis and oxidative stress in obese Zucker (*fa/fa*) rats [89]. Many health problems such as obesity, insulin resistance and oxidative stress have been linked to non-alcoholic fatty liver disease. On the other hand, the positive effects of consuming nopal are related to its antioxidant activity and ability to improve biomarkers of metabolic syndrome. Therefore, when obese rats were fed with a diet containing 4% nopal during 7 weeks, they exhibited a reduction in hepatomegaly and biomarkers of hepatocyte injury such as alanine and aspartate aminotransferases.

Even more, nopal consumption had a marked effect on hepatic steatosis with a higher serum concentration of adiponectin and a greater abundance of mRNA for genes involved in lipid oxidation/export and production of carnitine palmitoyltransferase-1. Nopal fed rats had significant lower levels of lipid peroxidation biomarkers and hepatic ROS compared to the control group. In addition, a lower postprandial serum insulin concentration and a greater liver phosphorylated protein kinase B (pAKT): AKT ratio in the postprandial state were observed in the nopal fed group. An increase in fatty acid oxidation and VLDL synthesis, and a decrease in oxidative stress and an improvement in liver insulin signaling are the main effects of nopal consumption on hepatic steatosis in obese Zucker (*fa/fa*) rats [89].

On the other hand, the effects on insulin function and glucose disposal have been attributed to the use of trivalent chromium in mammalian nutrition; furthermore, this element has been proposed to be important in mammals to maintain a balance in carbohydrate and lipid metabolism [90]. In this sense, some studies have validated such properties, finding a positive effect of chromium on fasting plasma glucose [91]. Evidence suggests that this single element may help in insulin signaling and improve systemic insulin sensitivity [92].

Chromium deficiency has been associated in acute and sub-acute syndromes reported in patients receiving total parenteral nutrition [93, 94]. Hence, this chromium deficiency was related to the development of diabetes and atherosclerotic disease [95, 96]. Therefore, the antihyperglycemic activity attributed to the presence of chromium (III) in nopal has also been investigated [97]. According to this study, nopal cladodes and pulp fruit extracts of *O. dillenii* were used to evaluate their effects on blood glucose concentration and glycemic curves of Sprague-Dawley rats. After acute administration, no significant differences were observed in the glycemic curve among nopal cladodes, fruit pulp and the control group. Besides, a slight decrease of fasting blood glucose was observed after 8 days of daily intake of nopal cladodes extract. Therefore, the possible role of Cr (III) present in high amounts in these vegetable foods was suggested to explain their antihyperglycemic activity [97].

6.3. Anticancer Activity

In 2005, a research group investigated the potential effect of nopal fruit extract on different cancer cell lines, namely ovarian epithelium cells (IOSE), ovarian cancer cell lines OVCA420, SKO3, HPVEG, the immortalized cervical epithelium cell line TCL-1; cervical cancer cell lines, HeLa and Me180; and bladder cancer cells UM-UC-6, T24 [98]. Cervical cancer cells were the most sensitive compared with ovarian and bladder cancer cells.

The inmortalized cervical epithelium cells and cervical cancer cells were inhibited in a range of 40 to 60% when 1% cactus pear solution was used. Similarly, an effect was observed on ovarian cancer cells, where the use of 5% of this cactus pear solution exerted growth inhibition in IOSE and OVCA420 cells, but a 10% solution was required to inhibit SKOV3 cell growth. In addition, bladder cancer cell growth was affected by the use of this nopal fruit extract; a 50% inhibition of these cancer cells was observed when a 1% concentration of this fruit extract was used. Therefore, cactus pear solution effect was dose and time dependent. In this sense, the IC_{50} (the concentration causing 50% cell death) in cervical and bladder cancer cells after a 5 day treatment with cactus pear solution was less than 2%. On the other hand, the IC_{50} varied from 0.8 to 1.5% in all cervical cancer cell lines tested; nevertheless, in bladder cancer cell lines UM-UC-6 and T24, the IC_{50} was 0.9 and 1.3%, respectively. Conversely, in IOSE, OVCA420 and SKOV3 ovarian cells the IC_{50} was 2, 0.8 and 8%, respectively. In brief, in all three cancer cell lines tested, the prickly pear solution induced apoptosis; the strongest effect was observed in cervical cancer cells against apoptosis induction. In other words, the apoptosis cell population increased by more than 50% when using 25% of cactus pear extract compared with untreated cells. The most sensitive effect was noticed in immortalized cervical epithelium cells, where apoptotic cells increased over 70% after treatment. This effect was slightly lower in ovarian and bladder cancer lines; for instance, apoptosis induction increased in cactus extracts from 40 to 50% in OVCA420 and SKOV3 cells, respectively. Wherefore, cactus pear extracts affected cell cycle in cancer cell lines starting at a 5% concentration and the effect of cactus on cell cycle was dose dependent, as revealed by the DNA content and cell cycle analysis. Finally, tumor growth was inhibited by the use of prickly pear cactus solution in nude mice compared to untreated animals or the ones treated only with water. Cactus pear solution effect on tumor growth inhibition in relation to tumor size was compared with N-(4-hydroxyphernyl)-retinamide (4-HPR), a chemopreventive agent used in other clinical trials such as in ovarian and bladder cancer [99]; in other words, the cactus pear inhibitory effect was similar to the one exerted by 4-HPR. In brief, the high antioxidant content of prickly pear fruits may be responsible for the observed effect, still, the exact mechanism for nopal fruits in cancer prevention was unclear, and this current study showed that cactus pear alter, the expression of certain genes related to cell growth and apoptosis [98].

One of the most dangerous gynecologic cancers is epithelian ovarian cancer. It presents a high mortality and recurrence rate (50-80%) despite surgery and aggressive treatments. A low percentage of patients (20-30%) survive 5 years. This situation is mostly due to the lack of adequate tools for an early diagnosis of cancer development and lack of effective treatment of the disease [67]. The drugs used to treat this disease such as the synthetic retinoid 4-HPR, require long-term administrations. The preventive effect will disappear once the treatment stops [99]. Hence, the use of drugs with less toxicity or the use of natural products to prevent cancer is urgently needed [100].

As we have described previously in this review, nopal fruits contain a high level of antioxidant compounds; and as it was stated earlier, they exhibited beneficial effects on different cancer cell lines [98]. Five years later, this same research group described the possible molecular mechanism for the prickly pear fruit effect on apoptosis of cancer cells [100]; thus, OVCA420 and SKOV3 were treated with 5 and 10% of prickly pear cactus fruit mixture extract, respectively.

ROS accumulation was observed in immortalized cells 2 days after being treated with 10% of the extract, at the same time cancer cells that were cultured with 5% and 10% experienced a dramatic ROS increase. Cancer cells exhibited a higher level of DNA fragmentation, along with other markers of disease such as the disturbed expression of ROS sensitive (NF-kB, c-jun/c-fos) and apoptotic related (Bax, Bad, caspase 3, Bcl2, p53 and p21) genes. NF-kB and p/SANPK/JN expressions decreased after 3 days of treatment; even so p-AKT was up-regulated. Apoptosis in cancer cells was significantly induced by the use of nopal fruit extract. A significant inhibitory effect of the extract was observed in cancer cell growth by the accumulation of ROS at an intercellular level possibly activating a cascade of reactions that lead to apoptosis [100].

Due mainly to the elevated phytochemical content of nopal cladodes and fruits, its potential use as an anticancer agent appears to be rising fast over the last decade. In this sense, *O. humifusa* fruits have been analyzed for their polyphenol and flavonoid contents as well as for their anti-carcinogenic effects on human breast cancer. As expected, *O. humifusa* showed high concentrations of total polyphenol as well as flavonoid. Effects of the water extracts of *O. humifusa* on the proliferation, G1 arrest and apoptosis of the MCF- 7 human breast cancer cells were also examined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays; G1 cycle arrest and apoptotic effect of *O. humifusa* were analyzed by flow cytometry [101].

When MCF-7 cells were treated with different concentrations of hexane, ethyl acetate and water extracts of *O. humifusa* fruits, the last samples significantly decreased viable cell numbers in a concentration dependent manner. A G1 arrest in MCF-7 cells was induced as well. The overall results indicate that water extracts of *O. humifusa* fruits might inhibit MCF-7 human breast cancer cell proliferation and induce G1 arrest [101].

In another study, nine 9 prickly pear juices were used to analyze their phenolic composition, antioxidant capacity and *in vitro* cancer cell citotoxicity on four different mammalian cell lines that were used for the analyses, namely mammary (MCF-7), prostate (PC3), colon (Caco2) and hepatic (HepG2) cells; normal fibroblast (NIH 3 T3) was used as control [102].

The prostate and colon cancer cell viability were the most affected, in particular by the Moradillo fruit juice. Nevertheless, this cultivar juice also diminished the growth of fibroblast control cells. Prostate cancer cells proliferation was diminished by the Rastrero cultivar; in addition, this fruit juice was effective against all four cancer cell lines investigated, which could correlate with the high antioxidant capacity observed, comparable to pomegranate juice. On the other hand, the colon cancer cells proliferation was affected by the use of Gavia cultivar juice, and affected prostate and hepatic cancer cell growth. *O. robusta* and *O. rastrera* juices were the only ones capable of diminishing the viability of hepatic cancer cells. Nevertheless, the authors suggest to investigate which are the anticancer compounds with higher activity and if the *in vitro* results observed correlate with *in vivo* studies [102].

Mycotoxins are natural occurring food contaminants. They may cause severe health problems such as endometrial adenocarcinomas and hyperplasia [103] as well as severe liver lesions that subsequently may develop hepatocarcinoma [104, 105]. Nopal cladodes (*O. ficus-indica*) were fed to Balb/c mice affected by mycotoxin exposition. Treatment with nopal cladodes restored favorably the number of polychromatic erythrocytes (citotoxicity evaluation) at a very low dose (25 mg nopal cladodes/kg bw). At increasing nopal concentrations, a significant prevention *versus* zearalenone genotocixity was observed. DNA fragmentation test in treated animal cells revealed a significant restoration of DNA after cladode administration in a dose dependent manner. The authors attributed this effect to the presence of multiple antioxidant compounds [106].

Glioblastoma, is one of the most common type of malignant tumors in the neurological system; thus, Hahm *et al.* [107] evaluated the effect of *O. humifusa* extracts over U87MG human glioblastoma cells. Hence, different plant parts (fruits lacking seeds, seeds, roots and cladodes) were used for this study from which hexane and water extracts were obtained. The extracts from this nopal species (*O. humifusa*) exerted a dose dependent effect on cell proliferation, arresting and suppressing its growth. In a similar way, the hexane soluble fraction obtained from fruits, cladodes and roots was effective on U87MG cells decreasing their proliferation by 49, 55 and 52%, respectively. After analyzing the cell cycle of the glioblastoma cells, a marked increase in G1 phase cells was observed, which was followed by a decrease in the S and G2/M phase cells indicating that the water partitioned fraction of *O. humifusa* might exert an effect in U87MG cell arrest in the G1 phase. Finally, no significant differences were observed in the number of apoptotic cells, yet non apoptotic cells increased in 2% [107].

Hepatocellular carcinoma (HCC) or primary liver cancer is caused mainly by infections (hepatitis B and C viruses). Other causes may include fungi toxins known as aflatoxins; being aflatoxin B1 (AFB1) the most potent among them, which exhibits hepatotoxic and hepatocarcinogenic properties. Nopal cladode extract was evaluated on Balb/c mice, analyzing the effects on genotoxicity, oxidative stress and cell death pathway induced by a sub-chronic treatment with AFB1 [108]. An increase in malondialdehyde in mice liver was observed after the exposure to AFB1; pre- and post-administration of nopal cladode extract along with AFB1 significantly reduced this effect. Early markers of oxidative stress were monitored as well; cladode extracts reduced significantly the levels of Hsp 70 and Hsp 27, demonstrating the efficacy of this plant extract against oxidative damage which is most likely due to its high antioxidant content.

Similarly, the antiproliferative effects of betanin, a pigment of the betacyanin type that has been isolated from *O. ficus-indica* fruits, were evaluated on human chronic leukemia cell line (K562) [109]. In this sense, the betanin nopal extracts exerted an effect in the proliferation of K562 cells, in a dose and time dependent manner with an IC_{50} of 40 mM. Additional studies revealed an effect on apoptotic markers such as DNA fragmentation patterns typical of apoptotic cells, as well as chromatin condensation, cell shrinkage and membrane blebbing. In addition, an effect of betanin treatment was observed in the induction of cytochrome c release into the cytosol, cleavage of poly (ADP) ribose polymerase (PARP), Bcl-2 down-regulation and reduction in membrane potentials. Even more, confocal microscopic studies revealed that treated cells suggest the entry of betanin into them. Consequently, these results demonstrate that betanin, a nopal fruit natural pigment, induces apoptosis in K562 cells throughout a central pathway that is mediated by the release of cytochrome c from mitochondria into the cytosol and PARP cleavage [109].

6.4. Other Nutraceutical Effects Exerted by Nopal

One of the most common metabolic bone diseases is osteoporosis which reduces bone strength, elevating the risk of fractures. The stage where peak bone mass is attained, differs due mainly to factors such as bone type; however, the peak bone mass is reached at about 25 years old, and after this average age, this mass tends to decrease gradually [110]. Interestingly, a relation between a higher risk of fracture and a bone mass decrease has been observed in obese children; this phenomena is mainly due to the fact that bone mineral density (BMD) and bone mineral content (BMC) are lower in obese children than in their normal counterparts [111, 112].

Nonetheless, an appropriate mineral intake such as Ca^{2+} and Mg^{2+} in the growth period can increase BMD and BMC, thus reducing the risk of fracture, and ultimately, the risk of developing osteoporosis or fractures in elder individuals [113, 114, 115]. In this sense, the effect of *O. humifusa* supplementation on bone density and related hormone secretion in growing male rats was investigated [3].

The rats in the control group were given a control diet and those in the experimental group were given 5% *O. humifusa* added to the control diet for 8 weeks. As a result, animals treated with nopal exhibited a higher osteocalcin level than the control group. Osteocalcin is a major non collagenous protein present in the bone matrix and it is considered a bone formation marker. This protein is synthesized by and released from osteoblasts [116]. This marker is also correlated with Ca^{2+} levels having this molecule high affinity for calcium and promotes absorption of hidroxiapatite (biological form of calcium in the body) in the bone matrix, leading to bone mineralization [117]. In this same way, intake of sufficient minerals such as Mg^{2+} and Ca^{2+} promotes an increase in ostecalcin [118]. Thereupon, *O. humifusa* with a high Mg^{2+} and Ca^{2+} content might have a positive effect on bone formation. At that same time, the parathyroid hormone in the experimental group was significantly lower than that of the control group. Serum $Ca^{2+}$is well known to be regulated by this hormone and vitamin D. When serum Ca^{2+} is low, increased parathyroid secretion stimulates calcium mobilization through bone resorption by osteoclasts [119]. Hypocalcemia can be caused by an inadequate intake in the diet, which leads to osteoporosis, due to an increased bone resorption as well as decreased bone mass [120]; consequently, repletion of calcium supplementation might have a beneficial effect on bone metabolism.

Likewise, it has been shown that 1000 mg/day calcium and 800 IU/day vitamin D supplementation significantly reduces parathyroid levels and increases bone mineral density in the context of Mg^{2+} deficiency in females over the age of 65 [121].

In this sense, *O. humifusa* can provide sufficient Ca^{2+} and might exert beneficial effects on the structural strength of bone; furthermore, it can arrest parathyroid secretion suppressing calcium mobilization from bones. Correspondingly, a reduction of bone formation and increase of bone resorption might be caused by the impairment of mineral intake as well as by Mg^{2+} and Ca^{2+} deficiency and could play a negative role in bone growth. According to the results observed, the supplementation with 5% of *O. humifusa* resulted in 2.2 times the level of Mg^{2+} and 1.3 times the level of Ca^{2+} in the experimental group, in relation to the control group. The mineral rich *O. humifusa* supplement likely exerted positive effects on bone metabolism through the suppression of parathyroid secretion as well as increased intestinal Ca^{2+} absorption due to the activation of vitamin D in kidney. In spite, of the fact that *O. humifusa* supplementation might exert positive effects on bone metabolism through the mechanisms described previously in this section, much more studies are needed to elucidate the role of this nopal species and its dietary supplementation on bone metabolism [3].

For more than a century, the peptic ulcer has been one of the most frequent causes of surgery. This disease presents high morbidity and mortality rates [122]. The two main causes of gastric ulcer are: infection with *Helicobacter pylori* and the use of non-steroid anti-inflammatory drugs [123]. Even more, excessive ethanol consumption may induce gastric ulcers [124] by causing vascular damage and gastric cell necrosis, which leads to ulcer formation [125]. The free radicals can exert a harmful effect on gastric cells; to cope with this, these cells present an enzymatic antioxidant defense system. But excessive generation of free oxygen radicals, resulting from ethanol consumption, enhances the lipid peroxidation process and attenuates the activities of the antioxidants defense system [126].

Adverse reactions are observed due to long term utilization of synthetic drugs to treat gastric ulcers [127]. Traditional use of *O. ficus-indica f.* inermis flowers in Tunisian medicine is based on their diuretic activity, their capacity to expulse renal calculus and to cure ulcer. Hence, a study was conducted to investigate if a 50% methanolic extract from the flowers of this nopal species might exhibit antioxidant activity and whether it might prevent ethanol induced ulcers [59]. The total phenolic content was determined (159.76 ±0.32 mg GAE/g of extract). The radical-scavenging activity of nopal flower extract was tested using a methanolic solution of the "stable" free radical, DPPH and compared with ascorbic acid and (+)-catechin used as standards. The EC_{50} values obtained showed that the radical-scavenging activity of nopal flower extracts (EC_{50}=147±0.9 µg/ml) appeared significantly lower than that of (+)-catechin and ascorbic acid. So, the reducing potential of this flower extract increased along with an increase of concentrations and reached a maximum at 800 µg/ml. Consequently, these values were much lower than those of (+)-catechin and ascorbic acid. The maximum inhibition effect of the nopal flower extract on peroxide formation with linoleic acid emulsion increased in a dose dependant manner and reached the maximum at 200 µg/ml; the effective EC_{50} of nopal flower extract that can inhibit linoleic acid peroxidation (EC_{50}=140 ± 2.07) appeared significantly lower than that of (+)-catechin.

Animal groups pre-treated with nopal flower extract (250, 500 and 1000 mg/kg) and ranitidine (50 mg/kg) exhibited a reduction on gastric damage caused by ethanol, in a dose dependent manner. The rate of such reductions was the following: 50.34, 86.51, 94.43 and 96.59%, respectively. The reduction of the ulcer lesion, in the groups described above, was accompanied by a significant decrease of ulcer index in all groups in a dose dependent manner compared with the ethanol group. In this same way, nopal flower extract pretreatment greatly lowered the mucosal MPO (myeloperoxidase) activity, MDA, PC (carbonylated proteins) and restituted the activities of antioxidant enzymes such as (SOD), catalase (CAT) and GSH-Px in the ethanol ulcerated rats [59].

In a second approach, this same research group evaluated the efficacy *of O. ficus-indica f. inermis* fruit juice on reversing oxidative damage induced by chronic ethanol intake in rat erythrocytes [4]. Phenolic and flavonoid contents were assessed as well. HPLC analysis revealed high concentrations of phenolic acids and flavonoids in prickly pear juice. Ethanol treatment markedly decreased the activities of erythrocyte SOD, CAT, GSH-Px and the level of reduced glutathione (GSH). Changes in the erythrocytes antioxidant ability were accompanied by enhanced oxidative modification of lipids (increase of MDA level) and proteins (increase in carbonyl groups). Interestingly, pre-administration of either 2 ml/100 g bw or 4 ml/100 g bw of prickly pear juice to ethanol-intoxicated rats significantly reversed decreases in enzymatic as well as non-enzymatic antioxidants parameters in erythrocytes. Likewise, the administration of the juice significantly protected lipids and proteins against ethanol-induced oxidative modifications in rat erythrocytes. The beneficial effect of prickly pear juice can result from the inhibition of ethanol-induced free radicals chain reactions in rat erythrocytes or from the enhancement of the endogenous antioxidants activities [4].

On the other hand, the consumption of Se in high or moderate ways has an impact on cancer mortality which was significantly lower for total cancers such as lung, colon and rectum, bladder, esophagus, pancreas, breast, ovary and cervix in some counties among the US. As a matter of fact, the US National Academy of Sciences recommends ingestion of 55 µg Se per day and the World Health Organization recommends 40 µg Se per day. A supplementation of organic Se up to 200 µg per day is considered non toxic in adults. Some of the benefits associated with the consumption of Se in the diet are related with an improvement of immune responses, a reduction in viral or bacterial infections, reduction in the incidence of breast, prostate, lung and liver cancers, and reduction on the onset of many heart diseases. Interestingly, it has been associated with a reduction on Alzheimer´s and associated dementias [128, 129].

The organic form of Se, selenocysteine, is a fundamental part of an antioxidant enzyme selenogluthatione peroxidase which is present in animals and humans; this enzyme is very important in free-radical scavenging [130]. In this sense, a study was conducted in nopal cladodes and fruits from salt and boron tolerant cultivars, in order to analyze mineral content, total phenolics, vitamin C and the free radical scavenging of the antioxidant compounds found in fruits and cladodes of different nopal cultivars, which were grown in agricultural drainage sediment. According to the results found in this study, the Se accumulation pattern differed among the cultivars. Interestingly, the average Se content was found higher in fruits (38 fold) and in cladodes (170 fold) than in normal soil grown cultivars. The overall accumulation rate of both nopal fruits and cladodes was 3.9 and 15.4 µg Se/g dw, respectively, when they were grown in drainage sediment. In this sense, these results demonstrate that nopal can constitute a novel source of Se and other important mineral nutrients.

Alike, the phenolic concentrations found in nopal fruits where higher for those grown in drainage sediment than thoes grown under normal soil conditions. On the other hand, the cultivation of nopal in normal soil conditions and drainage sediment soil did not exerted any effect in the vitamin C content of prickly pear fruits [131].

The effect of a methanolic extract of *O. ficus-indica* on neuronal injury was examined [132]. A neuroprotective effect was observed against neuronal injury induced by N-methyl-D-aspartate (NMDA), kainite (KA) and oxygen-glucose deprivation (OGD) in cultured mouse cortical cells, in a dose-dependent manner. In addition, the *O. ficus-indica* butanol fraction significantly reduced NMDA, and induced delayed neurotoxicity by 27%. The *in vivo* assay was conducted on gerbils which were treated with methanolic extracts every 24 h, 3 days or during 4 weeks and after the last dose an ischemic injury was induced. In addition neuronal cell damage in the hippocampal CA1 region was evaluated quantitatively at 5 days after isquemic injury. Neuronal damage was reduced by 32 and 36% after gerbils were fed with doses of 4 g/kg (3 days) and 1.0 g/kg (4 weeks). In brief, the use of *O. ficus-indica* might help in alleviating the excitotoxic neuronal damage induced by global ischemia.

The effect of nopal (*O. ficus-indica* cultivar Saboten Makino) on allergy inhibition has been evaluated as well [133]. Thereupon, the inhibition effect of a glycoprotein isolated from this nopal cultivar was evaluated on the activities of allergy mediators in compound 48/80 which stimulated mast cells. The overall anti-allergy potential of nopal was analyzed on ICR mice (*in vivo* study) and on RBL-2H3 cells (*in vitro*). Some allergy related factors were analyzed, and finally the authors suggested that the isolated glycoprotein is a natural compound that might block the antiallergenic signal transduction pathway [133].

On the other hand, the effects of nopal on long term memory have been studied. According to Kim *et al.* [134], the use of an n-butanolic extract of *O. ficus-indica* cultivar Saboten Makino during 7 days on mice increased significantly the latency time in the passive avoidance task relative to vehicle treated controls. In addition, other markers were increased after 7 days of butanolic extract administration. These markers were: the expression levels of brain-derived neurotrophic factor (BNDF), the phosphorylated extracellular signal-regulated kinase (pERK) ½ and the phosphorylated cAMP response element binding protein (pCREB). Consequently, the administration of this butanolic extract might enhance long term memory; even more, it was suggested that this effect can be mediated in part by the ERK-CREB-BNDF signaling and the survival of immature neurons.

7. APPLIED BIOTECHNOLOGY IN THE *OPUNTIA* GENUS

As we have mentioned in previous sections of this review, nopal has gathered much attention from the scientific communities around the world due mainly to its nutraceutical potential; additionally, other investigations are leading research on micropropagation and other *in vitro* tissue culture techniques, for many purposes, such as massive production of ornamental cultivars of nopal, as well as for other types of cultivars. Likewise, some tissue culture techniques are strictly linked to their further use in regeneration protocols after genetic transformation. In this sense, genetic transformation techniques such as *A. tumefaciens* or biolistics have been described. Unfortunately, in the case of biolistics no complete regeneration of transformed plant has been achieved so far. Finally, a recent contribution of nopal in biotechnology is for the isolation of enzymes with commercial value such as cellulases, xyloses and cyclodextrin glycosyltransferases (Fig. **1**).

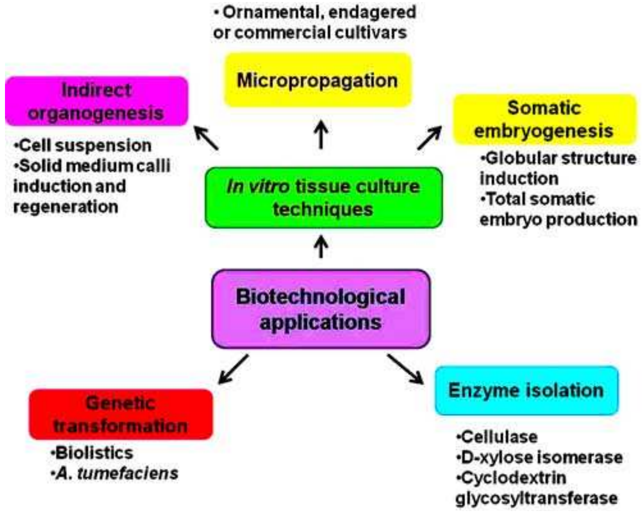

Fig. (1). Different biotechnological application for *Opuntia* spp. developed during the last decades.

7.1. *In vitro* Tissue Culture

De novo shoot organogenesis relies on somatic cell totipotency (the capacity to regenerate *in vitro*, the entire plants from single somatic cells) and constitutes the most common way to obtain *in vitro* plant regeneration. It has been demonstrated that *in vitro* plant regeneration takes place by two main pathways: *de novo* organogenesis and somatic embryogenesis, both of which are dependent on plant growth regulator (PGR) perception, cell division and dedifferentiation to acquire organogenetic competence, organ initiation and development [135]. The classical finding of Skoog and Miller [136] showing the importance between the ratio of auxin and cytokinin is still the guiding principle of *in vitro* organogenesis. Wherefore, higher cytokinin content *versus* low auxin levels results in shoot organogenes, meanwhile, when the opposite situation takes place (high auxin, low cytokinin) root development is triggered [135]. Among common *in vitro* culture techniques, micropropagation has proved to be successful in different cacti genera, namely *Cereus, Equinocereus, Ferocactus, Mammillaria* and *Opuntia* [5]. Even more, for the *Opuntia* genus many propagation systems have been developed [6].

7.2. Micropropagation

One of the first reports on *Opuntia* plant micropagation was described in the late 1980´s in *O. amyclaea* where axillary proliferation was used [137]. The best response in terms of shoot development was observed using 6-bencilaminopurine (BA), a cytokinin, after 25 days of culture where the best concentration was BA 10^{-5} M. Still, above these values the axillary shoot formation was inhibited and in contrast, in the absence of BA the number of axillary buds formed was significantly reduced. Rooting was observed with the aid of different IBA (indole-3-butyric acid) concentrations where roots appeared after 7 days and even more auxin application was not crucial for root development since they started to form in spite of the absence of auxin [137].

Another study conducted on *O. ellisiana* Griff revealed *in vitro* propagation conditions for this plant [138]. This *Opuntia* species is one of the slowest growing of all spineless nopal species, but one of its virtues is to present high water use efficiency (162 kg H_2O/kg dry matter). The areole explants were exposed to BA and IBA, where the highest shoot formation was observed; hence, the plantlet survival was 100%.

Micropropagation has been proved to be of much use in commercially cultivated materials, which was demonstrated with the work of Garcia-Saucedo *et al.* [5] where three main cultivated materials (Milpa Alta, Villanueva and Blanco sin Espinas) were successfully micropropagated. Wherefore, all materials responded well at 0.5 μM BA and rooting was observed utilizing 5.5 μM IBA. Consequently, this is an interesting application of *in vitro* culture techniques on *Opuntia* genotypes used as vegetable sources for human consumption. On the other hand, *in vitro* micropropagation can help in the massive production of ornamental nopal species with commercial value. Such application was described for *O. lanigera* Salm-Dyck [139]. In brief, different variables were analyzed including explants orientations, type of cytokinin and concentrations, as well as the spraying of GA_3 (giberellic acid) after transplantation. The highest shoot length was observed when applying DAP [6(γ,γ-dimetilalilaminopurine)] *versus* kinetin and BA. Better yet, the application of GA_3 after transplantation increased spine-hair length.

7.3. Somatic Embryogenesis

Early studies on the establishment of somatic embryogenesis protocols for the *Opuntia* genus were described by Pinheiro da Costa *et al.* [140]. Seeds were disinfested and a mechanical isolation of zygotic embryos was done under sterile conditions; 2,4-D (dichlorophenoxyacetic acid) was used alone or in combination with kinetin or abscisic acid (ABA). The globular structure obtained did not display the typical characteristics of somatic embryos. Nevertheless, with the addition of kinetin and 2,4-D the proper globular structure induction was achieved. Consequently, the use of kinetin seems to be critical for the development of these structures. In another report, somatic embryogenesis and plant regeneration for nopal was achieved [141]. Shoot apices were isolated from *in vitro* grown shoots, and transferred to induction medium that was supplemented with picloram (4-amino-3,5,6 trichloro-2-pyridinecarboxylic acid). Picloram and BA were used for embryo maturation and germination. Embryo structures appeared after 2 weeks as globular-shaped structures having a white opaque color, and after 30 days they differentiated to more advanced developmental stages. Picloram and BA resulted in the maturation and plant recovery of embryo clusters; the overall conversion frequency was 12.5%.

7.4. Indirect Organogenesis

One of the first reports concerning indirect organogenesis in nopal was described by Llamoca-Zárate *et al*. [142]. Cotyledon and hypocotyls sections were used as explants source. They were transferred to medium supplemented with different combinations and concentrations (2,4-D, kinetin and picloram). After incubation in darkness, the calli obtained were transferred to friable callus medium and grown in a cell suspension culture. Calli induction was observed between 14 and 21 days of culture. Finally, the authors suggested that a pre-requisite for calli induction was the use of picloram and casein hydrolysate in the culture medium. Yet, plant regeneration was not described.

In a more recent report, a regeneration protocol for *O. ficus-indica* (L.) cultivar Blanco sin Espinas by means of indirect organogenesis was described by Angulo-Bejarano and Paredes-López [6]. The explants were transferred to MS medium with different combinations of 2,4-D and BA. Calli induction and regeneration was achieved in medium with an almost 1:1 combination ratio between tested PGRs. New regeneration was observed when calli was transferred to MS medium plus BA; in addition, shoot elongation and rooting were observed in medium without plant regulators. One hundred percent acclimatization was observed among greenhouse conditions transferred plantlets with no differences with mother plants. According to these authors, this protocol can be used for plant regeneration after a genetic transformation event.

7.5. Genetic Transformation Events in Nopal

7.5.1. Particle Bombardment

Among the first attempts to achieve plant genetic transformation is the one by Llamoca Zárate *et al*. [143, 144] where the transient gene expression of two genes (*nptII* and *uidA)* was observed after particle bombardment on friable calli of nopal cell suspensions. The particle bombardment conditions used were 7.5 cm from flying disk to tissue and 1200 psi, and explants selection was done on 100 mg/L kanamycin. Accordingly, gene integration was analyzed by histochemical and photometric analysis for GUS activity. Nevertheless, no stable integration of the foreign genes was demonstrated and no transgenic plantlet regeneration was achieved. This same research group reported the transient gene expression on shoot apical meristems from nopal as well [144]. Three different plasmids were used for plant transformation. The conditions for the biolistic process were tungsten particles, 7.5 cm distance and a shooting pressure of 1200 psi. Successful delivery of foreign DNA was observed and GUS gene was successfully expressed in meristem cells. No blue staining cells were observed in control meristems. Finally, no stable transformation was reported.

Cruz *et al*. [145] reported particle bombardment in shoots apical meristems of nopal. These explants were bombarded with the pGA1 plasmid containing the *uidA* and the *Atahas* gene, which confers resistance to imazapyr. Biolistic conditions were used according to a previous report [146]. The transformation frequency obtained by the system reported here was 4.1%. More reliable studies are needed to confirm the foreign gene integration in the plant genome (Southern blot).

7.5.2. Agrobacterium tumefaciens

In this same way, a biological transformation process has been reported. Silos-Espino *et al*. [7] described an *Agrobacterium tumefaciens* genetic transformation protocol for nopal. Microinjection of a bacterial suspension was done on nopal areoles, the main meristematic tissue. To achieve regeneration, the resistant structures were dissected from the original explants to a fresh selective medium every 2 weeks for eight additional weeks; then, selected explants were transferred onto fresh regeneration medium supplemented with kanamycin. The transgenic nature of the explants was confirmed by PCR and Southern blot analysis. The overall transformation frequency was 3.2%. This is the first report of this nature in nopal and according to the authors it can be useful for nopal genetic transformation [7].

7.6. Enzyme Isolation and Characterization

Cellulase, also called endoglucanase, is a multi-subunit enzyme containing a catalytic core, cellulose binding domain and a flexible, heavy glycosylated linker region [147]. Nowadays, there is a growing interest and demand for renewable energy sources, which in turn has led to a demand on cellulose degrading enzymes, with potential application in the ethanol production [148]. Apart from the use in the biofuel industry, cellulases are also widely used in food industry processing; for instance, in coffee, as well as in the textile industry and in laundry detergents. The main obstacles for the use of cellulases at a commercial level are the low activity and elevated costs associated with the production of these enzymes [149]. Accordingly, there is growing demand fornew cellulose sources.

In this sense, Shyamala *et al*. [150] reported the identification of four endoglucanase temperature isoforms that were purified from the cladodes of *O. vulgaris*. The isoforms had an optimum catalytic activity at 30, 50, 70 and 90 °C, and their apparent molecular mass was 150, 20, 74 and 45 kDa, respectively. Two isoforms were thermostable and exhibited optimum activity at pH 4.5 and 7. Therefore, these enzymes were capable of working under acidic as well as under neutral conditions. In addition, thermostable enzymes tolerated higher temperatures with a longer half life to the catalytic function and inhibited microbial growth. Finally, the authors suggested that due to overall characteristics they might be employed in various industrial applications.

The isomerization between D-xylose and D-xylulose is reversibly catalyzed by an enzyme called D-xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5). This interesting enzyme can also act as a glucose isomerase converting D-glucose into D-fructose [151]. Hence, it is extensively utilized in the industrial production of high fructose corn syrup and ethanol from hemicelluloses [152]. Commonly known xyloses are from bacterial (*Streptomyces* spp. and *Bacillus* spp.) or fungal (*Aspergillus oryzae*) origin [153,154]; nevertheless, xyloses from plant origin have been isolated well [155]. With this in mind, Ravikumar *et al.* [156] reported the isolation of a thermophilic xylose isomerase from *O. vulgaris* which can be utilized in the production of high fructose corn syrup. In this way, two thermostable isoforms with an optimal activity at 70°C (T_{70}) and 90°C (T_{90}) were reported. This last isoform exhibited high efficiency under assay conditions by converting glucose to fructose. In view of this, the authors suggested that these overall properties exhibited by this enzyme make it a potential candidate to be applied in the high fructose corn syrup sweetener industry.

A 25% of the world enzyme market is represented by the amylases which are fully recognized industrial enzymes [157]. Amylases catalyze the conversion of starch and related polysaccharides into smaller polymers composed of glucose units [158]. The α-amylases (α-glucan-1-4-glucanohydrolases; EC. 3.2.1.1) hydrolyze the internal α-1,4 glycosidic links at random to produce less viscous solutions with lower molecular weight products [159]. The cyclodextrin glycosyltransferases (CGTases; EC 2.4.1.19) are members of the α-amylases family and convert starch and related substrates into cyclodextrins (CDs) through cyclization and an intramolecular transglycosylation reaction. These enzymes have potential applications for medical and analytical chemistry [160]. In this way, they are used in the baking industry since the incorporation of the enzyme into bread increases volume and delay the process of staling during storage [161]. Another application is the production of CDs (cyclodextrins). In fact, CDs have the capacity to encapsulate hydrophobic molecules with a potential use in the cosmetic, pharmaceutical, food and textile industries [162]. Normally, these enzymes are found in bacteria and Archaea [163] but there are no reports about plant CGTases. Ennouri *et al.* [164] reported the extraction and purification of an amylase from *O. ficus-indica* seeds. Accordingly, an increase in specific activity of 113 fold was observed. The apparent molecular mass of the enzyme is 64 kDa. This enzyme exhibits optimum activity at pH 5 and at 60°C. Under these conditions, the specific activity is 245.5 U/mg. The enzyme was activated by $Co^{2+}$and Mg^{2+} and strongly inhibited by Mn^{2+} and Fe^{2+}. The extracted enzyme belongs to the exo type of amylases and is classified as β-cyclodextrin glycosyltransferase, since it generates mainly β-cyclodextrin from starch. It shows high thermal stability and a wide range of pH stability, making it a promising prospect for industrial and food applications [164].

8. FOOD INDUSTRY APPLICATIONS

Nowadays, people ingest foods not just to cover their nutritional necessities; they also request healthy, natural and convenient foods with biological activity. Due to the increasing demand of convenient foods by consumers, several efforts are being carried out with the purpose of improving the functional or nutraceutical message of products by changing chemical composition, among other strategies.

In this sense, cladodes of *O. ficus-indica* have been used as a source of dietary fiber to replace wheat flour in the preparation of bakery products; dietary fiber enhances the physicochemical and technological properties of wheat flour and at the same time reduces the risk of degenerative diseases. Cakes made with blends containing 5% cladode flours did not differ significantly from the control. Thus, in terms of their handling properties, this cladode flour was incorporated in the formulation of cakes to increase their nutritional and nutraceutical value [165].

In the same year, Moreno-Álvarez *et al.* [166] evaluated the partial substitution of wheat flour by cactus pear stem flour from *O. boldinhgii* Britton et Rose. In this research, the formulations of blends composed of wheat flour (WF) and cactus pear stem flour (SF) with 95 and 5%, and 90 and 10%, respectively, had the best baking behavior. They showed a high acceptability in color, odor, flavor and texture. The agro-industrial exploitation of these products may allow the use of a marginal species with adequate nutritional value, in addition to reducing the cost of making pastry and bread products.

Furthermore, the pasting and physicochemical properties of instantaneous corn flour enhanced with prickly pear fibers were evaluated. The incorporation of 4% nopal powder, rich in total fiber, ash, and Ca to instantaneous corn flour increased the insoluble fiber and the ratio Ca/P in the final products such as tortillas and snacks [167].

Two years later, Guevara-Arauza *et al.* [168] evaluated the biofunctional activity of bars and tortillas enriched with nopal after their intake by healthy volunteers. The addition of nopal improved the polyphenols and fiber content in both tortillas and bars. Over 21 days of daily supplementation with tortillas enriched with nopal, the human oxidative status increased. In addition, triglycerides, glucose, and cholesterol concentrations decreased in plasma. Likewise, the supplementation of nopal improved the water activity, suggesting a lower risk of microbial growth; also, enzyme and chemical reactivity diminished, which would offer a better preservation of the products during storage.

The ice cream is poor in dietary fibers and natural antioxidants. El-Samahy *et al.* [169] designed a new product of ice cream using cactus pear pulp as a good fruit substitute. The pulp was concentrated up to 30° Brix and then added to basic ice cream mix. The sensory evaluation of the resultant ice cream indicated that the sample with 5% cactus pear pulp was very desirable as compared to the control. Based on its low acidity, high sweetness, nutritive value and attractive stable colors, cactus pear fruit may be a suitable source of natural additives or substituted material for production of various products like ice cream, among others.

The elaboration of edible films has been receiving attention, due to the rising interest for decreasing environmental contamination produced by plastics, and the necessity to prolong the shelf life of foods. In this sense, Espino-Díaz *et al.* [170] developed an edible film created by *O. ficus-indica* mucilage. They found that these films exhibited very good tensile strength and water vapor barrier properties.

Cai *et al.* [171] investigated the extraction, separation, purification and structural analysis of the polysaccharide from *O. milpa alta*; its three isolated fractions were examined using HPLC chromatography. The results showed that all fractions were mainly composed by rhamnose, arabinose, xylose, mannose, glucose, and galactose. The results showed that these polysaccharides contain safe compounds, which may be used by food and in pharmaceutical products.

The use of spray drying to process *O. ficus-indica* mucilage generates a powdered stable product of low higroscopicity, without carrier agents. The characterized powder had a polydisperse particle size distribution with agglomerate structure and a glass transition temperature of 45°C. The water adsorption performance of the powder indicated a weak adsorbate-adsorbent interaction. Thus, this study may be useful in developing cost-effective commercial procedures of *O. ficus-indica* mucilage as powdered food additive [172].

Likewise, a method to produce a red-purple food colorant from *O. stricta* fruits by spray drying was developed. *O. stricta* fruit juice is a conceivable font of betacyanin pigments which can be used as a natural red-purple food colorant. A remarkable feature for the use of betalains relies on its antioxidant capacity, which can be related with health benefits. Using dried glucose syrup as drying aid and fixing the spray drying process variables, a high color strength non-sticky powder was produced [173].

Sáenz *et al.* [174] reported the microencapsulation of bioactive compounds of the pulp and ethanolic extracts of cactus pear (*O. ficus-indica*); they were encapsulated either with maltodextrin or inulin. According to these authors the microcapsules produced represent an interesting food additive to be used in functional foods, due to both the presence of antioxidants and red colorants.

Medina-Torres *et al.* [175] produced microcapsules of gallic acid using spray drying with an extract of *O. ficus-indica* mucilage, which functioned as an encapsulating agent. The mucilage from *O. ficus-indica* is a promising and interesting alternative due to its emulsifying properties; multiple applications have been developed for this material. The usefulness of this heteropolysaccharide relies on its physicochemical properties, emphasizing its flow characteristics and electrolyte thickener capacity. Thus, the nopal mucilage microcapsules symbolize a promising food additive for inclusion into various food products.

9. NOVEL APPLICATIONS

Traditionally, cladodes have served as sources for vegetables, for medicinal and cosmetic purposes, and as forage. Application of cacti species for water treatment is rather recent compared to other natural coagulants such as nirmali or *Moringa oleifera*. The most commonly studied cactus genus for water treatment is *Opuntia*. Besides this genus, other cactus species involving *Cactus latifaria* have been also used as natural coagulants [176]. The elevated coagulation capability of *Opuntia* is attributed to the presence of mucilage, which is a complex and viscous carbohydrate deposited in cactus outer and inner pads that has high water retention capacity. Aluminum sulfate (alum) is a common coagulant used in wastewater treatment and can achieve 90-99% microbial elimination. Though, alum reacts with natural alkalinity present in the water, leading to pH reduction; it demonstrates low coagulation efficiency in cold waters and produces large sludge volumes. Thus, there is a need to propose and develop new treatment technologies for emerging communities [177].

Recently, Pichler *et al.* [178] proposed a low-cost technology to be implemented for turbidity reduction in drinking water, based on the mucilage extracted from a common cactus *O. ficus-indica*. These authors tested different flocculating agents: aluminum silicate, sodium hydroxide, aluminum sulfate and mucilage. The results showed that mucilage performs at the same efficiencies of aluminum sulfate at doses 300 times smaller. Mucilage is derived from a renewable source and its removal involves simple organic degradation; important advantages compared to $Al_2(SO_4)_3$, which involves a mechanical or chemical recovery process to prevent further contamination problems. This technology may have the potential to be applied in large-scale water treatment systems, principally when considering the tendency to “green” chemistry solutions.

Furthermore, the roles of guar, locust bean gum, and *Opuntia* mucilage, a by-product of ready-to-eat nopal, were tested as coagulant-flocculant aids in the treatment of a high-load cosmetic industry wastewater. These biopolymers showed conductivity and turbidity removals as high as 20.1 and 67.8%, respectively. The biopolymers produced an increase in the pH value, which is good for the quality of the produced water; and cactus mucilage removed between 75.9 and 63% of the oil and greases. Finally, it was concluded that the higher the biopolymer concentration, the higher the sludge metal content. This suggests that biopolymers may be adsorbing metals [179].

The use of nopal (*O. streptacantha*) cladodes biomass as raw material for Pb^{2+} removal from contaminated waters was analyzed. The amount of Pb^{2+} adsorbed by nopal increased with pH from 3.0 to 5.0. The lower adsorption suggests that Pb^{2+} and H^+ compete for the same adsorption site. The increase in the quantity of Pb^{+2} adsorbed with the pH could be explained by the increase in the density of negative charge on nopal surface due to ionization of COOH groups present in the mucilage. Nopal resulted in the effective Pb^{2+} removal (>90%) from contaminated solutions up to 0.241 mM. The useful implication of this investigation is the development of an economic and effective technology in which the nopal does not undergo any chemical or physical pretreatment; thus, it is a good choice for Pb^{2+} elimination from contaminated waters [180].

On the other hand, the employment of nopal powder and mucilage has been tested for improvement of lime mortars for restoration and edifying natural stone buildings. Lime mortar is a combination of water, lime, and river sand; it has been used in building materials since very early times. In the last decade, a revival of the use of lime mortars in the restoration of historic buildings has occurred as it was noticed that the traditional cement has some inadequate properties and that it is non-compatible with natural stones. The growth of acicular crystals of aragonite due to the use of nopal may help to increase the consistency of the mortar and result in better compressive strength. Moreover, the carbonation front is remarkably improved by the supplement of nopal both as mucilage and as powder; in numerous cases of restoration this is an important factor to avoid mortar deterioration just after application due to mechanical erosion or rain [181].

10. CONCLUSION

There is a growing interest in healthier foods worldwide; in this sense nopal is gaining attention due to its high nutraceutical potential. The high levels of antioxidant compounds present in nopal as well as its high dietary fiber seem to be the two most important features responsible for this activity. Even though, the inclusion of nopal in the menu around the globe is still a challenge; evidence suggests that the current consumption of its cladodes or fruits can help to prevent the onset of type 2 diabetes and weight control. Even more, the new biotechnological tools that are currently been applied in nopal might help on its availability (*in vitro* tissue culture techniques) and on the understanding of metabolic pathways, regulation and overexpression of nutraceutical gene products (genetic plant transformation). Interestingly, the isolation, purification and characterization of industrially important enzymes, such as cellulase and α-amylases, set the basis for new enzyme isolation protocols and for new industrial application niches for nopal.

Hence, the isolated substances or the combined action of several components from nopal could be responsible for the variety of applications of this ancient crop. Nevertheless, its nutritional properties, and high potential for both health and food industry applications remains unexploited.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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ABBREVIATIONS

ABA = Abscicic acid
ABTS = 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ALT = Alanine aminotransferase
AFB1 = Aflatoxin B1
Atahas = Acetohydroxyacid synthase
BA = 6-bencilaminopurine
BNDF = Brain derived neurotrophic factor
BMC = Bone mineral content
BMD = Bone mineral density
CAT = Catalase
CDs = Ciclodextrins
DAP = [6(γ,γ-dimetilaminopurine)]
DPPH = Diphenylpicrylhydrazyl scavenging method
EC$_{50}$ = Half maximal effective concentration
FRAP = Ferric reducing ability plasma assay
GAE = Gallic acid equivalents
GA$_3$ = Giberellic acid
GI = Gastrointestinal fat binding
GSH = Glutathione
GSH-Px = Glutathione peroxidase
GUS = β-glucuronidase protein
HCC = Hepatocellular carcinoma
HDL = High density lipoprotein
IBA = Indole-3-butyric acid
IC$_{50}$ = Half maximal inhibitory concentration
IDC = Ionic dialyzable calcium
IDF = Insoluble dietary fiber
KA = Kainite
kDa = KiloDaltons
MDA = Malondialdehyde
MPO = Myeloperoxidase
MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
NMDA = N-methyl-D-aspartate
nptII = Neomycin phosphotransferase II gene
LDL = Low density lipoprotein
ORAC = Oxygen radical absorbancy capacity
OGD = Oxygen glucose deprivation
PARP = Poly (ADP) ribose polymerase
PC = Carbonylated proteins
pCREB = Phosphorylated CAMP response element binding protein
PCR = Polymerase chain reaction
(pERK)½ = Phosphorylated extracellular signal-regulated kinase
PGRs = Plant growth regulators
Picloram = 4-amino-3,5,6 tricholoro-2-pyridinecarboxylic acid
ROS = Reactive oxygen species
SOD = Superoxide dismutase
SDF = Soluble dietary fiber
STZ = Streptozotocin
TE = Trolox equivalents
uidA = β-glucoronidase gene
VCEAC = Vitamin C equivalent values
VLDL = Very low density lipoprotein
2,4-D = 2,4-dichlorophenoxyacetic acid
4-HPR = N-(4-hydroxyphernyl)-retinamide
K562 = Human chronic leukemia cell line

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