Secondary metabolites from Cactaceae with antifungal effect

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Cactaceae family grows and persists through anatomical and metabolic adaptations that allow them to develop at arid and semi-arid areas with adverse climatic conditions. Among those strategies, the production of secondary metabolites protrudes. Secondary metabolites are chemical compounds synthesized by plants to accomplish specific functions related with plant protection and species survival, their presence on cacti makes this family a potential source of phytochemicals with antifungal activity of plant and human importance. The present review compiles recent information of research related to different types of secondary metabolites (glycosides, phenolic compounds, alkaloids and terpenes) present in members of the Cactaceae family and that have demonstrated some type of activity against fungi, including *in vitro* and *in vivo* studies, as well as their structure-activity relationship and action mechanisms. The presence of a variety of phytochemicals in plants from the Cactaceae family makes it a potential source of compounds with antifungal activity that can be used in favour of well-being and human health.

Keywords: Cactaceae; secondary metabolites; antifungal activity.

1. Introduction

The term cactus refers to a group of approximately 1,600 species in 130 genera, which includes the most conspicuous elements of the Western hemisphere, all them included in the Cactaceae family. The greatest diversity of this family is recorded in Mexico, with 660 species, and 518 endemic species, followed by Brazil, Argentina, Bolivia and Peru [1-5].

The family is subdivided into four subfamilies: Pereskioideae, Opuntioideae, Maihuenioideae and Cactoideae [5]. The most important genera are *Opuntia* and *Nopalea* due to their uses as food, fodder, medicinal and ornamental plants; as well as to their abundance throughout the Americas and Mediterranean central area, Europe, Asia, Africa, and Australia [3, 6-7]; however, numerous species of cacti belonging to different genera have different uses around the world, and are increasingly appreciated for their health benefits [5, 7-8]. Cacti have long attracted special attention due to their peculiar biology; they are abundant, diverse, and able to thrive under environmental conditions that produce significant physiological challenges through the seasons [9]. Their capacity to develop successfully in hot subtropical deserts all over the world [10] is possible due to the development of anatomical adaptations such as a spinous succulent body (water-storage tissue) with a thick waterproof epidermis which is covered by a waxy cuticle [5, 7, 11].

Additionally cacti possess a number of interesting metabolic characteristics that were developed to allow stems to function as the main photosynthesizers with Crassulacean Acid Metabolism, which let stomata open only at night, thus conserving water because much less water vapour is lost during cooler night hours, due to these characteristics, cacti are commonly known as succulent plants [4-5, 11]; as well as they possess a biochemical potential to synthesize different kinds of secondary metabolites which contributes to their survival [12].

For millennia human has used healing powers from plant species. The presence of secondary metabolites in plants makes them natural sources of remedies, used as natural medicines by local population in diseases treatment including leishmaniasis, malaria, schistosomiasis, fungal and bacterial infections [13]. Plants are an important alternative for health organizations and pharmaceutical industries in active ingredients search to create synthetic antifungals; currently, from one quarter to one-half of all pharmaceuticals dispensed in the United States have plant origin, world trade in medicinal plants is now more than 43 billion dollars and is predicted to reach to 5 trillion dollars in 2050 [13-14].

Fungi comprise a major part of biodiversity, from around 100,000 known fungal species, more than 400 species are known as animal and plant pathogens [15]. Worldwide occurrence of fungal infections, has been dramatically increased in the last 20 years, due to a continuous increase mainly among immunocompromised hosts, they produce serious invasive mycoses in individuals submitted to organ transplants, cancer, and diabetes mellitus [13-14]. Superficial mycoses are among the most frequent forms of human infections (those involving the skin and mucosal surfaces) not only in immunocompromised host, but also in healthy individuals, being estimated to affect more than 20-25% of the world's population [14, 16]. Dermatophytes are the most common cause of skin infections and they can achieve this due to virulence factors such as their ability to adhere and invade keratinized tissues [17]. New data indicate that relative proportions of organisms causing nosocomial bloodstream infections have changed over the last decade, with *Candida* species now firmly established as one of the most frequent agents. *Candida albicans* is part of human flora but under some circumstances in susceptible individuals it can cause systemic and superficial infections. Virulence factors of this opportunist microorganism include: its capacity to host adhesion, secretion of degradation enzymes, its ability to change morphologically and to form biofilms [17]. The distribution of dermatophyte infections and their causative agents varies

with geographical region and is influenced by a wide range of factors [18-19]. Nowadays antifungal drugs are essentially limited to three chemical classes: polyenes (amphotericin B), azole drugs (imidazoles, fluconazole, itraconazole, voriconazole and posaconazole) and echinocandins [20-21]; isavuconazole has been described as a new extended-spectrum triazole [22-23]. These agents display several limitations that can lead to complications; for example, amphotericin B was during nearly 30 years the only drug, and it is one of the few drugs that kill fungal cells, but can cause significant nephrotoxicity in patients [14], with a rapid development of fungal resistance (specially to azoles and to flucytosine), drug-drug interactions, fungistatic but not fungicidal mode of action. Thus, there is an urgent need for developing new antifungals with a broad spectrum and with fewer dose-limiting side effects [24-25]. On the other hand, plants can experience fungal infections too. Fungal plant pathogens comprise an important group of microorganisms that causes significant economic losses in agriculture around the world, such as they can infect any tissue at any stage of plant growth [15]. Plant diseases control depends upon the application of chemical fungicides, despite their potentially toxic effects on non-target organisms and the environment [26-27]. Although effective, their extensive use for several decades has disrupted biological control by natural enemies and has led to new pathogen strains that are resistant to fungicides [28]. Despite the huge amount of information about fungal plant pathogens, there is a limited commercial fungicide developed from a new knowledge approach. The absence of fungicides that can act in more than one site of action is a direct consequence of fungal resistance, which is common among currently used agrochemicals [29-30]. The aim of this review is to present the state of art of antifungal activity of secondary metabolites present in Cactaceae family members since, to our knowledge, there is not condensed information on this topic.

2. Production of secondary metabolites in Cactaceae

Plants are a rich source of secondary metabolites with potential or actual use as flavours, fragrances, pesticides, pharmaceuticals and antimicrobials. About 12,000 of these aromatic substances and their derivatives have been isolated and it represents roughly 10% of the total [14]. Cactaceae family is a source of secondary metabolites like alkaloids, carotenoids, betalains, triterpenes and sterols [31-32]. The production of those chemicals, in some cases occurs in the whole plant; in some other circumstances, there may be a selective production of specific secondary metabolites in any plant organ [33]. Many phytochemicals are synthesized in roots, which can then be stored *in situ* or transported to other organ plant. In other hand inside cells, sesquiterpenes, triterpenes and sterols occur in the endoplasmic reticulum, whereas monoterpenes originate in plastids; and amines and alkaloids in mitochondria [32, 34].

In addition to contribute to plant reproduction [12, 35], secondary metabolites of cacti serve as plant defence mechanism against predators (microorganisms, insects and herbivores). Chemically, most of secondary metabolites are aromatic substances such as phenols and their oxygen-substituted derivatives; others contribute to plant odour characteristics, such as terpenoids; quinones and tannins are responsible for plant pigmentation [12].

An important group of secondary metabolites in cacti are phenolic compounds, an extensive group of chemical antioxidants. A large quantity of those compounds has been detected in Cactaceae members, including phenolic acids such as gallic, cumaric, 3,4-dihydroxybenzoic, 4-hydroxybenzoic, ferulic and salicylic [36]. The presence of flavonoids, has been described in several species from *Opuntia* and *Cereus jamacaru*, some of them are quercetin, kaempferol and isorhamnetin glycosides; iso-quercitrin, isorhamnetin-3-O-glucoside, nicotiflorin, narcissin and rutin [37]. Recently pinostrobin, β-sitosterol, a mixture of β-sitosterol/stigmasterol, 13^2-hydroxyphaeophytina, phaeophytin A, sitosterol 3-O-β-D-glucopyranoside/stigmasterol 3-O-β-D-glucopyranoside, kaempferol, quercetin, the new substance 7′-ethoxy-trans-feruloyltyramine and trans-feruloyltyramine have been found, all of them in *Pilosocereus pachycladus* and *P. arrabidae* [38].

Betalains are the most characteristic pigments in this family, being *Opuntia* genus fruits important exponents. Their biosynthesis depends on the ability of plants to form betalamic acid which condenses preferentially with cyclo-DOPA or amino acids in nonenzymatic reactions, leading to red-violet betacyanins or yellow betaxanthins. While the latter compounds contain no glycosidic groups, betacyanins are mostly glycosides [39]. Betalains are found in only ten families of the Caryophyllaleae order [31, 40].

The Cactaceae family is also characterized by synthesize different kinds of alkaloids (nitrogen-containing compounds). *Lophophora williamsii*, popularly known as “peyote”, produces more than 50 different alkaloids, including both phenethylamines and tetrahydroisoquinolines, which are responsible for a hallucinogenic effect like lysergic acid diethylamide [41]. *Coryphantha* genus produces several alkaloids chemically similar to epinephrine. *Coryphantha macromeris* species posesses an alkaloid called macromerina, a drug with approximately 20% of mescalina power. *Mammillaria* genus contains entheogenic substances, such as tetrahydroisoquinolines [42].

Other group of secondary metabolites found in the Cactaceae family are triterpenes and sterols (steroids with several carbon rings and alcohol side chains) [11, 43]. Opuntisterol and opuntisteroside, both with a 5β configuration, together with the known sterols β-sitosterol, 7-oxositosterol, 6β-hydroxystigmast-4-en-3one and daucosterol have been isolated from the aerial parts of the cactus *Opuntia dillenii*. Even though opuntisterol and opuntisteroside do not have a double bond at C-7, their discovery presents the possibility of finding molecules with the same 5β configuration, similar to ecdysteroids, in other plants of Cactaceae [44]. The presence of steroids (24-alkyl-Δ5-sterols, mainly sitosterol) has

been identified in eight different cacti species, from seven different genera: *Echinopsis tubiflora*, *Pereskia aculeata*, *Hylocereus undatus*, *Notocactus scopa* var. *marchesii*, *Epiphyllum* sp., *Schlumbergera bridgesii*, *Opuntia comonduensis*, and *Opuntia humifusa*. Other steroids were identified, including stigmasterol, sitostanol, and avenasterol [41, 44].

3. Antifungal activity of Cactaceae

Research on secondary metabolites biological properties has been mainly focused to anticancer properties [5, 7-8]; particularly for Cactaceae members, there have been no exhaustive studies on their implication in antimicrobial areas, specifically as antifungals. Despite all this, current knowledge on properties of phytochemicals has greatly contributed to solve health problems [33]. As described above, plants are the first option for investigations due to their large number of antifungal metabolites with a unique structural diversity. Plant metabolites have advantages to microbial metabolites regarding their low molecular weights, which makes them suitable for antifungal drugs formulation.

Secondary metabolites with antifungal activity from Cactaceae have been mainly tested as crude extracts obtained with different solvents, consisting of a complex mixture of numerous components. Water has been employed to obtain volatile compounds and essential oils, as well as polar secondary metabolites. Crude extracts composition varies according to solvent nature, hence biological activity could change. Either major or trace compounds might give rise to biological activity; possible synergistic or antagonistic effects of compounds also play an important role in microorganism inhibition [45]. The most studied fungi are human pathogens (*Candida*, *Microsporum* and *Trichophyton*), plant pathogens (*Cladosporium*, *Alternaria*, *Phoma*, *Rhizoctonia* and *Fusarium*), and food spoilage (*Aspergillus* and *Penicillium*). A variety of test methods (agar well or disc diffusion, micro or macro-dilution, mycelium growth inhibition) and doses (1 to 500 mg/mL; 75% or whole extract) have been examined [46-52, 55, 58-59, 61-62, 64-68, 71-73, 79]. All the antifungal study variables complicate the comparison of the results among different research works (Table 1 and 2).

Opuntia has been the most studied genus for antifungal compounds. Fruits, flower, stem (cladodes), even root extracts have been tested against a variety of human pathogenic, phytopathogenic and food spoilage implicated fungi. Kumaar et al. [46] found antifungal activity against six different fungal organisms of methanolic extracts (1000 μg/mL) from *Opuntia dillenii* fruits. Alkaloids, flavonoids, tannins and glycosides were detected. Shafiei et al. [47] studied the antimicrobial effects of methanolic extracts from *Opuntia stricta* fruit, extensively used in traditional medicine, results showed that methanol extract have antifungal effect against *Candida albicans* (40 mg/mL). Moosazadeh et al. [48] analysed the chemical composition and antifungal activity of *Opuntia stricta* fruit essential oil. Nineteen compounds were identified in the oil by Gas Chromatography-Mass Spectrometry (GC-MS), with thymol (42.7%) as the dominant component with antifungal activity against *Candida albicans* at low (2.5 to 40 mg/mL) concentrations, which was attributed to the high content of thymol. While Bergaoui et al. [49] reported antifungal activity in volatile compounds extracted from *O. lindheimeri* fruits only against *Alternaria solani*.

For *Opuntia* flowers, Ammar et al. [50] examined the antifungal activity of hexane extracts from *Opuntia ficus-indica* and *O. stricta* in four different flowering stages, against *Aspergillus niger* and *Candida lipolytica*. Exclusively the extract from post flowering stage (flowers closed and dry) of *O. stricta* inhibited both fungi; extracts from *O. ficus-indica* did not present activity. Chemical composition of hexane extracts analysed by GC-MS, revealed the presence of secondary metabolites belonging to carboxylic acids (28–97%), terpenes (0.2–57%), esters (0.2–27%) and alcohols (<1.8%). Ennouri et al. [51] analysed the chemical composition of hexane extracts from *Opuntia ficus-indica f. inermis* flowers at four flowering stages, and their antifungal activities against two fungi strains. Results revealed the presence of secondary metabolites belonging to carboxylic acids, terpenes, esters, and alcohol classes. Linoleic acid (9,12 octadecadienoic acid) and camphor were present in the four flowering stages with low sesquiterpene concentration.

Only full-flowering stage extract presented antifungal activity against *A. niger*. Weak antifungal effects of hexanic extracts from full-flowering stage (flower fully opened) may be due to some components, such as linoleate ethyl, being detected only in this stage. Chahdoura et al. [52] evaluated the methanolic extracts of *O. microdasys* flowers from three different vegetative stages against eight fungi species, including human and plant pathogens as well as food spoilage implicated. Extracts obtained from the vegetative stage (green closed petal flowers) were the most effective inhibiting fungal growth (1.25 mg/mL to 10 mg/mL). *Aspergillus versicolor* and *Penicillium funiculosum* were the most susceptible fungal species, whereas *P. ochrochloron* showed the highest resistance against the *O. microdasys* extracts. Antimicrobial activity was attributed to phenolic compounds present in methanolic extracts [53-54]. Phenolic compounds may interact with microorganism's cell membrane or cell wall through hydrogen bonds, causing changes in membrane permeability and cell destruction. Prabhakaran et al. [55] obtained ethylacetate fraction from *Opuntia stricta* flowers ethanolic extract, this fraction showed antifungal activity (50 mg/mL) against *Curvularia lunata* and *Candida albicans*. Volatile compounds from *O. lindheimeri var. linguiformis* flowers presented moderate inhibitory activity against *Fusarium oxysporum f. spp niveum*; and extract from *O. microdasys* flowers weakly inhibited *Rhizoctonia solani* [49].

Table 1. Studies on antifungal activity of *Opuntia sp.* extracts against different fungal species.

Opuntia species	Solvent used to extraction	Fungal species	Fungus kind	Analysis of extract	Secondary metabolites in extract
Fruits					
Opuntia dilleni	Methanol	*Aspergillus niger*	HP, PP	PPS	Alkaloids, flavonoids, tannins, triterpenes
		Candida albicans	HP		
		Monilinia fruticola	PP		
		Auricularia polytricha	PP		
		Chaetomella raphigera	PP		
		Arthrobotrys oligospora	NP		
Opuntia stricta	Methanol	*Candida albicans*	HP	NA	NA
Opuntia stricta	Essential oil	*Candida albicans*	HP	GC-MS	19 compounds: thymol and n-octane
Flowers					
Opuntia ficus-indica *Opuntia stricta*	Hexane	*Aspergillus niger* *Candida lipolytica*	HP, PP HP	GC-MS	50 compounds: carboxylic acids, terpenes, esters, alcohols, aromatic compounds
Opuntia ficus-indica f. inermis	Hexane	*Aspergillus niger* *Candida lipolytica*	HP, PP HP	GC-MS	26 compounds: linoleic acid, sesquiterpenes, camphor
Opuntia microdasys	Methanol	*Aspergillus fumigatus*	HP, FS	NA	Probably, polyphenolics
		Aspergillus ochraceus	FS		
		Aspergillus versicolor	FS		
		Aspergillus niger	HP, PP		
		Penicillium funiculosum	PP		
		P. ochrochloron	FS		
		P. verrucosum var.cyclopium	FS		
		Trichoderma viride	PP		
Opuntia stricta	Ethanol	*Curvularia lunata*	PP	NA	NA
		Candida albicans	HP		
Stems/cladodes					
Opuntia sp	Water, lanolin, cocoa butter	*Rhizoctonia solani*	PP	SP	Hydrolysable and condensed tannins
Opuntia ficus-indica,	Water, ethanol, lanolin, cocoa butter	*Phytophthora cinnamomi*	PP	SP	Hydrolysable and condensed tannins
Opuntia sp	Tris-HCl Buffer	*Aspergillus flavus*	FS, HP	NA	NA
Roots					
Opuntia sp	Tris-HCl Buffer	*Aspergillus flavus*	FS, HP	NA	NA
Various					
O. lindheimeri var. linguiformis (leaves, flowers and fruit) *O. macrorhiza (leaves and flowers)* *O. microdasys* var. *pallida (leaves)*	Steam distillation	*Alternaria solani*	PP	GC-MS	39 components. Acids pentadecanoic, hexadecanoic and nonadecanoic; butyl tetradecanote, (E)-3-Butyldiene phthalide, octacosane, nonacosane, phytol
		Botrytis cinerea	PP		
		Fusarium solani f. sp. cucurbitae	PP		
		F. oxysporum f. sp.niveum	PP		
		Pythium ultimum	PP		
		Rhizoctonia solani	PP		

PP=Phytopathogen; HP=Human pathogen; FS=Food spoilage implicated; NP= Nematophagous. PPS= Preliminary phytochemical screening; GC-MS = Gas Chromatography-Mass Spectrometry; SP = Spectrophotometric; NA= Not analysed. References: 46-52, 55, 58-59, 61.

Opuntia cladodes (leaves, pads or stems) have been investigated for antifungal activity [56-57]. Castillo et al. [58] studied water, lanolin and cocoa butter extracts from *Opuntia sp*. against *Rhizoctonia solani*; presented low inhibitory effect (below 40% inhibition) with a tannin concentration near 500-3000 ppm. In a subsequent research, the authors evaluated antifungal activity of these extracts against *Phytophthora cinnamomi* [59], water extract containing 4000 ppm of polyphenols showed the highest fungal inhibition effect (100%). In both investigations, the use of alternative organic solvents permitted the extraction of a variety of polyphenols from plants; all them with a chemical constitution and biological activity according to the solvent applied. In general, it was observed that the polyphenols obtained from plant

extracts using different solvents have effects on mycelium growth inhibition of *P. cinnamomi*, being ethanolic extracts 20 times better than water and 5 times better than lanolin extracts on *P. cinnamomi* growth inhibition [58-59]. As described above, naturally occurring pesticide compounds are synthesized by the plant as defence system, and it could include not only low molecular weight secondary metabolites but also antimicrobial proteins products, as reported by Santana et al. [60], who isolated a stable, ion dependent and chitin-binding lectin from *Opuntia ficus indica* cladodes which was able to affect the growth of *Colletrotrichum gloesporioides*, *Candida albicans*, *Fusarium oxysporum* and *Fusarium solani*; the lectin showed high activity against *C. albicans*, reducing the fungal growth in 59%. Frisby [61], evaluated the antifungal activity of stem and root from *Opuntia sp*. Tris-HCl buffer extracts presented strong inhibition of fungal growth against *Aspergillus flavus*. After removing soluble, low-molecular-weight materials from extracts by dialysis (<3,500 Da), the activity was lost, the author discussed the possible implication of more than one type of soluble metabolites in the antifungal activity, some of them with molecular weights lower than 3,500 Da. Bergaoui et al. [49] reported the composition and antifungal activity of volatile compounds extracted from different aerial organs (leaves, flowers and fruits) of *Opuntia* species. (*Opuntia lindheimeri* var. *linguiformis*, *Opuntia macrorhiza* and *Opuntia microdasys*) against six fungal species frequently isolated in Tunisian crops, fruits or soils. Eight compounds were commonly found in different extracts (pentadecanoic, hexadecanoic and nonadecanoic acids; butyl tetradecanote and (E)-3-Butyldiene phthalide; octacosane and nonacosane; and phytol). Extracts from leaves exhibited the strongest antifungal activity against *Alternaria solani*.

Cactaceae family members have been screened for natural alternatives to control fungal infections. Table 2 presents a summary of antifungal studies involving secondary metabolites found in cactus family as active compounds. Zapata et al. [62] used mid sections of *Cereus deficiens* stems to ethanolic degreased (EDE), ethanol non-degreased (ENDE) and aqueous (AE) extracts. Polyphenols, tanins and saponins in EDE were detected, all fungi were inhibited, mainly by ethanolic extracts. *P. infestans* and *S. rolfsii* were the most susceptible strains. The authors suggest that the biological activity of extracts could be due to the presence of essential oils and polyphenolics in the extracts, based on bibliographic references [63].

Rodriguez et al. [64] analysed the phytochemical profile of methanolic extracts from *Ariocarpus kotschoubeyanus* and *A. retusus*. The extracts contained compounds from the three main groups of secondary metabolites: isoprenoids (terpenes, saponins), phenolic derivatives (phenols, phenolic acids, flavonoids) and alkaloids. *A. retusus* methanolic extract (500 mg/mL) inhibited *Microsporum gypseum* growth in a similar extent to the standard ketonocazole (60 mg/mL). The antifungal effect may be due to saponins content in the extract, since these molecules have shown biological activities such as antimicrobial, antitumor, cytotoxic, ichthyotoxic, anthelmintic, spermicides and anti-inflammatory. On the other hand, the methanolic extract from *A. kotschoubeyanus* inhibited the growth of *M. gypseum* and did not present effect on *M. manum*. In a subsequent investigation [65] stem and root of *A. retusus* extracts were obtained, stem extracts (500 mg/mL) inhibited *Trichophyton tonsurans* and *Microsporum cookei* growth.

Soto-Cabrera et al. [66] evaluated the activity of methanolic extracts of *Stenocereus sp*. The major compounds were betulin, β-sitosterol, β-amirin and saponins. Biological tests showed moderate activity against *C. albicans* (0.03mg/mL). Soto-Cabrera et al. [67] evaluated the antifungal activity of three extracts from *Stenocereus stellatus* as well as their phenolic content and phytochemical profile by GC-MS. All extracts showed inhibitory activity against *C. albicans* (31 µg/mL) and *Rhizopus sp.* (15 µg/mL). *A. oryzae* and *P. notatum* presented MIC values higher than standard (125 µg/mL). Ethyl acetate extract presented the highest concentration of total phenolic compounds (50.78 mg eq. gallic acid/g extract) and total flavonoids (115.12 mg eq. catechin/g extract). In the chromatographic analysis, β-sitosterol, β-amyrine, betulin and some other molecules were identified in extracts, and their presence could explain partially the inhibition of fungi strains.

Treviño et al. [68] determined the antifungal activity of methanolic extracts from *Stenocereus pruinosus* and *Echinocereus stramineus*. Phytochemical analysis showed the presence of different compounds from two of the three main groups of secondary metabolites: phenolic derivatives (phenols, phenolic acids, flavonoids) and alkaloids. Isoprenoids like terpenes and saponins were only detected in the *S. pruinosus* extract. Only *S. pruinosus* methanolic extract presented fungicidal activity (500 mg/mL) against *Microsporum gypseum* and *M. canis*. Alkaloids, triterpenes and saponins are biologically active compounds correlated with antifungal activity. Frisby [61], evaluated the stem and root from *Echinocereus reichenbachii* against *Aspergillus flavus*. Aqueous crude extracts from *E. reichenbachii* root presented strong inhibition of fungal growth. After removing soluble, low-molecular-weight materials from the crude extracts by dialysis (<3,500 Da), the activity was lost.

Pereskia genus is considered the least advanced from Cactaceae family, its leaves are widely used as emollients, in skin wound healing; and some species had shown anti-inflammatory, antioxidant, antifungal, antimicrobial and cytotoxic activities, and a variety of secondary metabolites [69-70]. Souza et al. [71] evaluated three leaf extracts from *Pereskia aculeate* phenolic content. Extracts showed different antifungal activity, *Aspergillus versicolor* was the most susceptible strain; its growth was inhibited by the three extracts tested, while *A. niger* was resistant to chloroform and methanolic extracts. Even not determined, sterols such as sitosterol, were suggested to present the antifungal activity.

Gomez-Flores et al. [72] investigated *in vitro* antifungal activity of fresh and dry *Nopalea cochenillifera* pads. They reported that methanolic extract from fresh pads, and the three extracts from dry pads possess antifungal activity against

Table 2 Studies on antifungal activity of Cactaceae family members.

Cactaceae species (tissue)	Solvent used to extraction	Fungal species	Fungus kind	Analysis of extract	Secondary metabolites in extract
Cereus deficiens Otto & Dierf (S)	Ethanol, water	*Phytophthora infestans*	PP	PPS	Essential oils, saponins, polyphenols and tannins
		Fusarium oxysporum, F. sp. lycopersici, F. sp. Cubense, F moniliforme	PP		
		Lasiodiplodia theobromae	PP		
		Sclerotium rolfsii	PP		
		Colletrichum gloeosporioides	PP		
		Alternaria solani	PP		
		Rhizoctonia solani	PP		
		Bipolaris maydis	PP		
Ariocarpus kotschoubeyanus (W) *Ariocarpus retusus* (W)	Methanol	*Microsporum gypseum*	HP	PPS	Sterols and methylsterols, sesquiterpenlactones, coumarins, saponins, flavonoids and alkaloids
		Microsporum nanum	HP		
Ariocarpus kotschoubeyanus (W) *Ariocarpus retusus* (S, R)	Methanol	*Trichophyton tonsurans*	HP	PPS	Sterols and methylsterols, sesquiterpenlactones, coumarins, saponins, flavonoids and alkaloids
		Microsporum canis	AP		
		Microsporum cookei	HP		
Stenocereus sp. (S)	Methanol	*Candida albicans*	HP	GC-MS	Betulin, β-sitosterol, β-amirin and saponins
		Aspergillus niger	HP, PP		
Stenocereus stellatus (S)	Hexane, ethyl acetate, methanol	*Candida albicans*	HP	GC-MS	Polyphenols, flavonoids, β-sitosterol, β-amyrine, betulin
		Aspergillus niger	HP, PP		
		Aspergillus oryzae	FS		
		Penicillium notatum	FS		
		Rhizopus sp.	HP, PP		
Stenocereus pruinosus (W) *Echinocereus stramineus* (W)	Methanol	*Microsporum gypseum*	HP	PPS	In both cacti: terpenes, phenols, phenolic acids, flavonoids, alkaloids. Saponins, only en *S. pruinosus*
		Microsporum canis	AP		
		Microsporum nanum	HP		
		Microsporum cookei	HP		
Echinocereus reichenbachii (S, R)	Tris-HCl buffer	*Aspergillus flavus*	FS, HP	NA	NA
Pereskia aculeata (L)	Petroleum ether, chloroform, methanol	*Aspergillus versicolor*	FS	SP	Total phenolic compounds
		Aspergillus niger	HP, PP		
		Penicillium citrinum	PP		
Nopalea cochenillifera (C)	Hexane, chloroform, ethanol	*Candida albicans*	HP	NA	NA
Nopalea cochenillifera (C)	Ethanol	*Candida glabrata*	HP	SP	Total polyphenol and flavonoids
		Candida albicans	HP		
		Prototheca zopffi	PP		
		Cryptococcus neoformans	PP		
		Saccharomyces cervisiae	FS		
		Malassezia furfur	HP		
Mammillaria huitzilopochtli (Callus cells in culture)	Ethylacetate (culture medium) Dichloromethane: methanol, 9:1(cells)	*Cladosporium sp*	PP	NA	Bioactive compounds with phytoalexin type effect
		Phoma sp	PP		
		Alternaria sp	PP		
		Rhizoctonia sp	PP		
		Fusarium sp	PP		
		Fusarium moniliforme	PP		
		Helminthosporium sp	PP, HP		
		Phaecilomyces sp.	PP, NP		

S= Stem; W= Whole plant; R= Root; L= Leaves; C= Cladodes. PP=Phytopathogen; HP=Human pathogen; FS=Food spoilage implicated; AP= Animal pathogen; NP= Nematophagous. PPS= Preliminary phytochemical screening; GC-MS = Gas Chromatography-Mass Spectrometry; SP = Spectrophotometric; NA= Not analysed. References: 61-62, 64-68, 71-73, 79.

Candida albicans. Necchi et al. [73] evaluated the *in vitro* antifungal activity of ethanol extract from the same cactus and its content of total phenolic compounds. The results showed inhibitory activity over six fungal strains. MIC ranged from 0.625 mg/mL to 2.5 mg/mL. *Candida albicans* was the most sensitive, while *C. glabrata* and *Malassezia furfur*,

were the most resistant strains. Ethanolic extract presented 29.62 and 7.63% of polyphenols and flavonoids, respectively.

Plant cells are biosynthetically totipotent, which means that each cell in culture retains complete genetic information and hence can produce a wide range of chemicals found in the parent plant. Plant cell cultures are an attractive alternative source to whole plant production of high-value secondary metabolites Biotechnology offers an opportunity to exploit cells, tissues, organs or entire organism by growing them *in vitro* and genetically manipulate them to get the desired compounds [74]. It has been an important tool in Cactaceae family propagation studies [75-76], as well as secondary metabolites production [40, 77-78]. Among several strategies to enhance secondary metabolites production in plant cell cultures, the use of *Fusarium moniliforme* homogenates as elicitor was tested by Robles-Zepeda et al. [79] to study its effect on the production of antifungal metabolites in *Mammillaria huitzilopochtli* suspension cell cultures. The extract obtained from growth medium and cactus cells was evaluated for antifungal activity. The extract showed inhibition over all tested fungi (100 μg/mL), except for *Phoma sp.* who was completely inhibited at 400 μg/mL. Authors suggest the presence of bioactive compounds with phytoalexin type effect which could be used eventually against *Phoma*.

Conventional antifungal drugs cause serious mammalian cytotoxicity, partly through the intracellular production of reactive oxygen species (ROS), and because of fungi are eukaryotic organisms that share diverse metabolic profiles with animal and plant cells; therefore, several antifungal agents discovered to be potentially active against pathogenic fungi have failed to survive during testing process because the fungicide target site is found in another organism, causing toxicity. With the rapid emergence of fungal resistance, a strong demand for antifungal agents with a new mode of action has arisen. One of the modern pathogenic fungi research challenges, is to find out new modes of action that provide improved fungicide activity against health important target, combined with the protection of environmental and public safety [80-82]. Antifungal compounds not only serve as drugs or templates for drugs, in many cases, they lead to the discovery and better understanding of targets and pathways involved in the disease process [83]. Several different cellular targets of conventional antifungal drugs have already been identified (Table 3). Examples include cell wall/membrane integrity, mitochondrial respiration, cell division, signal transduction, and macromolecular synthesis [84].

Caspofungin acetate; micafungin and anidulafungin, are molecules which inhibit the synthesis of glucose homopolymer in β-(1,3)-D-glucan, an essential component of cell wall of many fungi, but absent in mammals. The inhibition of β-(1,3)-D-glucan synthase interferes with fungal cell wall synthesis and its integrity, leading to osmotic instability, lysis of the wall and death of the fungal cell. Since January 2008, micafungin has been approved for *Candida* infections prophylaxis in patients undergoing hematopoietic stem cell transplantation. Glucan synthase is not present in mammalian cells and therefore is an attractive target for antifungal activity. Strobilurin class fungicides act broadly by targeting vegetative growth and fungal life cycle. These compounds, structurally based on natural products, target mitochondrial electron transport within the respiration chain and interfere with the ubiquinol cytochrome C oxidoreductase [83]. On the other hand, fungal action modes of only a few secondary metabolites from plant compounds are well known, and there is not information about antifungal compounds from Cactaceae, but the research is growing, so that, we should not reject the possibility to develop, in the future, commercial fungicides from natural products with good perspectives in commercial market.

Even though individual antifungal compounds from Cactaceae have not been studied, some secondary metabolites from other sources had been identified and their mechanisms of action have been proposed. Phenolic compounds have been shown to inhibit enzymes by reacting with the sulfhydryl groups of amino acids [12]. Quinones, flavones, flavonoids, tannins and flavonols form complexes with the nucleophilic amino acids of proteins which leads to their inactivation. Flavones are phenolic structures containing one carbonyl group. The possible mechanism of action of flavones and flavonoids is hampered by conflicting findings. Flavonoids lacking hydroxyl groups on their β-rings are more active against microorganisms than are those with the two OH groups; this finding supports the idea that their microbial target is the membrane. However, several authors have also found the opposite effect, the more hydroxylation, the greater the antimicrobial activity. The latter finding reflects the similar result for simple phenolics. It is safe to say that there is no clear predictability for the degree of hydroxylation and toxicity to microorganisms. Quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism. As with all plant-derived antimicrobials, the possible toxic effects of quinones must be thoroughly examined [12, 33]. It has been shown that phenolic alcohols (thymol, carvacrol, eugenol) are the strongest inhibitors of enzymatic processes. This is attributed to its lipophilic characteristic and its free OH groups [12, 52]. Many phytopathogenic (except for biotrophic) fungi secrete hydrolytic enzymes that diffuse into host cells prior to the advance of microorganisms, which can be inhibited by free radicals of oxidized phenols that function as nonspecific inhibitors; such as tannins, cyanidin, delphinidin and malvidin anthocyanindins [12, 33]. Highly aromatic planar quaternary alkaloids such as berberine and harmane action mechanism are attributed to their ability to intercalate with DNA [12].

Table 3 Cellular targets of conventional antifungal drugs and secondary metabolites.

Antifungal compounds	Cellular targets
Conventional antifungal drugs	
- Caspofungin acetate - Micafungin - Anidulafungin	Cell wall/membrane integrity: - Inhibit the synthesis of the glucose homopolymer β-(1,3)-D-glucan
- Strobilurin class	Mitochondrial electron transport: - Interfere with the ubiquinol cytochrome C-oxidoreductase
Secondary metabolites	
Phenolic compounds: -Phenolic alcohols (thymol, carvacrol, eugenol)	- Cell membrane or cell wall union through hydrogen bonds - Inhibition of enzymatic processes - Union to surface-exposed adhesins, cell wall polypeptides and membrane-bound enzymes
Alkaloids	- Intercalate with DNA
Terpenes	- Damage to biomembranes
Saponins	- Disintegration of the membrane
Proteins and polypeptides	- Degradation of cell wall polymers, cell membrane channels - Degradation of ribosomes - Inhibition of DNA synthesis

References 12, 33, 52, 83, 85-86.

Terpenes action mechanism is not fully understood but it has been proved that terpenes are primarily responsible for the antimicrobial activity of essential oils; and their antimicrobial effect is based on the ability to damage biomembranes. Depending on their lipophilic characteristics they interact with membrane enzymes and interfere with vital processes such as osmosis, synthesis of sterols and phospholipids [12]. Saponins antifungal activity of is due to their ability to form complexes with the sterols in the membranes of the fungi which causes its disintegration [12, 85].

And finally, antifungal proteins and polypeptides have very varied mechanisms of action, including degradation of cell wall polymers, membrane channels, degradation of ribosomes, and inhibition of DNA synthesis. There are many proteins whose mode of action is not yet known [86]. To our knowledge there is no research works on isolated individual secondary metabolites from Cactaceae with proved antifungal activity, therefore there are no reports on the structure-activity relationship. Furthermore, there are no reports on *in vivo* studies of the application of components with antifungal activity from Cactaceae.

4. Conclusion

The Cactaceae family can be considered an important source of bioactive substances with antifungal activity and excellent candidates for the development and formulation of new generation antifungal agents with fewer side effects, a broader action spectrum and lower cost than the current ones. More research needs to be developed to identify the bioactive components and evaluate their future applications.

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