THE MEXICAN MEDICINAL PLANTS WITH ANTIFUNGAL PROPERTIES ARE AN ECONOMIC AND HEALTH OPPORTUNITY AREA

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Summary

The traditional knowledge on Mexican medicinal plants is taken into account for their possible application in mycoses control. The medicinal plants are easy accesses and low cost, their use responds to the social, economic and cultural needs. In the context of the economic potential benefit of production and commercialization of medicinal plants, we are discussing the relevance of plant antifungal derivatives, particularly the extract features, its active compounds, its mechanism of action and the potential they offer for to mycosis control. The attention is focus in the search of secondary metabolites with high antifungal potential against plant and animal pathogenic fungi, from Mexican endemic plant; *Artemisia ludoviciana* Nutt, *Enterolobium cyclocarpum* (Jacq) Griseb, *Heliopsis longipes* “A. Gray” Blake, *Satureja macrostema* “Bent” Briq and *Tagetes lucida* Cav.

Key words: Antifungal compounds, developed rural, mycoses

Economic Potential of Medicinal Plants

The knowledge of pharmacological and clinical aspects of medicinal plants used by the autochthonous Mexican culture is an opportunity for to strengthen the social ties of the Mexican to their rural community and land, due to potential bear medicinal plants as an extra source of money as a result of an increase in demand for medicinal plants and of expansion of this economic sector.

Although no reliable statistic data exist of the formal commerce of vegetal species and the amount of medicinal plants that are commercialized in Mexico, some indices could be considered for the planning of the economic opportunity that medicinal plants offer rural communities, some of them are: 1) Dry medicinal plants that are commercialized in the popular markets of the main cities of Mexico. After interviewing the managers of local medicinal plant stores, it’s been estimated that in Morelia city (Michoacan, Mexico) (Figure 1), the cost of 500 kg of dry medicinal plants per month is US$1,400.00.

Figura 1. Mexico map. Mexico have thirty two Federal States and Michoacan State (black) is localized on West Central on southern coast of Pacific Ocean between at 17°54'34" to 20°23'37'' North latitude and between at 100°03'23" to 103°44'09'' West longitude.

The plants used for tea infusions and for extraction of their active principles in large scale such as *Tagetes erecta* (cempasuchil, native name) or *Capsicum* spp (chile, native name) were not considered. 2) The supply of medicinal plants for the increase number of companies and formally establishes associations related with the commercialization of medicinal plants for national and international use. 3) The increasing requirement of medicinal plants is important in the economic sector as well as for the public health. In the economic, a conservative estimation of the actual market price is of eight million of dollars annually. Meanwhile the importance to Mexican public health is founded on the elevated consumption of natural and homeopathic products, formulated with medicinal plants. This socio-economic situations have gained interest of the Mexican federal government, that have made an effort to regulate this activity, instituting entities that regulating and maintaining vigilance of socio-economic sector of medicinal plants. 4) It is been estimated that more than 85% of medicinal plants that are commercialized in the country, they are obtained from their natural habitat. A lot of them are native from central and south Mexico (location of Michoacan State), some of them could be exhausted with the subsequent loss of tradition and good sustainable manage of medicinal plants practiced by the local traditional medical. Also, these indexes show that in Mexico the domestication, production and commercialization of medicinal plants is an economic resource scarcely exploit in a country of great ethno-medicinal tradition and that in the pass it has not been considered as an economic opportunity for the improvement of the habitants of the rural areas, meanwhile nowadays it is an economic sector that could co-aid the economy of a developing country as Mexico. In addition to the potential economic benefits visualized for the systematic production of medicinal plants, other aspects not considered in Mexican laws should be taken in account, such as the ecologic benefit due to the halt of disturbance of the natural locations of propagation, conflicts with the culture lands due to the relative abundance, the potential threaten of extinction, the inconvenient of the seasonal culturing of the specie, the official transacting and the diplomatic actions with the owners of the Mexican fields towards subtraction and culture of the wild species.

Role of Medicinal Plants in Mexican People

Humankind has found in plants a cure for countless diseases, facts that have been registered in ancient texts of different cultures, including the ones from Mesoamerica. Nowadays, in rural areas of Mexico it has been found, that the proportion between herbal therapist and medical doctors is four to one, which indicates the use of traditional medicine as the best alternative to access to health service for this population. Furthermore, modern medicine practice began recently in many rural Mexican areas, where the people rarely have the economic resources to pay for costly modern synthetic drugs. This is why the population continues to trust in regional medicine plants and other sources of traditional medicine.

It has been estimated that 85% of the Mexican population uses plants with medical purposes as well as food ingredients. In other countries such as Pakistan, it is estimated that 80% of the population depends on medicinal plants for treatment, comparatively in China where 80% of the population rely on the same resource. Since past century it was evidence those technologically advanced countries as United States of America, 60% of the population uses medicinal plants to control certain diseases, meanwhile in Japan there is a greater demand for medicinal plants then for official modern medicine [1]. Summing, the overall interpretation is that the use of medicinal plants as a health source is independent to the technological development of the country.

It is known that Mexico posses approximately 23,000 to 30,000 vascular plant species, that represent a flora greater that of United States of America and Canada together, and of equal magnitude as the one of ex Soviet Union. For Michoacan State, it is not known the exact number of plant species [2], but in a conservative manner it is believe the existence of 4,000 species, many of which are use in traditional medicine [3]. Due to the great biodiversity and geographic location of México, it has been available a large amount of botanic material with therapeutic purposes that since early times has been use empirically. Is for this, the need for scientific studies that demonstrate the benefit of plants what tradition has known since long time.

Reasons to Search Plant Metabolites to Treat Mycosis

Folk medicine uses a big amount of medicinal plants to treat a broad array of diseases such as diarrhea, mycoses, zoonosis and flu; other high social and economical impact diseases such diabetes, hypertension, heart disease and cancer. However, to show the economic potential of medicinal plants in Mexican society development, it will be necessary to consider an example, those plants that have been selected carefully and wisely for centuries of observation; being our focus those plants that have been use to treat mycoses.

The mycoses have increased alarmingly due to the emergence of resistance to the conventional antifungal agents, the persistence of the compound in the organism and in the environment, the undesirable collateral effects, and the increase prevalence of immunocompromised patients; factors that affect our methods to have an effective control of the mycoses in humans, other vertebrates and plants [4].

Not so long ago, synthetic fungicidal agents were considered agents with low grade toxicity. However, in 1986, the National Academy of Science of the United States, determined with respect of pesticide residues in food, that fungicidal compounds are the chemical agents with the greatest carcinogenic potential when compare with insecticides and herbicides together [5]. Furthermore, the problem of the resistant pathogens to these chemical compounds has worsen to such degree that in data from 1993, more than 150 species were resistant, especially fungi [6]. For this reason, interest has emerged on the identification of the compounds of vegetal origin with antifungal properties, but with different mechanism of action then the conventional compounds. Also, it has increased the need of the development of new strategies to prevent the fungi infections with the usage of environment friendly preparations and formulas with the pure antifungal active form or the use of different extracts from different solvents (water, ethanol, chloroform, ethyl acetate and hexane), essential oils and dusts.

Plants produce oxygen, nutrients and a great variety of useful products in the industries such as food, textile, cosmetic and pharmaceutical. Plant products and secondary metabolites are use as artificial sweeteners, flavors, biocides, pigments and medicines, all of them have properties known for more than 20,000 years. The employment and ability to manage the knowledge of the chemical strategies of plant survival developed from the interaction between the microorganisms and predators, has demonstrated the potential of this natural source in the search of new molecules with bactericidal, fungicidal and insecticidal activity. Interestingly, the pharmaceutical analysis has validated those plants that tradition has uses based on a method of trial and error, demonstrating their efficacy, although some have shown to be innocuous and others potentially dangerous.

A reduce number of vegetal species has been studied as fungicides, since there are at least half a million flower plants that have not been studied and whose active principles could be decisive in the control of diseases caused by fungi.

Nevertheless, a small number of substances of vegetal origin have been exploit by modern medicine, mostly due to the discovery of antibiotics from fungi in the 1950's, and the development of synthetic fungicidal agents [7].

The advantages of the antifungal of vegetal origin is the specificity of their mechanism of action against pathogens, their ability to rapidly inactivate under environmental conditions, and the fact that they do not harm beneficial organisms or mammal cells such as humans [8].

Antifungals

The ideal antifungal should be of broad spectrum, low cost, non-toxic for the host, and with the capacity to be administered through different routes, without resistance induction and with adequate pharmacokinetic properties. Commercial antifungal include different chemical structures and mechanism of action. The classification of antifungal agents could be by its chemical structure (polyenes, azoles, allylamines, lipopeptides, morpholine derived, pyridone, benzofurane, thiocarbamate, essential oils, etc.), or by its origin (substances produced by living organisms or chemical derived), or by its action spectrum (broad or limited spectrum), or by its mechanism of action (fungistatic or fungicidal), or due to the route of administration or employment on the host and for their toxicity and the selectivity of their action [9]. The treatment of mycosis was developed from the usage of antifungal considered as "first generation" such as heavy metals, to the "second generation", obtained synthetically. For the treatment of severe infections, the antifungal are limited to amphotericin B, fluconazol, itraconazol and 5-fluorocytosine (Table 1) [10]. For the control of these microorganisms, antifungal of systemic and topical action are used. The former such as amphotericin B, an antibiotic of the chemical class of the macrolides, whose activity depends on the joint to the sterols fraction in particular of ergosterol of the fungi membranes, those resulting in an increase in membrane permeability.

There are two chemical groups of azolic antifungal, the imidazoles (clotrimazole, miconazole, ketoconazole, econazole, butoconazole, oxyconazole and sulconazole), and the triazoles (terconazole, itraconazole and fluconazole); All of them can be use systemically and orally.

These antifungal agents are important as inhibitors of synthesis of ergosterol -a component the fungi cellular membrane of fungi- inhibiting the proliferation of organisms as Ascomycetes, Deuteromycetes and Basidiomycetes [11]. Tetraconazole is a systemic fungicidal compound and is important for its great efficacy, broad spectrum and optimal selectivity, since its active radical, tetrafluoro ethyl esther, does not act on phytosterols or gibberellins in treated plants [12].

Some triazoles of the most recent generation, can affect phytosterols and gibberellins production, causing phytotoxic effects as plant size reduction (Table 1). Due to its differentiated systemic behavior, with a favorable equilibrium between liposolubility and hydrosolubility, it expresses an optimal tissue distribution in vegetal plants, providing a uniform and complete protection [13].
Three main difficulties exist when searching a new antifungal: 1) both fungi and host cells are eukaryotic and share similar biochemical characteristics, thus the great possibility of causing adverse effects in the host. 2) The diversity of fungi species with multiple check points and genetic instability [14]. 3) Co-evolution of pathogen to avoid the fungicide action and to survive.

Table 1. Generations of antifungal compounds used commercially, action spectrum, effect and origin.

ANTIFUNGIC	**ACTION SPECTRUM**	**EFFECT**	**ORIGIN**
1ª **GEN**			
Flucitocine	Analogous to nucleic acid precursors Inhibitor of pyrimidins synthesis	Fungicide	Synthetic
Heavy metals	Toxic to metabolism and respiration Barrier against sporangia and zoospore germination	Fungicide	Synthetic
Metaloids	Keratolytic	Fungicida weak	Synthetic
Sulphonamides	Inhibitors of DNA synthesis and Dihydrofolate synthase	Fungistatic	Plant
Sulphur derivates	Inhibitor enzymatic e.g. Lipoxygenase and antagonist of different receptor proteins	Fungicide	Plant
Tonalftate	Blocking the ergosterol synthesis by to inhibit the epoxydation of escualene	Fungistatic	Synthetic
2ª **GENE**			
Allyl amines	Blocking the ergosterol biosynthesis and accumulate scualene	Fungicide of broad spectrum at low concentration	Synthetic
Azoles	Blocking the ergosterol synthesis, they are inhibiting the enzyme lanosterol demethylase dependent of cytochrome P_{450}	Fungistatic at low concentration and fungicides at high dosis	Synthetic
Benzofuranes	Mitotic inhibitor, affecting the DNA replication and chitin synthesis like to griseofulvin action	Fungiestatic	Fungal
Lipopeptides	Altering the plasmatic membrane to lyses	Fungicide	Fungal
Morfoline derivates	Inhibition of sterol synthesis, modifying the membrane permeability. Inhibition of assemble of oomicete cell membranes	Fungicide	Synthetic
Piridone	Induce the depletion of electrolytes on fungus and reduce the nucleic acid and protein synthesis	Broad spectrum Fungistatic	Synthetic
Polyenes	They are joining to ergosterol to forming ionic channel on fungic membranes affecting the membrane permeability, it lost K^+, monosaccharides and other metabolites	Fungistatic at low concentrations and fungicide at high concentrations	Fungal
3ª **GENE**			
Estrobilurin	Affecting the mitochondrial respiration.	Fungicide	Sinthetic
Tetraconazol	Inhibition of sterols synthesis, inhibitors of 14-α-lanosterol demethylase dependent of cytochrome Fungicide P_{450}	fungicide	Synthetic and semi synthetic

Due to the underlying absence of an ideal antifungal drug and considering the variables that can affect its pharmacological activity, *in vitro* as well as *in vivo*, it is necessary to continue the search of new antifungal agents. Antifungal of vegetal origin emerge as an alternative of health and economic potential.

Plant with Antifungal Properties

Early reports of substances produced by plants with high antifungal potential: Maruzzella [15] studied over 119 essential oils of vegetal origin of which 59 were effective against fungi, Magboul *et al*. [16] and other groups of researches have demonstrated that dermatophytes and other pathogen fungi are sensible to compounds produced by plants such as sesquiterpene lactone, present as the active principle in the Asteraceae family. There are many works with plants from the Asteraceae family that show effect against pathogen fungi, the most important belonging to the genus *Achillea*, *Ageratum*, *Artemisia*, *Aster*, *Blumea*, *Caesulia*, *Centaurea*, *Crepis*, *Inula*, *Solidago*, *Parthenium*, *Pentanema*, *Tagetes* and *Tanacetum*. Plants from other fifty families demonstrate also antifungal characteristics, the best being the Fabaceae and Brassicaceae [17, 18, 19].

Plant Secondary Metabolites Against Fungi that Affect Human Health

The most important human diseases caused by fungi are the dermatomycosis, superficial mycosis, subcutaneous mycosis and profound mycosis. These diseases are caused by known fungi as *Trichophyton rubrum* (Ascomycetes), *Candida albicans* (Basidiomycetes), *Sporothrix schenckii* (Deuteromycetes), *Cryptococcus sp* and *Aspergillus sp*, that infect skin, subcutaneous tissue and inner organs as lung, bones, joints, muscle and genitourinary tract. Recently new pathogens have been detected, among them some opportunistic yeasts (*C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, C. dubliniensis, Saccharomyces sp* and *Rhodotorula sp*), Hyalohyphomycetes as *Aspergillus, Fusarium* and *Scopulariopsis*; species of Zygomycetes as *Absidia, Mucor* and *Rhizomucor*. Also the Phaeohyphomycetes as *Alternaria, Bipolaris, Curvularia, Pneumocystis carinii*; and others capable of causing sinusitis, cutaneous infection, endophthalmitis, pneumonia, and rhinocerebral mycosis. The vegetal metabolites as vernolepin, vernodaline, taraxasterol, parteniol, gayuolane, artemisinin and some flavones, have shown toxicity against *A. niger*, *Trichoderma viride* and *C. albicans*, and dermatophytes [20]. Zheng *et al.* [21] proved the antifungal effect of isolated flavones of *Artemisia giraldi* against filamentous fungi *A. flavus* and *T. viride.* The plant *A. mexicana* possess a strong activity against *C. albicans* [22]; the sesquiterpene lactones of *Vernonia amygdalina* inhibit the growth of *A. niger* and *C. albicans* [16]; extracts of *Rhubarb* are effective against species of *Trichophyton*, *Microsporum* and *Epidermophyton* [23]. Reports of other plant metabolites against fungi have demonstrated adverse effects on human health (Table 2). Interestingly it has even been reported that vegetal extracts of some plants strengthening the effects of chemical synthetic fungicides [24].

Table 2. Plant species and plant secondary metabolite against fungi that affecting the human health

PLANT SPECIE	METABOLITE	FUNGUS TEST	REFERENCE
Achillea fragrantissima	Terpineol	*C. albicans*	[27]
Ageratum conyzoides	Essentials oil	*Epidermophyton floccosum* *Microsporum cani* *Trichophyton mentagrophytes*	[28]
Artemisia giraldi	Flavones	*A. flavus* *Trichoderma viride*	[21]
Azadirachta indica	Triterpenes, Azadirachtin	*M. nanum* *T. rubrum* *T. mentagrophytes*	[29]
Camellia sinensis	epicatequin-3-O-galate (ECG)	*C. glabrata* *Clavispora lusitatiae* *C. laurentii*	[30]
Citrus bergamia	Bergaptene, Limonene	*Candida* spp	[31]
Drimys brasiliensis	sesquiterpen drimane	*E. floccosum* *T. rubrum*	[32]
Oxalis erythrorhiza	3-Heptadecyl-5-methoxy-phenol	*M. canis* *M. gypseum* *T. mentagrophytes* *T. rubrum*	[33]
Parthenium argentatum x P. tomentosa	Partheniol, Guayulone	*A. niger*	[19]
Pelargonium graveolens	Chlorotalonyl	*Colletotrichum gloesporioides*	[34]
Piper regnellii	Conocarpan	*C. albicans*	[35]
Ranunculaceous bulbosus	Protoanemonin	*E. floccosum*	[36]
Richardia brasiliensis	Tannins	*C. albicans* *E. floccosium* *T. verrucosum*	[37]
Solidago virgaurea	Triterpenoids	*C. albicans*	[38]
Tagetes erecta	Essentials oil	*T. mentagrophytes*	[39]
Tribulus terrestris	Espirostanol	*C. albicans* *Cryptococcus neoformans*	[40]
Vernonia amygdalina	Vernodalin, Vernolepin, Taraxasterol	*A. niger* *C. albicans* *C. albicans*	[16] [16] [41]

Role of Plant Antifungals in Agriculture

There have been described more than 200 species of fungi that affect crops, their presence on the crops and deficient manage of pathogen represent great economic losses for the agricultural industry. Most of them belonging to eight fungi genera: *Alternaria, Aspergillus, Botrytis, Colletotrichum, Fusarium, Helminthosporium, Rhizoctonia* and *Verticillium* [25]. The problem has reached such amplitude that most of fungicides in the world –over 300 chemical products– are use for the control of plant fungi. This number is increasing each year due to the incessant research in this field. In most of the cases, the action of fungicides is preventive, being effective in the initial state of infection, when the hyphae have not penetrated the plant tissue.

The preventive fungicides are absorbed by the plant cuticle and they are not transported to the plant interior. In contrast with systemic fungicides or vegetal chemotherapeutics of the last generation, those are absorbed by the plant roots, leafs and seeds [26]. For to phytopathogenic fungi, triazoles as tetraconazole, a fungicidal from the last generation, has been used. Nevertheless, the benzene substitutes, thiocarbamates, ethylene-*bis*-ditiocarbamates, thiophthalimides, benomyl, cycloheximide, triforine and organometallic compounds of cooper, mercury, tin and cadmium, they are the most widely consume in the world on the last century.

However, all of them share great disadvantages, owed to their capacity to pollute the environment, with phyto and zootoxic effects [42].

Some metabolites produced by plants have been employed with success in the control of phytopathogenic fungi. For example, anethole extracted from seeds of *Pimpinella anisum (Umbelliferae)* has shown antifungal activity against *A. niger*, when applied with sesquiterpene dialdehyde polygodial isolated from several plants. This is an example of synergistic antifungal effect, where two or more compounds exhibit grater potency than that of one alone [43]. Also, there have been isolated two isoflavonoids, the colutequinone and the colutehydroquinone from the root cortex of *Colutea arborescens*, which have shown activity against 38 strains of *Aspergillus sp*. This fungus was also sensible to nyasol, a compound obtained from *Anemarrhena asphodeloides* [44,45]. Commercial formulations of extracts and essential oils of *Cassia sp.* mixed with *Capsicum* sp. and *Sinapis* sp., were applied in soil with melon crop, demonstrating antifungic activity against the fungus causing of fusarium wilt of melon, *F. oxysporum* f. sp. *melonis* [46]. Other studies indicate a fungitoxic effect of vegetal extracts against *Aspergillus* spp, *Botrytis cinerea, Colletotrichum* spp, *Helminthosporium oryzae, Pythium ultimum, Rhizoctonia solani, Sclerotium rolfsii,* among others (Table 3).

On the other hands it has been demonstrated that plant derivates used in agriculture contain active substances compatible with the environment, they are innocuous to humans since no residues or toxicity are generated.

As an example, soy lecithin, that contains phosphatidylcholine and a fungistatic lipid. We can also mention neem oil (*Azadirachta indica)*, a product with systemic action that has demonstrated efficacy in the prevention and control of soil fungi as *Olpidium* spp and oomycete *Phytophthora* spp [59].

Table 3. Plant species and secondary metabolites used in the control of phytopathogen fungi and fungi-like organisms.

PLANT SPECIE	METABOLITE	FUNGI/FUNGI LIKE ORGANISMS TEST	REFERENCE
Ageratum conyzoides	Chromenes	*Fusarium* spp *Rhizoctonia solani* *S. rolfsii*	[47]
Ananas comosus var. Cayenne	Cinnamic acid, Ferulic acid, Fumaric acid	*Pythium* sp	[48]
Chrysanthemum coronarium var. Spatiosum	Crisancorin	*Botrytis cinerea* *Physalospora piricola*	[49]
Clytostoma ramentaceum	Ursolic acid	*A. niger* *F. oxysporum*	[50]
Curcuma longa	Ethyl *p*-methoxy cinnamate	*H. oryzae*	[51]
Holmskioldia sanguinea	Wogonin	*B. cinerea*	[52]
Lawsonia inermis	Lawsone	*H. oryzae*	[53]
Parthenium hysterophorus	Terpenoids, Partenin	*Aspergillus sp* *Alternaria solani* *H. maydis* *Sclerotium rolfsii*	[54]
Reynoutria sachalinensis	Emodin	*Fusarium* spp	[55]
Ruta graveolens	Epoxyde of rutacridone	*B. cinerea* *Colletotrichum fragariae* *C. gloeosporioides* *C. acutatum* *F. oxysporum*	[56]
Wedelia biflora	Methyl kaurenoic acid	*Pythium ultimum*	[57]
Zingiber officinale	Aqueous extract	*A. niger* *A. flavus* *F. oxysporum*	[58]

Some Mexican Plants with Antifungic Properties.

Mexico possesses a privilege location on the terrestrial surface, and works as a bridge between two subcontinents. Its orography and large coasts make a diverse and ecological unique environment capable of sheltering numerous of vegetal species. Great effort has been made on floweristic inventories, and ecological and botanic studies [60, 61, 62]. Understanding of these pharmacologic and clinical aspects of the autochthon Mexican culture constitutes an important source of information in the search of new active medicinal agents [63]. A total of 4,000 plant species are used by 62 ethnic Mexican groups, but only 1% of these plants have been exhaustively studied, as for the rest, their active principle and possible beneficial effects on human health and agriculture remain unknown [60].

It has been found that numerous Mexican plant species contain antifungal metabolites, specially the Asteraceae, Labiatae, Fabaceae and Brassicaceae families, that through analysis, their fungicidal activity has been determine. In recent time it has been demonstrated the fungicidal effect of the mixture of affinin and bornyl decatrienate [extracted from *Heliopsis longipes* "A. Gray" Blanke (chilcuague)] and tymol epoxide [metabolite obtained from *Helenium mexicanum* H.B.K (cabezona)]. At *in vitro* bioassays the mixture inhibits growth of *C. lindemuthianum*, thus justification the use of this metabolite as antifungal agent in bean plants [64, 65] (Table 4).

Table 4. Some Mexican plants and their metabolites that affecting to fungi and fugi-like organisms growth

PLANT SPECIE	METABOLITE	FUNGI/FUNGI LIKE ORGANISMS TEST	REFERENCE
Asclepias curassavica	Terpenes, Cardenolides	*C. albicans*	[66]
Bouvardia ternifolia	Essentials oil	*R. solani*	[67]
Chenopodium ambrosiodes	Terpenes	*R. solani*	[68]
Eupatorium aschenbornianum	Benzofuranes	*T. mentagrophytes* *T. rubrum*	[69]
Flourensia cernua	Essentials oil	*R. solani*	[67]
Heliopsis longipes	Affinin, Bornyl decatrienate	*C. lindemuthianum* *C. gloesporioides* *P. cinnamomi*	[70] [65] [64]
Larrea tridentata	Resine	*Pythium* sp	[71]
Origanum majorana	Essentials oil	*P. infestans* *R. solani*	[67]
Persea americana Mill.	(E,Z,Z)-1-Acetoxy-2-hydroxy-4-oxo heneicosa-5,12,15-triene	*C. gloeosporioides*	[72]
Tagetes lucida	6,7-dimethoxy-4-methyl cumarine, Escoparon	*F. moniliforme* *F. sporotrichum* *R. solani*	[73]

As a result, our group study selected five native species from west Mexico: *Artemisia ludoviciana* Nutt. (estafiate), *Enterolobium cyclocarpum* (Jacq.) Griseb (parota), *Heliopsis longipes* A. Gray Blake (chilcuague), *Tagetes lucida* Cav (santamaría) and *Satureja macrostema* Benth. Briq. (nurite).

The selection was based on the historic use of these plants in traditional medicine of the Mexican north central and western region, and the lack of knowledge of their fungicidal properties, except for the chilcuague, whose antifungal effects have been determined early [74,75,76].To study the antifungal properties of these plants, three different extracts were tested (aqueous, chloroformic methanolic and ethyl acetate) obtained from the green area (steam and leafs), root or flower of each plant.

All the extracts were tested against five wild strains of fungi: *C. lindemuthianum, C. albicans, S. schenckii, S. cerevisiae* and *M. circinelloides*. Data show that the greatest antifungal effect they stopped fungi growth was induced by the chloroformic methanolic extracts from steam and leafs. The above is true except for *H. longipes,* in which the root extract was more effective. The maximal antifungal effect was obtained from the chloroformic methanolic extract from the air part of the “estafiate” similar to that display by “chilcuague” roots (Table 5).

Table 5. Effect inhibitory of chloroformic-methanolic extracts from four Mexican medicinal plant on fungal growth. The fungal susceptibility to extracts was done by the classic paper disk-agar diffusion assay as reported by Raahave [77]

PLANT SPECIE	FUNGI TEST	INHIBITION (%)
Artemisia ludoviciana (Leaves and stem)	*C. albicans*	53
	C. lindemuthianum	50
	M. circinelloides	61
	S. cerevisiae	53
	S. schenckii	55
Heliopsis longipes (Roots)	*C. albicans*	43
	C. lindemuthianum	33
	M. circinelloides	69
	S. cerevisiae	0
	S. schenckii	43
Satureja macrostema (Leaves and stem)	*C. albicans*	45
	C. lindemuthianum	41
	M. circinelloides	0
	S. cerevisiae	60
	S. schenckii	37
Tagetes lucida (Leaves and stem)	*C. albicans*	50
	C. lindemuthianum	35
	M. circinelloides	37
	S. cerevisiae	50
	S. schenckii	81

In the same way, the maximal inhibitory effect of the extract of *A. ludoviciana* against the growth oomycete *P. cinnamomi* was observed. Secondary metabolites are other important aspect in searching of antifungal agents from plant, some of them inhibiting the catalytic activity of enzymes of metabolic pathways belongs to fungal vital functions such as folate synthesis. Folic acid and derivates (folates) are essential cofactor vitamins in enzymatic reactions of fluxes through C_1 metabolism in all organisms. All of them carry out a critic cellular function in array enzymatic reactions of different process vitamin dependent, where the vitamins as folate are essential factors.

Their biochemical understanding is partial to cause of complex cellular process, with reference to human being, he have 30,000 genes and about of 3,800 enzymes in catalog at present, and 22 % of them needing a cofactor to make catalytic process, a molecular companion that in many cases is a vitamin as folate. In general the B2, B6 and B12 vitamins and folates are involved in enzymatic process of DNA methylation, phosphorilates compounds synthesis, synthesis and to recycling folates. On the other hand failure in folate intake cause an increase in uracyl incorporation into DNA with a increase in chromosomes lysis and DNA hypermethylation both double and single strand. It was observed an uracyl excess into cell with folate deficiency, too [78].

However the folates are synthesized by microorganisms and plants but not for vertebrates. The folates are essential molecules for organisms because they have not a folate pathway complete.

This characteristic appealing to search selective plant antifungic, in this context it was observed that hardwood aqueous extract from *E. cyclocarpum* have components that *in vitro* assay inhibiting the dihydrofolate reductase (DHFR) using crude extracts cells free from fitopathogen filamentous fungi same that Trimetoprim a conventional DHFR inhibitor (Table 6). So far no effective antifungic based on DHFR inhibition or other enzymes of folate pathway is used in therapy. The known inhibitors are neither potent nor selective. However some pure plant secondary metabolites that *in vitro* inhibiting the DHFR, could be the molecular template to chemical modification with aim to increase potency and selectivity.

Table 6. *In vitro* effect of aqueous extract obtained of hardwood *E. cyclocarpum* on dihydrofolate reductase (DHFR) from fitopathogen fungi. Enzymatic activity was measurement for the method reported by Toth [79]

Fungi	**Specific activity (μmol/min/mg)**		
	Control	**Trimetoprim (mg)**	**Aqueous extract (mg)**
C. acutatum	98.5 ± 9.2	0.2 ± 0.04	1.4 ± 0.2
C. lindemuthianum AFG1	38.2 ± 3.9	1.5 ± 0.4	3.6 ± 0.3
C. lindemuthianum AFG2	27.9 ± 4.6	0.8 ± 0.1	4.6 ± 0.2
C. lindemuthianum AFG3	67.6 ± 9.1	0.2 ± 0.1	0.8 ± 0.2
C. lindemuthianum 75A	122.4 ± 12.3	2.1 ± 0.5	3.9 ± 0.3
C. lindemuthianum 75B	173.3 ± 14.9	0.8 ± 0.2	7.6 ± 0.7
F. oxysporum	180.8 ± 23.4	1.8 ± 0.4	3.2 ± 0.2
Rhizoctonia sp.	62.8 ± 9.0	0.8 ± 0.2	0.9 ± 0.1

Conclusions

The search of efficient antifungal is an actual need, since fungicides currently used have not been efficient in the eradication of mycoses. This fact generates an alternative to use the medicinal plants or their pure metabolites to inhibit fungal growth without harming host.
The plant species *A. ludoviciana*, *E. cyclocarpum*, *H. longipes*, *T. lucida* and *S. macrostema*, all of them are not including the list of species in extinction risk of Mexican official policy [80], and could be collecting from wild habitat for to domestic and commercial uses.However is necessary to continue the investigation of ecology, physiology and culture techniques of this plant species for to not to lose this natural resource. The limited action of Mexican health system into rural zone, a low rural wage and idiosyncrasy of Mexican people will increase the use and trade of medicinal plants. The domestic production of plant medicinal can get to buffering to healthcare needs and to be an extra income for to poor population.

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