Phytochemicals: new weapons against new enemies

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Phytochemicals exhibiting various degrees of antimicrobial activity occur in plant stems, leaves, barks, flowers, and fruits. The advent of antibiotic resistant microbial pathogens has imposed the need to search for new weapons to fight against them. In this sense, natural phyto-antimicrobials appear to be safe when compared to new synthetic antimicrobial agents, attending to the consumers' demands for "more natural, greener and cleaner" drugs. Central and South America offer a wide variety of plants comprising a diverse ecological niche where to find these compounds. The aim of this chapter is to review the bibliography in order to describe natural phyto-antimicrobials that can be found in this vast scenario. The emphasis is placed on target microorganisms so as to give an easy and quick way of finding specific Central and South American plant-derived phytochemicals for each pathogenic microflora.

Keywords Americas; natural phytochemicals; microflora

1. Introduction

Among the flora of different regions of the world, Latin America represents one of the wealthiest sources of material with pharmacological activity due to its biodiversity [1]. It possesses a very high number of vascular plants (85,000 plants) [2] and there is a recent evidence that neotropical forests located in Latin America possess the highest diversity of plants in the world [3]. In addition, some factors critically distinguish the medicinal plants of Latin America. (A) This region possesses a huge unexplored biodiversity [4]; (B) there is a rich tradition of use of medicinal plants [5, 6]; (C) the ethnopharmacological knowledge has been tightly kept or transmitted by the many indigenous populations still living in this region [4, 7-15] and (D) these resources have been poorly studied.

Most plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganisms. More than 10,000 plant secondary metabolites of antimicrobial importance have been isolated. Among secondary metabolites, the main group belongs to phenols, in which are found tannins, flavonoids, quinones; followed by terpenoids, essential oils, alkaloids, lectines and polypeptides and other mixtures [16, 17]. These active compounds from plants may have antiseptic or antibacterial function [18]. In many cases the mechanism of action of these plant extracts and essential oils is well known: such as disturbance or disintegration of the cell structures and membrane, destruction or inactivation of genetic material, destabilization of the proton motive force, electron flow, active transport and coagulation of the cell content [18-22].

Microorganisms have developed resistance to many antibiotics probably due to the indiscriminate use by health professionals and the general population. This situation has generated an immense clinical problem in the treatment of infectious diseases, resulting in a huge public health problem. These problems have revived the interest in studying new points of view from plants with already known antimicrobial properties [23], as well as in the search for new species of plant able to provide new natural antimicrobial compounds. The aim of this chapter is to review the bibliography in order to describe natural phyto-antimicrobials that can be found in Latin America. The emphasis is placed on target microorganisms (Gram positive and Gram negative bacteria, molds and yeasts) so as to give an easy and quick way of finding specific Central and South American plant-derived phytochemicals for each pathogenic microflora.

2. Gram positive bacteria

Gram positive bacteria are those that are stained dark blue or violet by Gram staining. Gram positive organisms are able to retain the crystal violet stain because of the high amount of peptidoglycan in the cell wall. Gram positive cell walls typically lack the outer membrane found in Gram negative bacteria.Outside the cytoplasmic membrane of the gram positive bacteria the fundamental polymer is peptidoglycan, which is responsible for the maintenance of cell shape and osmotic stability. In addition, typical essential cell wall polymers such as teichoic or teichuronic acids are linked to some of the peptidoglycan chains. Generally, because their structural characteristic, gram positive bacteria are more sensitive to phytochemical or natural products with antimicrobial properties than gram negative bacteria.

2.1. *Staphylococcus* spp.

The *Staphylococcus* genus includes at least 40 species. Most are harmless and reside normally on the skin, for example *S. epidermidis*, and mucous membranes of humans and other organisms. *Staphylococcus aureus* is a bacterium that is carried on the skin or in the nose of approximately 25% to 30% of healthy people without causing infection. Most of these skin infections are minor, are not spread to others, and usually can be treated without antibiotics. However, nowadays there are more resistant strains because of the abusive and indiscriminate use of synthetic antimicrobial compounds. Being an aerobic, undemanding and fast growing bacterium, *S. aureus* is widely utilized as target microorganism in antimicrobial assays. It has been documented [24-26] that *S. aureus* is one of the bacteria most susceptible to plant extracts. Many studies reported that plant extracts of several vegetal species growing in the center and the South of America are active against this genus. For example, Oliveira et al. [27-28] described that essential oils of *Lippia* species that are used in a Brazilian community had good antibacterial activity against *S. aureus* ATCC 25923 and MRSA (methicillin resistant *S. aureus*). Toledo et al. [29] demonstrated that *Annona coriacea, Curatella americana*, *Kielmeyera lathrophyton*, *Plathymenia reticulata* and *Sclerolobium aureum* showed antimicrobial activity, which are commonly employed as medicines by the population of Brazil. Among the aforementioned species, the extracts from *K. lathrophyton* showed the best antibacterial activity against Gram-positive bacteria (minimum inhibitory concentration (MIC) = 250 µg/ml against *S. aureus* ATCC 25923). Moura Costa et al. [30] evaluated several plants that are used in the Rio das Cobras indigenous reserve (Brazil) and they concluded that the aqueous extracts of the leaves of *Campomanesia eugenioides* and aqueous and organic extracts of the leaves of *Cordia americana* as well as the aqueous extract of its bark had activity only against *S. aureus*. In Peru, Kloucek et al. [31] analized six species traditionally used for treating health conditions likely to be associated with microorganisms. These plants are used in Callería district in the Ucayali Department of Peru. All plants possessed significant antimicrobial effect, however, the extract of *Naucleopsis glabra* exhibited the strongest activity against Gram-positive bacteria (MICs ranging from 62.5 to 125 µg/ml). Bussmann et al. [32] reported on the antibacterial activity of ethanolic and aqueous extracts belonging to 140 plant species used in Northern Peru. *Bacharis sp.*, *Borago officinalis*, *Croton lechleri*, *Eugenia obtusifolia, Hypericum laricifolium* and *Phoradendron cf.* were the most actives against *S. aureus* ATCC 25923. Most of these species are traditionally used to treat wound infection, throat infections, serious inflammations, and post-partum infections. In Puerto Rico, Meléndez et al. [33] worked with 172 different plant extracts and they found 14 species with antibacterial properties. Two members of the *Rutaceae* family*, Citrus aurantifolia* and *C. aurantium* were the most effective against *S. aureus,* followed by *Punica granatum, Phyllanthus acidus* and *Crescentia cujete*. Salvat et al. [34] described the results of a screening for in vitro antimicrobial activity in the extracts of 39 plant species from the provinces of Chaco and Formosa (Argentine). It can be observed that all of the "active" extracts were able to inhibit *S. aureus*, either normal strain ATCC 8095 or antibiotic resistant strain INEI 2213. Extracts of *Astronium balansae*, *Geoffroea decorticans*, *Prosopis kuntzei* and *Peltophorum dubium* demonstrated bactericidal activity against *S. aureus*. *Baccharis species* are widespread in South America. Several species are used in Argentinian traditional medicine, Feresin et al. [35] reported the isolation, structural elucidation, and antimicrobial effect of p-coumaric acid derivatives, labdane diterpenes, and flavones from *B. grisebachii* collected in the Provincia de San Juan, Argentina. The labdane diterpene showed inhibition in both *Staphylococcus aureus* (methicilline resistant and sensible strains) with MICs of 125 µg/ml. Zampini et al. [36] in their study with plant species traditionally used in the "Puna" or "Altiplano" of Argentine for ailments related to bacterial infections, showed that ethanol extracts (tinctures) of aerial parts of *Baccharis boliviensis*, *Fabiana* and *Parastrephia* showed the highest levels of antibacterial activity on methicillin, oxacillin and gentamicin resistant *Staphylococcus* with MIC values from 20 to 150 µg/ml. In a recent study, Torres et al. [37] evaluated hydroalcoholic and aqueous extracts of twenty climber species from the *Bignoniaceae* family that grow in the north of Argentina for in vitro antibacterial activity against Gram-positive and Gram-negative strains. Among all plants tested, the tinctures of *Adenocalymma marginatum*, *Amphilophium vauthieri*, *Arrabidaea caudigera* and *Cuspidaria convoluta* showed the most promising antimicrobial properties. This result would support their use for the treatment of skin infectious, mainly caused by *Staphylococcus* strains. *Acmella ciliata* is a native weed of northern South America known by its aliphatic alkamide content and used as an anesthetic and analgesic for toothache and sore throat. Their essential oils and their volatile fractions contain a high proportion of sesquiterpenes such as β-trans-caryophyllene, the major component. Essential oils showed a strong antimicrobial activity against *Staphylococcus aureus* and *S. epidermidis* [38].

2.2. *Enterococcus* spp.

Enterococci are cocci that often occur in pairs or short chains, and are difficult to distinguish from streptococci on physical characteristics alone. Two species are common commensal organisms in the intestines of humans: *Enterococcus faecalis* (90-95%) and *E. faecium* (5-10%). Important clinical infections caused by *Enterococcus* include urinary tract infections, bacteraemia, bacterial endocarditis, diverticulitis, and meningitis. Sensitive strains of these bacteria can be treated with ampicillin, penicillin and vancomycin. *Enterococcus faecalis* is a saprophytic component of the enteric flora and causes severe comorbidities from peritonitis, intra-abdominal abscess and endocarditis. *Enterococcus faecalis* have been reported to have intrinsic resistance to cephalosporins, aminoglycosides, beta-lactams and vancomycin (glycopeptide). Enterococci were reported as the second most common cause of nosocomial infections in the US. Enterococcal meningitis is a rare complication of neurosurgery. New epidemiological evidence has shown that enterococci are major infectious agent in chronic bacterial prostatitis. Enterococci are able to form biofilm in the prostate gland making their eradication difficult. Zampini et al. [36] demonstrated that *Baccharis boliviensis* and *Fabiana bryoides* were more active than the other plant species on multiresistant *E. faecalis*. The Argentinean highland plant *Parastrephialucida* is used in traditional medicine as an antiseptic and anti-inflammatory crude drug was analized by D´Almeida et al. [39], who concluded that the antimicrobial constituents led to 12 phenylpropanoids and two simple phenolics. Most of the compounds occurring in the active fractions were E-caffeoyl or E-cinnamoyl esters included prenyl and phenethyl derivatives. The MIC values of the most active fractions ranged between12.5and 200 mg/mL against reference strains and local isolates of *Staphylococcus aureus* and *E. faecalis*. Joray et al. [40] obtained extracts from 51 native and naturalized plants from central Argentina which were evaluated for their in vitro inhibitory activity on pathogenic bacteria with the aim of selecting the most active ones as new sources of effective antibiotics. Extracts from *Achyrocline satureioides*, *Flourensia oolepis*, *Lepechinia floribunda*, and *Lithrea molleoides* were the most potent, with MIC and MBC values ranging from 0.006 to 2 and 0.012 to 10 mg/mL, respectively, on both gram-positive and negative bacteria. The antibacterial activity-guided isolation of *A. satureioides* ethanol extract showed 23-methyl-6-O-desmethylauricepyrone (1) to be the most active compound. This compound showed inhibitory effects against *S. aureus* and *E. faecalis* with MIC and MBC values of 0.002 and 0.008 mg/mL. Kloucek et al. [31] showed that the extract of *Naucleopsis glabra* exhibited the strongest activity against *S. aureus*, *S. epidermidis* and *E. faecalis* (MICs ranging from 62.5 to 125 µg/ml). The extracts of *Astronium balansae*, *Geoffroea decorticans* and *Peltophorum dubium* were able to inhibit *E. faecium* [34].

2.3. *Bacillus* spp.

Bacillus species can be obligate aerobes or facultative anaerobes, having catalase activity. Ubiquitous in nature, *Bacillus* includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. Two Bacillus species are considered medically significant: *Bacillus anthracis*, which causes anthrax, and *B. cereus*, which causes a foodborne illness similar to that of *Staphylococcus*. *Bacillus cereus* is a sporulating foodborne pathogen capable of causing diarrheal and emetic syndrome diseases [41]. Numerous natural plant extracts and essential oils have shown antimicrobial activities against various foodborne pathogens [42-44]. The best activity against these bacteria was observed for the crude extracts of *Kielmeyera lathrophyton*, against *B. subtilis* (MIC = 500 µg/ml) [29]. Xanthones isolated from the bark of *Kielmeyera variabilis* showed antibacterial activity against *S. aureus* and *B. subtilis* [45].

3. Gram negative bacteria

This bacterial type is more complex than Gram positive, both structurally and chemically. Gram negative cell wall structure contains two external layers to the cytoplasmic membrane. Immediately after the cytoplasmic membrane there is a thin peptidoglycan layer. The area between the external surface of the cytoplasmic membrane and the internal surface of the outer membrane is referred to as the periplasmic space. This space is a compartment containing a variety of hydrolytic enzymes, which are important for the breakdown of large macromolecules involved in cell metabolism. In the case of pathogenic Gram negative species, many of the lytic virulence factors such as collagenases, hyaluronidases, proteases, and beta-lactamase are in the periplasmic space. *Enterobacteriaceae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, together with the non-lactose fermenting bacteria, such as *Pseudomona aeruginosa* and *Acynetobacter baumannii*, are environmental bacteria usually found in soil, water and human habitats. In the human body they are localized in the gastrointestinal tract [46]. The study of these bacteria is always related to risk infections that affect public health. According to the Atlanta CDC (Center for Disease Control and Prevention, USA), the ESKAPE group, namely *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella species*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* species, can cause 2/3 fraction of sepsis associated to medical care. *Escherichia coli* is the one that fulfil the third remaining part, which also includes *K. pneumoniae* that produces betalactamases of extended spectra [47].

3.1. *Pseudomonas aeruginosa*

The bacterium is a highly relevant opportunistic pathogen that causes disease in both animals and humans. It is typically found in soil, water, skin flora, and most man-made environments, since it requires minimal amounts of oxygen for growth, thereby allowing it to colonize a multitude of both natural and artificial environments. *Pseudomonas aeruginosa* is notorious for causing a number of clinical conditions. The organism is able to infect heart valves of intravenous drug users and prosthetic heart valves causing endocarditis. Respiratory infections caused by *P. aeruginosa* occur in individuals with a compromised lower respiratory tract or a compromised systemic defense mechanism. Primary pneumonia occurs in patients with chronic lung disease and congestive heart failure. Moreover, *P. aeruginosa* causes bacteremia (presence of bacteria in the blood) primarily in immunocompromised patients. Also cause meningitis and brain abscesses. Urinary tract infections caused by *P. aeruginosa* are usually hospital-acquired and related to urinary tract catheterization, instrumentation or surgery. This bacterium is the predominant bacterial pathogen in some cases of external otitis including 'swimmer's ear'. *Pseudomonas aeruginosa* is one of the most common causes of bacterial keratitis, and has been isolated as the etiologic agent of neonatal ophthalmia (inflammation of the eye). *Pseudomonas aeruginosa* has also been implicated in folliculitis and unmanageable forms of acne vulgaris. *Ilex paraguariensis* "erva mate" or "yerba mate" is a tree native to the south region of South America. The extract of *I. paraguariensis* obtained by supercritical fluid presented antimicrobial activity against *S. aureus* (inhibition zones between 7 and 18 mm) and *P. aeruginosa* (inhibition zones between 6 and 11 mm), both at concentrations of 100 mg ml^{-1} to 25 mg ml^{-1} [48]. *Urtica dioica* var. *lepthofylla* is native from Ecuadorian mountains to the north of Argentina. It is used to treat stomachache, to treat rheumatic pain and for colds and cough [49]. Ethanol extracts of this plant inhibited *P. aeruginosa* and *S. aureus* [50]. Among twelve endemic species of the genus *Chresta* from the region of "Cerrado Brasileiro" (Brazil), *C. scapigera* was found to have antimicrobial activity. Its hexanic extracts were active and inhibited the growth of *S. aureus*, *K. rhizophila*, *S. mutans*, *E. coli* and *P. aeruginosa*. The bioactive compounds were mainly flavonoids and triterpenoids [51]. Another study about Brazilian medicinal plants currently used in the folk medicine showed that *Piper regnellii* presented a moderated activity on *P. aeruginosa* (MIC of 250 $\mu g.ml^{-1}$) and the extract is active against *E. coli* (MIC 1000 $\mu g.ml^{-1}$). The antimicrobial activity in species of the *Piperaceae* family has been found to be attributed to amides, essential oil, lignans, phenylpropanoids, alkaloids, neolignans and chromene [52].

3.2. *Klebsiella* spp.

This bacterium is a type of Gram-negative bacteria that can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Increasingly, *Klebsiella* bacteria have developed antimicrobial resistance, most recently to the class of antibiotics known as carbapenems. They are normally found in the human intestines (where they do not cause disease). They are also found in human stool (feces). In healthcare settings, *Klebsiella* infections commonly occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for *Klebsiella* infections. Healthy people usually do not get *Klebsiella* infections. Zampini et al. [53] examined multiresistant bacteria under the influence of ethanol extracts from 11 Argentinean plant species (*Baccharis boliviensis*, *Chiliotrichiopsis keidelii*, *Chuquiraga atacamensis*, *Fabiana bryoides*, *F. densa*, *F. punensis*, *Frankenia triandra*, *Parastrephia lucida, P. lepidophylla*, *P. phyliciformis* and *Tetraglochin cristatum*). They observed growth inhibition in at least one of the following tested strains: *K. pneumoniae*, *E. faecalis*, *E. coli*, *Enterobacter cloacae*, *Morganella morganii*, *P. aeruginosa* and *Proteus mirabilis*. In a study of *Ocimum tenuiflorum* L. "albahaca blanca", a species growing in Cuba, its ethanolic extract presented antimicrobial activity against *Enterobacter aerogenes, Serratia marcescens* and *K. pneumoniae,* in falling order of sensibility [54]. *Juglans cinerea* "nogal blanco americano" grows in North America and its bark extract was active against *Pseudomonas aeruginosa* 187, *Salmonella typhiumurium*, and *K. pneumoniae* [55].

3.3. *Escherichia coli*

Most strains of *E. coli* are harmless, but many others can cause diseases. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses. This is the most prevalent causative agent of urinary tract infection in the community, representing over 95% of the isolates. In nosocomial infections prevalence is lower, but still the most isolated agent. *Lippia alba* is a plant distributed from Central to South America, as well as in Africa. Its ethanolic extract presented antimicrobial activity against *E. coli* and clinical isolates of *Proteus mirabilis* and *Morganella morganii* [56]. *Cymbopogon martinii* "palmarosa", a highly common plant in Brazil, presents a wide spectrum of action against three ETEC (enterotoxigenic *E. coli*) and two EPEC (enteropathogenic *E. coli*) serotypes, whereas Java citronella grass (*Cymbopogon winterianus*) inhibited one EPEC and two ETEC serotypes [57]. The concentration responsible for the microbial inhibition varied between 100 and 500 $\mu g.ml^{-1}$ [58]. *Baccharis dracunculifolia* oil "alecrim-do-campo" at a 10µL dose, prevented microbial growth of *E. coli* and *P. aeruginosa* in antimicrobial assays [59]. Ethereal fraction of spikes of *Rumex conglomeratus* "ruibarbo" was active against *E. coli* ATCC 25922 [60]. Methanolic extracts of *Verbesina enceliodes* had activity against *E. coli*, *Salmonella typhimurium* and *Proteus* spp. [61]. Hydroalcoholic extracts of Brazilian medicinal plants was found that *Psidium guajava* afforded extract with moderate activity against gram-positive bacteria and on the gramnegative bacterium *E. coli*. Also, the extracts of *Arctium lappa*, *Tanacetum vulgare* and *Mikania glomerata* presented some degree of activity against both gram-positive and gram-negative bacteria. *Eugenia uniflora* presented moderate activity on both *S. aureus* and *E. coli* [52].

3.4. *Vibrio cholerae*

It is a Gram-negative bacterium well-known for its ability to cause cholera, severe bacterial infection in humans. *Vibrio cholerae* is also motile due to its polar flagellum and makes ATP (an energy molecule) by aerobic respiration if oxygen is present, but is also capable of switching to fermentation - facultative anaerobe. Species belonging to the genus *Vibrio* are distinguished from *Enterobacteriaceae* by their flagella, as well as by being oxidase positive. *Vibrio cholerae* causes diarrhea in humans by a mechanism that involves close adherence to epithelial cells in the small intestines where they secrete an enterotoxin. The cholera toxin, affects the mucous membranes of the epithelial layer of the intestines, which causes diarrhea. In fact, untreated cholera has a 50% to 60% mortality rate. *Lippia graveolens* "orégano mexicano or salvia de castilla" is a shrub found in America. Comparison of the survivals curves for the organisms studied showed that the effect of the essential oil on the bacterial population of *S. aureus* and *V. cholerae* was bacteriostatic at MIC dose (62.5 µg.ml^{-1}), but a bactericidal effect is observed at MBC dose (125 µg.ml^{-1}) within first eight hours [62]. In Peru the population uses *Tagetes pusilla* "anís serrano", known from Mexico to northern Argentina, to relieve stomach upset. Its essential oil showed an excellent effect on *V. cholerae* inhibition (91mm halo). *Cymbopogon citrates* "hierba luisa", is an herbaceous plant of universal distribution. Its essential oil has good effect against *Salmonella typhi* ATCC 6539 (34 mm halo) and against *V. cholerae* (36 mm halo) [63].

3.5. Acinetobacter spp.

Acinetobacter sp. is a group of bacteria commonly found in soil and water. While there are many types or "species" of *Acinetobacter* and all can cause human disease, *A. baumannii* accounts for about 80% of reported infections. *Acinetobacter* infections rarely occur outside of healthcare settings. This bacterium causes a variety of diseases, ranging from pneumonia to serious blood may also "colonize" or live in a patient without causing infection or symptoms, especially in tracheostomy sites or open wounds. An investigation work in Guatemala showed that *Byrsonima crassifolia* species "nance" had good antibacterial activity against strains *P. aeruginosa* and *A. baumanii* as well as *Psidium guajava* specie "guayaba" was active against *A. baumannii* [64]. *Lippia alba* is known as "salvia morada" in Argentine or "pronto alivio" in Colombia and this species is native in Central America and South America. The essential oils of *L. alba* in Brazil displayed significant inhibitory activity against Gram negative bacteria as *A. baumannii* (with inhibition halos of 5 mm in chemotype citral and 7 mm in chemotype carvone) and *E. coli* showing inhibition zones higher than positive control (halo of 17 mm in both chemotypes) [65].

3.6. *Helicobacter pylori*

It is a Gram-negative bacterium with a form of curved S-shaped, which also has a non-cultural coccoid form with rounded ends. This pathogen possesses five to seven distinct flagella and grows best when cultured at 37°C. Interestingly, in resting individuals, 37°C is the stomach's temperature - the targeted organ; however, the bacteria are able to withstand temperatures as low as 25°C. *Lippia alba* is used for the treatment of multiple gastro-intestinal ailments. In the current in vitro study of the aqueous extracts from *L. alba* leaves against three isolated *Helicobacter pylori* cultures, it was determined that there was an antibacterial with bactericide minimum concentration (BMC) of 0,5 mg / ml [66]. The antibacterial activity of 53 medicinal plants used traditionally in Mexico was studied on various strains of *H. pylori* and showed greater inhibitory effects with aqueous extracts of *Artemisia ludoviciana* subsp. *mexicana*, *Cuphea aequipetala*, *Ludwigia repens* and *Mentha piperita* [67]. In a mexican study of Yucatec-Mexico, found four plants: *Casimiroa tetrameria*, *Dostenia contrajerva*, *Jatropha gaumeri* and *Piscidia piscipula* submitted CMI against *H. pylori* of 10 mg.ml^{-1} or less [68].

4. Molds and Yeasts

There is a clear and urgent need for the discovery of new alternative compounds for antifungal therapy since only a limited number of drugs derived from five antifungal classes is available [69], some of which show toxicity, produce recurrence or lead to the development of resistance due, in part, to the intensive prophylactic use of antifungal drugs [70]. Plants provide unlimited opportunities for the isolation of new antifungal compounds because of the unmatched availability of chemical diversity [71-72]. The signs or symptoms which give support to a traditional antifungal use are mainly related to skin or mucosal conditions, which are produced by dermatophytes (*Epidermophyton*, *Microsporum* and *Trichophyton* spp.). Other frequent traditionally described fungal-related conditions are leucorrhoea (mainly produced by *Candida* spp.) and respiratory diseases, which can be of fungal origin (caused by species of *Aspergillus* or *Cryptococcus*). The yeast fungus, *Cryptococcus neoformans*, has been identified as the fourth most common cause of life-threatening infection in AIDS patients. *Candida albicans*, a yeast that is a normal commensal in the microbial flora of the skin, the oral cavity and the vagina, commonly becomes pathogenic when immunological defenses are suppressed by treatment or disease. Potentially fatal infections with *C. albicans* and other species of *Candida* are also known [73]. Furthermore, infections by fungi that exist saprophytically in plants, moss or soil occur in tropical and subtropical regions where people walk with bare feet. Inoculation through cuts and abrasions commonly results in a localized primary lesion. Cutaneous sporotrichosis, which is caused by *Sporothrix schenckii,* presents a nodular or pustular lesion which later ulcerates; lymphatic system may also be involved if the disease continues after weeks or months. Chromomycosis, which is caused by fungi of several genera, including *Fonsecaea* and *Cladosporium,* is a chronic infection of exposed areas such as the lower leg [74].

4.1. Dermatophytes

In a multidisciplinary and multinational study (Argentina, Bolivia, Brazil, Colombia, Costa Rica, Guatemala and Panama) carried out by Svetaz et al. [75], many Latin American plants with reported antifungal-related ethnopharmacological uses were described. Among 114 plant species, the ethanolic extracts of many showed antifungal activity (MIC≤250 µg.ml^{-1}) in at least one of the fungal species *Microsporum gypseum* C 115; *Trichophyton rubrum* C 113; *Trichophyton mentagrophytes* ATCC 9972; *Epidermophyton floccosum* C 114. Those plants were: *Gomphrena pulchella* Mart. subsp. *rosea*, *Schinus areira*, *Aristolochia argentina*, *Gaillardia megapotamica*, *Gochnatia glutinosa Mikania periplocifolia* and *Xanthium spinosum* from Argentina; *Socratea exorrhiza*, several species of *Piperaceae* and *Serjania elongata* from Bolivia; *Aleurites moluccana*, *Epidendrum mosenii*, *Drimys winteri* and *Curcuma zedoaria* from Brazil; *Virola koschnyi* and *Gynerium sagittatum* from Costa Rica; *Piper amalago*, *P.jacquemontianum* and *P. scabrum* from Guatemala; *Chiococca alba* from Panama. Chemical studies are needed to be conducted in order to isolate and elucidate structures involved in the antifungal activity of these species.

In this sense, exhaustive chemical studies reported the isolation of 3-(3,3-dimethylallyl)-*p*-coumaric acid and the flavonoids pectolinaringenin, nevadensin, and dimethoxy sudachitin from the exudate of the aerial parts of the perennial shrub *Baccharis grisebachii* from Argentina, with activity towards dermatophytic fungi and some bacteria [75- 77]. Further studies [35] reported that 3-Prenyl-*p*-coumaric acid and 3,5-diprenyl-*p*-coumaric acid were active towards *Epidermophyton floccosum* and *Trichophyton rubrum* with MICs of 50 and 100–125 µg.ml^{-1}, respectively. The diterpene labda-7,13-*E*-dien-2β,15-diol was found to be active towards *E. floccosum* and *T. rubrum* with MICs of 12.5 µg.ml^{-1} while the MIC against *Microsporum canis* and *T. mentagrophytes* was found to be 25 µg.ml^{-1}. The diterpene had also been described active towards *Microsporum gypseum* with a MIC of 50 µg.ml^{-1}, and showed inhibition in both *S. aureus* (methicilline resistant and sensible strains) with MICs of 125 µg.ml^{-1}.

Lippia alba, known as "erva-cidreira", and *Lippia alba* f. *intermedia,* known as "carmelitana", both species from Brazil, had been described by Oliveira et al. [27]. Its use in popular medicine can be explained, partly, by the bioactive volatile constituents. However, large variations in the composition of these oils have been observed, depending on the origin of the plant material, plant stage and the plant part selected for distillation of the essential oil [78]. This may be due to environmental effects, different extraction methodologies, as well as the genetic variability of these plants [79]. The variability is so common that Matos [80] suggested the division into different chemotypes. So far, at least 12 chemotypes have been described. The analyses of the essential oils allowed the identification of *Lippia alba* as a myrcene–citral chemotype (15% and 37.1%, respectively) and *Lippia alba* f. *intermedia* as a citral chemotype (22.1%), showing activity against *Trichophyton rubrum* T544. The authors attributed their antifungal activity to the high content of oxygenated monoterpenes, specially represented by aldehydes and alcohols.

Among forty-five aqueous, dichloromethane and methanol crude extracts of 14 plant species used in the traditional medicine of Paraguay, half of the plant species showed antifungal activity against *T. mentagrophytes* CECT 2795 and *M. gypseum* CECT 2908. The species showing the highest activity were *Acanthospermum australe*, *Calycophyllum multiflorum*, *Geophila repens* and *Tabebuia avellanedae* [11].

Through ethnobotanical surveys, Cáceres et al. [81] selected 44 plant extracts from Guatemala which had revealed antimycotic activity against the most common dermatophytes. Plants which exhibited anti-dermatophyte activity were: *Byrsonima crassifolia*, *Cassia grandis*, *Cassia occidentalis*, *Diphysa carthagenensis*, *Gliricidia sepium*, *Piscidia piscipula*, *Sambucus mexicana*, *Smilax regelii*, *Solanum americanum* and *Solanum nigrescens*. The most commonly inhibited dermatophytes were *E. floccosum* (43.2%), *T. rubrum* (36.0%), and *T. mentagrophytes* (31.8%); the less inhibited were *M. canis* (22.7%) and *M. gypseum* (24.0%).

In Peru, the leaves of *Cestrum auriculatum* ("hierba santa", "hierba santa hembra", "hierba hedionda") are used by the community of Pamparomas for the treatment of skin infections and allergies. The filamentous fungus *M. gypseum* and *Sporothrix schenckii* were inhibited by the ethanol extract of the leaves of *C. auriculatum*. *Iryanthera lancifolia* and *Ophryosporus peruvianus* proved to be the most effective in selectively inhibiting *T. mentagrophytes*. The report presented by Rojas et al. [83] was the first on the antimicrobial activity of these species.

4.2. *Candida spp.*

Brazilian plant extracts had been thoroughly studied as a natural source of phytochemicals against *Candida* sp. Oliveira et al. [27-28] postulated that the antimicrobial activity of essential oils of *Lippia alba* and *L. alba* f. *intermedia* was probably related to the high content of oxygen-containing monoterpenes (51.0–40.1%, respectively), represented mainly by aldehydes and alcohols, such as neral/geranial and nerol/geraniol. Other studies had demonstrated the antimicrobial activity of the essential oil from two chemotypes of *L. alba*. Linalool-chemotype was active against *C. albicans* [83]. *Candida albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019 were inhibited by the crude extracts of *Curatella americana*, *Sclerolobium aureum* and *Plathymenia reticulata.* Interestingly, the extracts were made with "cachaça", as the extractor liquid, because it is a common extraction method used in Brazil, mainly in the region where the species were collected. Cachaça is a sugar-cane brandy containing 38–54% v.v^{-1} ethanol at 20°C, very popular among Brazilians [29]. A study carried out by Moura Costa et al. [30] to evaluate the antimicrobial activity and *in vitro* cytotoxicity of aqueous extracts (AEs) and hydroalcoholic extracts (HAEs) of some plants used as medicinal on the indigenous reserve in Rio das Cobras, Paraná, Brazil, showed that three species of Candida were inhibited. Antimicrobial activity against *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 28707 was found using the extracts obtained from the leaves of *Campomanesia eugenioides* and *Schinus terebinthifolius*. However, the HAE exhibited the best results, and the local population uses only the AE to treat their diseases, which presented low antimicrobial activity.

In Mexico, the antifungal activity of hydroalcoholic extracts from *Jatropha dioica, Euphorbia prostrata, Salvia texana, Colubrina greggii, Clematis drummondii* was tested against clinical isolates *C. albicans.* Minimum inhibitory concentrations ranging from 63 to 500 μg.ml^{-1} were found. By means of differential extraction of these extracts, 30 extracts of different polarity were obtained, 12 of which displayed activity in a range of concentrations between 16 and 63 μg.ml^{-1}. These results helped to highlight the importance of the application of ethnobotanic criteria in the search and selection of plants that may provide new opportunities for the treatment of refractory infections, such as those caused by *C. albicans* [84].

4.3. Miscellanea

People from Honduras, in general, are said to have a well-developed pharmacopoeia based largely on vascular plants. Many people still rely on medicinal herbs as their first line of defense against disease and discomfort. Results of an antimicrobial screening indicated that bioactivity could be detected in 35% of the Honduran medicinal plants tested. This fact reinforced the concept that the investigation of ethnobotanically used plants would reveal a substantial number of positive responses to *in vitro* screens [85]. *Piper aduncum* active compound 4-methoxy-3,5- bis (3%-methylbenzoic acid) inhibited three yeasts (*Candida neoformans* ATCC 32264, *C. albicans* NIH B311 and *S. cerevisiae* ATCC 9763), two filamentous fungi (*Aspergillus flavus* ATCC 9170 and *A. fumigatus* ATCC 26934) and one dermatophyte (*T. mentagrophytes* ATCC 9972).

A multinational study performed with the aim to screen Latin American plant extracts against two species of subcutaneous fungi (*Sporothrix schenckii* and *Fonsecaea pedrosoi*) showed that these fungi were inhibited by *Bourreria huanita* (MIC: 12.5 and 25 μg.ml^{-1}), *Gnaphalium gaudichaudianum* (MIC: 50 and 12.5 μg/mL) and *Thurovia triflora* (MIC: 25 μg/mL) [86].

A survey comprising seven countries from Latin America (Argentina, Bolivia, Brazil, Colombia, Costa Rica, Guatemala and Panama) [75] showed that 58 out of the 144 plants analyzed (40.3%) were active (MIC≤1000 μg.ml^{-1}) at least against one of the studied fungus (*Cryptococcus neoformans* ATCC 32264) ; 21 (14.6%) were active against yeasts (*Candida albicans* ATCC 10231, *C. tropicalis* C 131, *Saccharomyces cerevisiae* ATCC 9763); 13 (9.0%) against *Aspergillus* spp. (*A. flavus* ATCC 9170, *A. fumigatus* ATCC 26934, *A. niger* ATCC 9029); and 57 (39.6%) against dermatophytes (*Trichophyton rubrum* C 110, *T. mentagrophytes* ATCC 9972, *Microsporum gypseum* C 115 and *Epidermophyton floccosum* C 114). Among those plants, the best antimicrobial activity was displayed by *Zuccagnia punctata* from Argentina, *Piper glabratum* and *Serjania elongata* J.B. from Bolivia, *Drimys winteri* from Brazil; the MIC values of these plants extracts were ≤ 250 μg.ml^{-1}.

5. Concluding remarks

Herbal medicinal products are among the best-selling nutrition-related products of our time. The public assumes these products are both safe and effective, but there is little basis for this assumption, especially in a regulatory environment in which these products are essentially unregulated [88]. A wide range of phytochemicals has been studied as potentially therapeutic. Many of these chemicals have been identified based on chemical structure, *in vitro* activity, epidemiological evidence, and bioactivity screening programs. The fact that a chemical has interesting properties or that it demonstrates bioactivity in a screening protocol does not necessarily indicate that the chemical will be effective *in vivo* in humans. If a chemical is not bioavailable or it is heavily metabolized *in vivo*, it will be ineffective. The pharmacokinetics of phytochemicals in humans has been largely ignored. In order to understand the potential therapeutic effectiveness of a phytochemical, researchers will have to study its pharmacokinetics.

In summary, phytochemicals, components of plants in our foods, herbs and general environment, comprise an enormous variety of chemical structures from many chemical classes. In practical terms, a clear definition of biological activity and structural identification of the specific phytochemical agent responsible for this activity is the first requirement before undertaking pharmacokinetics studies. Only then can useful studies be made of characteristics and mechanisms for potentially useful manipulation to control or enhance activity. These studies would include absorption, distribution, metabolism and metabolites, effective half-life, site and mode of activity, inactivation, excretion, etc. Unfortunately, only a minority of phytochemicals with claimed biologic effects have been properly or adequately studied in this manner.

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