Antimicrobial activity of secondary metabolites and lectins from plants

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This review outlines the antimicrobial activity of secondary metabolites and lectins, compounds usually associated to defense mechanisms of plants. Secondary metabolites are separated into nitrogen compounds (alkaloids, non-protein amino acids, amines, alcamides, cyanogenic glycosides and glucosinolates) and non-nitrogen compounds (monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, saponins, flavonoids, steroids and coumarins). Lectins are carbohydrate-binding proteins and their biological properties include cell-cell interactions. This chapter reports solvent organic extracts (mixture of secondary metabolites), isolated secondary metabolites and lectins from plants with antimicrobial activity against Gram-negative and Gram-positive bacteria as well as antifungal activity towards human and plant pathogens. Mechanisms proposed for antimicrobial activity of secondary metabolites and lectins against bacteria and fungi are also discussed. The effects of plant secondary metabolites and lectins on deleterious human and plant microorganisms indicates their perspectives of antimicrobial uses.

Keywords antibacterial activity; antifungal activity; plant lectins; secondary metabolites.

1. Secondary metabolites with antimicrobial activity

Solvent organic extracts contain a mixture of secondary metabolites including alkaloids, flavonoids, terpenoids, and other phenolic compounds; these molecules are associated to defense mechanisms of plants by their repellent or attractive properties, protection against biotic and abiotic stresses, and maintenance of structural integrity of plants. Polar solvents (such as organic acids), solvents of intermediate polarity (such as methanol, ethanol, acetone, and dichloromethane) and solvents of low polarity (such as hexane and chloroform) are used to extract plant secondary metabolites that differ in structure and polarity. Then extracts from the same plant material obtained with solvents of different characteristics have distinct biological properties. Extracts from aerial parts of *Salvia tomentosa* were evaluated for antibacterial activity and it was reported that non-polar extracts showed moderate activity and polar extracts were inactive [1].

Solvent organic extracts from aerial parts, bark, flowers, fruits, heartwood, leaves, twigs and root from medicinal plants have been investigated aiming to validate their ethnopharmacological use. Extracts from plants used to treat diarrhea (*Indigofera daleoides*, *Punica granatum*, *Syzygium cordatum*, *Gymnosporia senegalensis*, *Ozoroa insignis*, *Elephantorrhiza elephantina*, *Elephantorrhiza burkei*, *Ximenia caffra*, *Schotia brachypetala* and *Spirostachys africana*) contained agents against bacteria that cause gastrointestinal infections (*Vibrio cholerae*, *Escherichia coli, Staphylococcus aureus*, *Shigella dysentery*, *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii* and *Salmonella typhi*) and this strengths their usefulness in the treatment of diarrhea [2]. Extracts from *Calophyllum brasiliense* leaves (obtained with acetone), *Mammea americana* fruit peels (obtained with acetone and hexane) and dichloromethane extract of the resinous exudate from *Baccharis grisebachii* were also effective against methicilline-resistant and sensible *S. aureus* strains [3, 4]. Table 1 shows that solvent organic extracts may be antibacterial agent only on Gram-positive or both Gram-positive and Gram-negative bacteria. Differential sensitivity of Gram-positive and Gram negative bacteria to plant extracts may be explained by the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components; this makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da. The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier [5].

Different types of secondary metabolites have been identified as the active principles of antimicrobial solvent organic extracts (Table 2). The tannins methyl gallate and gallic acid from *Galla rhois* inhibit cariogenic (*Actinomyces viscosus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus salivarus*, *Streptococcus mutans* and *Streptococcus sobrinus*) and periodontopathic (*Porphyromonas gingivalis*) bacteria and the *in vitro* formation of *S. mutans* biofilms; authors suggested the use of these compounds to prevent the formation of oral biofilms [6].

Solvent organic extracts with antifungal activity against species that cause diseases in humans and plants have been reported. Dichloromethane extract of the resinous exudate from *Baccharis grisebachii* containing diterpene (labda-7,13*E*-dien-2β,15-diol) and coumaric acids (3-prenyl-*ρ*-coumaric acid and 3,5-diprenyl-*ρ*-coumaric acid) was active against *Epidermophyton floccosum*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and

Trichophyton rubrum [3]. Ethanolic extracts of *Caesalpinia mimosoides* showed potent activity against fungi (*M. gypseum* and *T. rubrum*); gallic acid (a tannin) was detected as the main principle of the extract [7]. A methanolic extract from *Myracrodruon urundeuva* containing cinamic derivatives, flavonoids, gallic acid, luteolin, and tannins showed antifungal activity on *Fusarium* [8]. The extract had important role in growth inhibition of *Fusarium lateritium* and *Fusarium oxysporum*, as evidenced by inhibition superior to commonly used antifungal Cercobin. *F. oxysporum* is a phytopathogen and opportunistic human pathogen.

Table 1 Organic solvent extracts from medicinal plants with antibacterial activity.

Medicinal use	Plant	Antibacterial activity	
		Gram + and -	Only Gram +
Respiratory disease	*Abuta grandifolia, Cordia alliodora*	X	
	Acacia nilotica, Caesalpinia pyramidalis, Cupania oblongifolia, Cupania platycarpa		X
Digestive disease	*A. grandifolia, Maytenus macrocarpa, Naucleopsis glabra, Annona cherimola*, *Calophyllum brasiliense, Ozoroa insignis*	X	
	Commiphora parvifolia, Ocotea glomerata, Simarouba amara, Talisia esculenta		X
Skin disease	*Guazuma ulmifolia, Solanum incanum*		X
	Lipia adoensis, Mammea americana, Eryngium creticum, Juglans regia, Lycium europeum, Micromeria nervosa	X	
Malaria	*Aegiphila lhotskiana, Hedychium coronarium, Simarouba amara*		X
Anti-inflammatory	*Annona salzmanni, C. pyramidalis, Pterodon polygalaeflorus*		X
	Schinus terebinthifolius	X	
Antirheumatic	*Annona muricata, Marsdenia altissima, P. polygalaeflorus, T. esculenta*		X
	C. alliodora, N. glabra	X	
Healing activity	*Pterocarpus rohrii, Plantago lanceolata, Pinus gerardiana*	X	
	A. nilotica, Syzygium jambolanum, Dipteryx micrantha, Andira inermis, Auxemma oncocalyx		X
Renal disease	*Sarcopoterium spinosum, Pistacia lentiscus, E. creticum, Retama aculeatus*	X	
	Indigofera spinosa, Cadaba glandulosa		X
Fever	*Anogeissus schimperi, Bauhinia thonningi, Cassia goratensis, Butyrospernum parkii, Boswellia dalzielli*	X	
Veneral disease	*Abutilon indicum, Vitex nigundo, Boswellia serrata, Commiphora mukul, Bixa orellana, Raphanus sativus*	X	
Eye disease	*Syzygeum guineense, Lippia adoensis, Zizyphus jujube, Capparis spinosa, Lycium europeum, Retama raetam, Zizyphus spina-christi, Albezzia lebbeck*	X	

Gram + and – means Gram-positive and Gram-negative bacteria and (X) means bacteriostatic or bactericide effects. References: [2, 4, 9-19].

Prenylated flavonoids purified from Asian medicinal plants *Broussnetia papyrifera*, *Echinosophora koreensis*, *Morus alba*, *Morus mongolica* and *Sophora flavescens* showed antifungal activity against *Candida albicans*; the authors highlighted the high potential use of them in Asian traditional medicine to treat infections [20]. A mixture of linear aliphatic primary alcohols isolated from cyclohexane extract of *Solanecio mannii* leaves was an antifungal agent on *C.*

albicans (minimal inhibitory concentration of 1.6 µg/ml) while fatty acid esters of diunsaturated linear 1,2-diols from cyclohexane extract of *Monodora myristica* fruits were active against on *C. albicans* and *Candida krusei* [21].

Table 2 Antimicrobial activity of secondary metabolites from medicinal plants.

Microorganism	Compound	Plant
Bacillus cereus, Bacillus subtilis, Micrococus kristinae, Staphylococcus aureus, Aspergillus flavus, Cladosporium sphaerospermum	3,5,7-Trihydroxyflavone (galangin)	*Helichrysum aureonitens*
B. cereus, S. aureus, Staphylococcus epidermis, Candida albicans, Cryptococcus neoformans	Benzoquinone and benzopyran	*Gunnera perpensa*
B. cereus, B. subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, S. aureus, C. albicans, C. neoformans	Helihumulone	*Helichrysum cymosum*
B. cereus, S. aureus	Carnosol and 7-O-methyl-epirosmanol	*Salvia chamelaeagnea*
B. subtilis, E. coli, K. pneumoniae, S. aureus	Anolignan B	*Terminalia sericea*
B. subtilis, E. coli, K. pneumoniae, S. aureus	Sesquiterpenoid	*Warburgia salutaris*
B. subtilis, E. faecalis, E. coli, P. aeruginosa, S. aureus, Aspergillus niger	Flavonoids	*Combretum erythrophyllum*
E. coli, S. aureus, C. albicans		*Erythrina burttii*
E. coli, *Salmonella typhimurium*, *Staphylococcus epidermis*, *S. aureus, C. albicans*, *Saccharomyces cerevisiae*		*Broussnetia papyrifera*, *Echinosophora koreensis*, *Morus alba*, *Morus mongolica* and *Sophora flavescens*
E. coli, S. aureus	Terpenoids	*Spirostachys africana*
E. coli, K. pneumoniae, S. aureus	Vernolide and vernodalol	*Vernonia colorata*
Epidermophyton floccosum, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum, S. aureus*	Diterpene and coumaric acids	*Baccharis grisebachii*
Actinomyces viscosus, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus salivarus, Porphyromonas gingivalis, Streptococcus mutans, Streptococcus sobrinus	Methyl gallate and gallic acid	*Galla rhois*
C. albicans	Alkaloids	*Aniba panurensis*
S. aureus	Naphtoquinones	*Tabebuia avellanedae*
A. niger, Botrytis cinerea	Saponin	*Astragalus verrucosus*
S. aureus, B. cereus, Clostridium perfrigens, E. faecalis, Micrococcus luteus, Aeromonas hydrophila, Enterobacter sakazakii, K. pneumoniae, E. coli, Enterobacter cloacae, P. aeruginosa, Vibrio vulnificus, Pseudomonas luteola, Chryseobacterium indologenes, C. albicans, A. niger, Penicillium sp.	Glucosinolates	*Aurinia sinuata*
Mycobacterium tuberculosis	Quassinoids	*Ailanthus altissima*
M. tuberculosis	Xanthones	*Canscora decussata*
Mycobacterium smegmatis, Mycobacterium intracellulare, Mycobacterium chelonae, Mycobacterium xenopi	Ferruginol	*Juniperus excelsa*
Mycobacterium avium, M. tuberculosis	Gingerols	*Zingiber officinale*

References: [2, 3, 6, 20, 22-39]

Essential oils are a bioactive mixture of complex compounds synthesized as secondary metabolites by buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark. The lipophilic nature of essential oils makes them permeable to cellular membrane; cytotoxic effects include cell alterations in plasma membrane, cytoplasm and nucleus [40]. *Lippia rugosa* oil containing geraniol, nerol and geranial as main components was able to inhibit *Aspergillus flavus* growth as well as the production of aflatoxin, probably due to interference on fungal cellular metabolism [41]. Antifungal activity on dermatophytes *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis* was also found in the essential oil from *Moringa oleifera* leaves [42]. *Salvia pisidica* and *Achillea ligustica* oils showed antibacterial activity against Gram (+) bacteria and were suggested as food preservatives and anti-cariogenic agent [43, 44]. The essential oil from *Salvia tomentosa*, composed of β-pinene (39.7%), α-pinene (10.9%) and camphor (9.7%), was highly active and showed minimal inhibitory concentration ranging from 0.54 mg/mL (C*lostridium perfringens*) to 72.00 mg/mL (*Moraxella catarrhalis*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*). The essential oil was not active on *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* [1].

Antimicrobial activity of phenolic compounds present in plants change according its structure; flavone, quercetin and naringenin were effective in inhibiting the growth of *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Staphylococcus epidermidis* while gallic acid inhibited only *P. aeruginosa*; rutin as well as catechin did not show any effect on the tested microorganisms [45].

The mechanisms of antimicrobial action of plant secondary metabolites are not fully understood but several studies have been conducted in this direction. Flavonoids may act through inhibiting cytoplasmic membrane function as well as by inhibition of DNA gyrase and β-hydroxyacyl-acyl carrier protein dehydratase activities [46, 47]; the isoflavone genistein was able to change cell morphology (formation of filamentous cells) and inhibited the synthesis of DNA and RNA of *Vibrio harveyi* [48]. It has been suggested that terpenes promote membrane disruption, coumarins cause reduction in cell respiration and tannins act on microorganism membranes as well as bind to polysaccharides or enzymes promoting inactivation [49-51]. Although a number of publications have focused on the isolation and identification of bio-active compounds, it is important to keep in mind that a single compound may not be responsible for the observed activity but rather a combination of compounds interacting in an additive or synergistic manner.

2. Antimicrobial lectins

Lectins are carbohydrate-recognizing proteins that bind to cells promoting hemagglutination and antimicrobial effect. Plant lectins have been isolated from bark, cladodes, flowers, leaves, rhizomes, roots and seeds. Alternatively, plant recombinant lectins have been expressed in heterologous systems [52]. Plant lectins can be glycosylated molecules and staining on polyacrylamide gel specific for glycoprotein can easily reveal the presence of glycan in the lectin structure; carbohydrate moiety characterization can be performed after lectin tryptic digestion in gel followed by enzymatic deglycosylation and mass spectrometric analysis [53]. The compact globular structures, molecular aggregation and glycosylation in general result in high structural stability of lectins [54, 55]; high temperature is a powerful denaturing agent leading to protein unfolding through breaking of hydrogen bonds that maintain protein structure and heated lectins can or not lose their biological properties.

Lectins are distributed in fucose, mannose, sialic acid, *N*-acetylglucosamine, *N*-acetylgalactosamine and glycan-complex groups according to carbohydrate specificity [56]. The selectivity of binding is achieved through hydrogen bridges, van der Waals and hydrophobic interactions between sugar and lectin site**.** The presence of multiple molecular forms of protein is a frequent phenomenon in some plant species; they may have distinct carbohydrate specificity, charge, mobility on polyacrylamide gel and biological property [57]. Molecular forms with different electrophoretic mobility which belong to the same species are called isolectins [58] and the term isoform was proposed for lectins belonging to the same species when heterogeneity of genetic origin was not well defined [59].

Hemagglutinating activity is the most commonly used assay for the detection of lectin in a sample due to the simplicity of implementation and ease visualization of agglutination. Aliquot of sample is serially diluted in microtitration plate before addition of erythrocytes and hemagglutinating activity (titer) is defined as the reciprocal of the highest dilution of sample promoting full agglutination of erythrocytes. The hemagglutinating activity occurs when the lectin binds to carbohydrate from erythrocyte surface promoting a network among them (Figure 1A); sometimes lectin is not detected due to steric hindrance in the lectin-carbohydrate interaction and previous enzymatic treatment of erythrocytes is needed to occur hemagglutination [60].

The hemagglutination assay allows the assessment of lectin stability to pH and temperature values and thus can determine the conditions to be used in the biotechnological application of lectin. Additionally the assay may reveal lectin carbohydrate specificity defined by carbohydrate that more effectively inhibits the hemagglutinating activity (Figure 1B). Alternative strategies to detect the carbohydrate specificity of lectins are surface plasmon resonance method using carbohydrate immobilized on a gold-coated glass prism and enzyme-linked adsorbent assay using monosaccharide-polyacrylamide conjugates on the microplates [61, 62].

Lectins can be extracted from plant tissue with water, 0.15 M NaCl or buffer solutions when it is necessary to control pH for the maintenance of hemagglutinating activity. The temperature and extraction time depends on stability and

solubility of the lectin and may vary from 4 to 27º C, from minutes to hours. Lectin can also be extracted using a reversed micelle system of the anionic surfactant sodium di(2-ethylhexyl)sulfosuccinate in isooctane; protein solubilization is strongly dependent on pH, concentration of surfactant and on the size of the micelle relative to that of the protein [63]. Lectin present in a mixture of proteins can be isolated by column chromatography that promotes separation due to differential migration of proteins adsorbed to the matrix. Disruption of interactions lead to the release of proteins in distinct fractions, dependent on the binding of each protein component of sample to the matrix. Presence of oil and pigments in vegetal tissues can interfere in lectin isolation by chromatography since non-specific adsorption of these contaminants on matrix constitutes an impediment to lectin-matrix interaction. Plant tissues with high oil and polysaccharide content can be previously treated before protein extraction aiming to eliminate contaminants; polyethylene glycol (PEG 8000) is effective in removing polyphenolic compounds [64].

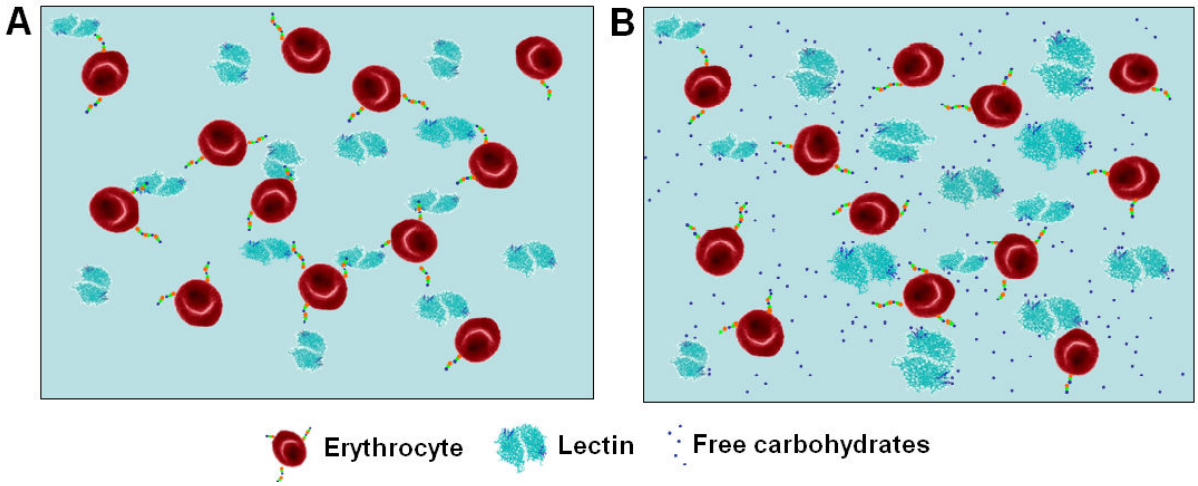

Fig. 1 Schematic representation of erythrocyte network promoted by lectin binding to surface carbohydrates (A) and inhibition of hemagglutinating activity by free carbohydrates (B).

The conditions used in the chromatographic steps (washing, lectin adsorption, and desorption) including volume and protein concentration of sample, pore size and matrix charge, column length, temperature and solution used for lectin desorption, flow velocity and volume of fraction collected are defined in order to increase yield and degree of purity. The choice of chromatographic method is performed according to lectin biochemical characteristics and isolation procedures can use one or sequential chromatographic processes.

Affinity chromatography is present in almost all purification procedures of lectin with defined specificity due to advantages such as high recovery and high specificity. The method provides a high degree of protein purification, in a single step, with maintenance of the biological activity. Polysaccharide matrices such as Sephadex, chitin and Sepharose consisting of glucose, N-acetylglucosamine and galactose units, respectively, are selected according to the specificity of the lectin to be isolated. Lectins that recognize glycoconjugates may be isolated by affinity chromatography on columns containing glycoproteins immobilized on Sepharose activated with cyanogen bromide [65]. A method was developed to immobilize egg proteins and the affinity matrix was efficient to purify lectins from extracts of *Phaseolus vulgaris* (complex saccharide binding), *Lens culinaris* (mannose and glucose binding), and wheat germ (sialic acid, acetyl-glucosamine, and its polymer binding) in terms of milligrams per gram of matrix [66]. Another alternative for lectin affinity isolation is the use of ferromagnetic levan particles, a composite of the carbohydrate levan from *Zimomonas mobilis* and magnetite. Lectins are eluted with 0.3 M monosaccharide solutions and recovered from particles by a magnetic field [67].

Cytotoxic effects of lectins may be revealed by antitumoral and antiviral activities and also by deleterious effect on microorganisms (Table 3); lectins of different carbohydrate specificities are able to promote growth inhibition or death of fungi and bacteria. Table 4 shows proposed applications of lectins for detection, typing, and control of bacteria and fungi that cause damage to plants and humans.

Antibacterial activity on Gram-positive and Gram-negative bacteria occurs through the interaction of lectin with components of the bacterial cell wall including teicoic and teicuronic acids, peptidoglycans and lipopolysaccharides; study revealed that the isolectin I from *Lathyrus ochrus* seeds bind to muramic acid and muramyl dipeptide through hydrogen bonds between ring hydroxyl oxygen atoms of sugar and carbohydrate binding site of lectin and hydrophobic interactions with the side chains of residues Tyr^{100} and Trp^{128} of isolectin I [68].

The inhibition of fungi growth can occur through lectin binding to hyphas resulting in poor absorption of nutrients as well as by interference on spore germination process [58]. The polysaccharide chitin is constituent of fungi cell wall and chitin-binding lectins showed antifungal activity; impairment of synthesis and/or deposition of chitin in cell wall may be the reasons of antifungal action [69]. Probably the carbohydrate-binding property of lectin is involved in the

antifungal mechanisms and lectins of different specificities can promote distinct effects. Plant agglutinins are believed to play a role in plant defense mechanism against microorganism phytopathogens [70].

Table 3 Plant lectins with antimicrobial activity.

Plant (tissue)	Lectin specificity	Antimicrobial activity
Araucaria angustifolia (seed)	GlcNAc	*Clavibacter michiganensis*, *Xanthomonas axonopodis* pv. *passiflorae*
Artocarpus incisa (seed)	GlcNAc	*Fusarium moniliforme*, *Saccharomyces cerevisiae*
Artocarpus integrifolia (seed)	GlcNAc	*F. moniliforme, S. cerevisiae*
Astragalus mongholicus (root)	Lactose/D-Gal	*Botrytis cincerea, Fusarium oxysporum, Colletorichum sp., Drechslera turcia*
Eugenia uniflora (seeds)	Carbohydrate complex	*Bacillus subtilis, Corynebacterium bovis, Escherichia coli, Klebsiella* sp., *Pseudomonas aeruginosa, Streptococcus sp., Staphylococcus aureus*
Gastrodia data (corms)	α-Man/ GlcNAc	*B. cinerea, Ganoderma lucidum*, *Gibberella zeae*, *Rhizoctonia solani*, *Valsa ambiens*
Hevea brasiliensis (latex)	Chitotriose	*B. cinerea, Fusarium culmorum, F. oxysporum* f. sp. *pisi, Phycomyces blakesIeeanus, Pyrenophora triticirepentis, Pyricularia oryzae, Septoria nodorum, Trichoderma hamatum*
Myracrodruon urundeuva (heartwood)	GlcNAc	*B. subtilis, Corynebacterium callunae, E. coli, Klebsiella pneumoniae, P. aeruginosa, S. aureus, Streptococcus faecalis.* *Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Fusarium decemcellulare*, *Fusarium lateritium*, *Fusarium fusarioides*, *Fusarium verticiloides*
Ophiopogon japonicus (rhizome)	Man	*Gibberella saubinetii, R. solani*
Opuntia ficus indica (cladodes)	Glc/Man	*Colletrotrichum gloesporioides*, *Candida albicans, F. oxysporum, F. solani*
Phaseolus coccineus (seeds)	Sialic acid	*Helminthosporium maydis*, *Gibberalla sanbinetti*, *R. solani*, *Sclerotinia sclerotiorum*
Phthirusa pyrifolia (leaf)	Fru-1,6-P2	*B. subtilis*, *K. pneumoniae*, *Staphylococcus epidermidis*, *S. faecalis* *F. lateritium, R. solani*
Pisum sativum (seed)	Man	*Aspergillus flavus*, *F. oxysporum, Trichoderma viride*
Sebastiania jacobinensis (bark)	Carbohydrate complex	*F. moniliforme*, *F. oxysporum*
Talisia esculenta (seeds)	Man	*Colletotrichum lindemuthianum*, *F. oxysporum*, *S. cerevisiae*
Triticum vulgaris (seeds)	GlcNAc	*Fusarium graminearum, F. oxysporum*
Urtica dioica (rhizome)	GlcNAc	*B. cinerea*, *C. lindemuthianum*, *Phoma betae*, *Phycomyces blakesleeanus*, *Septoria nodorum*, *Trichoderma hamatum*, *T. viride*

D-Gal: galactose; Fru-1,6-P2: fructose-1,6-biphosphate; Glc: glucose; GlcNAc: *N*-acetylglucosamine; Man: mannose.
References: [70-85].

Myracrodruon urundeuva Fr. All is broadly distributed in Brazil. Considered a hardwood, it is very resistant to degradation by microorganisms; its heartwood contains antimicrobial lectin [70]. The heartwood lectin inhibited Gram-positive (*Bacillus subtilis, Corynebacterium callunae, Staphylococcus aureus* and *Streptococcus faecalis*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria but was more effective on Gram-positive than on Gram-negative bacteria. The lowest minimal inhibitory concentration (MIC) was determined for *S. aureus* (0.58 μg/mL) and minimum bactericidal concentration (MBC) for this bacterium was 8.1 μg/mL; *K. pneumoniae* was the least sensitive microorganism (MIC of 9.37 μg/mL). The lectin is a chitin-binding protein with antifungal activity against *Fusarium* strains; the highest percentage of growth inhibition was obtained for *F. oxysporum* (60.8% ± 2.9) and similar inhibition was detected against *Fusarium decemcellulare* (51.1% ± 3.8) and *Fusarium fusarioides* (51.1% ± 1.9).

Phthirusa pyrifolia leaf lectin with a unique affinity for fructose-1-6-biphosphate showed antimicrobial activity [71]. Antibacterial activity was detected against *Klebsiella pneumoniae*, *Staphylococcus epidermidis* and *Streptococcus faecalis* but bactericide effect was only detected on *Bacillus subtilis* (MBC of 0.5 mg/mL). The lectin was an antibacterial agent more effective for Gram-positive than for Gram-negative bacteria and it was suggested that the

bacteria sensitivity was related to levels of peptideoglycan on the wrapper. The *P. pyrifolia* lectin was an antifungal agent on *Fusarium lateritium* and *Rhizoctonia solani* but did not affect the growth of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus arrhizus*, *Paecilomyces variotti*, *Fusarium moniliforme*, *Candida albicans*, *Candida burnenses*, *Candida tropicalis*, *Candida parapsilosis* and *Saccharomyces cerevisiae*.

Table 4 Application of lectins in the study of microrganisms

Application	Lectin source	Year
Antifungal agent	*Artocarpus incisa*, *Artocarpus integrifolia, Ophiopogon japonicus*, *Opuntia ficus indica, Phaseolus coccineus, Pisum sativum*, *Sebastiania jacobinensis, Talisia esculenta*	2002, 2006, 2007, 2008, 2009, 2010
Antimicrobial agent on bacteria and fungi	*Myracrodruon urundeuva*	2009
Biotinylated lectins applicable to large scale typing of *Staphylococcus epidermidis*	*Triticum vulgaris*, *Glicine max*, *Lens culinaris*, *Canavalia ensiformis*	1992
Clinical microbiology and therapeutic applications	*Eugenia uniflora, Phthirusa pyrifolia*	2008, 2010
Colloidal gold-labeled lectin for the direct microscopic observations of bacterial exopolysaccharides in Cheddar cheese matrix using transmission electron microscopy	*Ricinus communis*	2005
Fluorescein-conjugated lectins for rapid visualization of *Candida albicans, Aspergillus fumigatus* and *Fusarium solani*	*Canavalia ensiformis*, *Lens culinaris, Triticum vulgaris*, *Ulex europeus*	1986
Identification of *Mycobacterium* species (*M. tuberculosis*, *M. avium*) by different agglutination in a microtiter plate	*Canavalia ensiformis*, *Cladrastis lutea*, *Galanthus nivalis*, *Narcissus pseudonarcissus*, *Vicia fava*, *Vicia sativa*	2006
Lectin-magnetic microspheres to distinguish between bacterial species from aqueous suspensions	*Helix pomatia*	1996
Quartz crystal microbalance lectin-based biosensor to identify the presence of bacteria	*Canavalia ensiformis*, *Lens culinaris*, *Maackia amurensis*, *Triticum vulgaris, Ulex europeus*	2008
Selectivity in targeting to skin-associated bacteria by Con A-bearing liposomes (*Streptococcus sanguis* and *Corynebacterium hofmanni*) and WGA-bearing liposomes (*Staphylococcus epidermidis*)	*Triticum vulgaris*, *Canavalia ensiformis*	1995
Tool for studying bacterial infections and inflammatory processes	*Araucaria angustifolia*	2006

References: [70-78; 80-92]

A thermo resistant lectin isolated from *Eugenia uniflora* seeds demonstrated a remarkable non-selective antibacterial activity [73]; the lectin strongly inhibited the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella* sp. with MIC of 1.5 μg/mL while was less effective in inhibiting the growth of *Bacillus subtilis*, *Streptococcus* sp. and *Escherichia coli* (MIC of 16.5 μg/mL). Bactericide activity was mainly detected for *S. aureus, P. aeruginosa* and *Klebsiella* sp. (MBC of 16.5 μg/mL); the authors suggested the use of lectin for clinical microbiology and therapeutic purposes.

The antibacterial activity of *N*-acetyl-D-glucosamine-binding lectin isolated from *Araucaria angustifolia* seeds on phytopathogenic bacteria was revealed by reduction in the colony forming units. The lectin was more effective against the Gram-positive *Clavibacter michiganensis* (80% of reduction) than on Gram-negative *Xanthomonas axopodis* (60% of reduction). Electron microscopy revealed that treatment with *A. angustifolia* lectin promoted morphologic alterations including presence of pores in the Gram-positive bacteria membrane and bubbling on the Gram-negative bacteria cell wall [74].

Bark of *Sebastiania jacobinensis*, used by people as medicine to treat infections, contains antifungal lectin of glycan-complex carbohydrate specificity group [75]. The effect of lectin on growth of *Aspergillus niger*, *Candida albicans*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Fusarium moniliforme* and *Trichoderma viride* was investigated and the lectin was active only on *Fusarium* species. The lectin was not toxic for *Artemia salina* and embryos of *Biomphalaria glabrata* and it was suggested that this fact is interesting for perspective of its biotechnological use as antifungal agent.

A stable, ion dependent and chitin-binding lectin isolated from *Opuntia ficus indica* cladodes 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% [77].

Mannose-binding lectins with antifungal activity have been described. The lectin isolated from *Ophiopogon japonicus* rhizomes was an antifungal agent against the phytopathogens *Gibberella saubinetii* and *Rhizoctonia solan*i but not on *Penicillium italicum* [76]. The lectin of *Pisum sativum* seeds inhibited the growth of *Aspergillus flavus*, *Fusarium oxysporum* and *Trichoderma viride* [81].

3. Conclusion

Plant tissues contain secondary metabolites and lectins with antibacterial and antifungal activities and thus are sources of natural bioactive molecules to control pathogens that cause diseases in plants and humans. The ability of lectin selectively to bind microrganisms makes them potential tools to study pathogen species.

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