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EXPLORING THE ANTIMICROBIAL PROPERTIES OF PLANT LEAF LECTINS (A REVIEW)

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Abstract - *This review portraits the antimicrobial properties of lectins isolated from a few plants. Lectins are proteins that specifically bind to carbohydrates and their special property is that they cause agglutination or precipitation of carbohydrate-containing molecules. Lectins being widely distributed in nature are resistant to most microbial communities and hence their optimization and usage to treat microbial infections are necessary. Lectins are abundantly found mainly in leguminous plants. This review gives a brief idea of lectin extraction from leaves of various plants and its antimicrobial activities. Certain compounds present in plants that possess antimicrobial properties against various bacteria and fungi can be used in various fields such as in agriculture, horticulture, medicine etc. which mainly reduces the use of chemical antimicrobial agents which results in the production of more natural products cost-effectively.*

Key Words: **lectins, carbohydrate binding sites, hemagglutination, antibacterial activity, antifungal activity**

1. INTRODUCTION

An enormous number of diseases have been caused by bacterial and fungi which contribute to the increased mortality rate as the use of commercial antibiotics has elevated, harmful pathogens develop resistance towards these frequently used drugs such as penicillin, methicillin, and oxacillin. The intensive use of antimicrobial agents in humans, animals, and plants favors the emergence of microorganisms' proof against these medications. Hence lately much importance is given to natural sources such as medicinal plants as they contain components that are fatal to pathogens. There is excellent interest in analytic antimicrobial substances from natural sources. Proteins and peptides with antimicrobial activity are present molecules found in microbes, plants, animals, and humans, that act by interfering with the microorganism growth by completely different mechanisms.

Lectins are natural carbohydrate-binding proteins that can mediate the identification of micro-organisms through the interaction with complex carbohydrates on microbial surfaces [1] promoting host-pathogen communications, immune defense activation and cell-to-cell signaling.

Lectins contain two or more catalytic sites for the binding of specific sugars such as mono or oligosaccharides. These proteins are widely distributed in nature and have been isolated from microorganisms, plants, and animals [2]. Cells possessing carbohydrate units on their surface have binding sites for lectins of other cells. Lectins bind to carbohydrates of other cells through weak interactions, when the number of lectins and carbohydrate groups from other cells increases, the interaction becomes stronger and agglutination of cells or precipitation of polysaccharides or glycoconjugates can be seen. Earlier studies conclude that lectins present in plants, defend them against pathogens, herbivores, and predators. This potential property has paved the way for advanced studies of their role in the control of microorganisms and pest insects.

Besides, antitumor activities, cytotoxic as well as vasorelaxant effect, and immunomodulatory actions are some properties among the wide range of biological activities described for plant lectins. These lectins play a role in immunological defense against pathogens, inhibit adhesion and migration of microbial cells and block viral infections [3].

2. LECTIN CHARACTERIZATION

Lectins are mainly characterized based on their sugar-binding specificity. Lectins can bind to monosaccharides and oligosaccharides or just oligosaccharides. According to their specificity, lectins have been classified as glucose/mannose, N-acetylglucosamine, galactose/N-acetylgalactosamine, and fucose-binding specific [4]

fava bean lectin showed specificity for mannose and glucose residues [5]. A lectin from the corn coleoptyle

interacts with galactose or N-acetylgalactosamine [6]. A commercially available lectin named *Aleuria aurantia* lectin (AAL) is known for its high affinity for α-1, 6-fucosylated oligosaccharides and it is used to estimate the extent of α-1,6-fucosylation on glycoproteins and to fractionate glycoproteins [7]. *Lotus tetragonolobus* lectin is specific to fucose [8]. Lectins that are specific to Sialic acid were found in invertebrates such as from the Indian horseshoe crab [9], marine crab Scylla serrata [10], lobster, tunicalase, fungus *Hericium arinaceum* [11] and leaves of mulberry [12].

Lectins can be detected by hemagglutination assay where lectins bind to RBC's causing visible clumps. Lectins can be extracted using various buffers such as PBS [13], tris buffer [14], or just saline [15] (0.2M NaCl) and precipitated using various saturations of ammonium sulfate for example 50% saturation was used to precipitate the crude extract of *Aloe vera* L. leaf pulp lectins [13]. The precipitated lectins can be dialyzed and purified using a sequence of chromatographic techniques such as gel filtration chromatography using Sephadex G-50 column [16], ion-exchange chromatography on a DEAE cellulose column [16], and affinity chromatography [15]. Once the pure lectin is obtained after chromatography, SDS-PAGE or native gel electrophoresis is usually carried out to check the molecular size of the lectin along with some standard proteins as references.

Most lectins are active at different temperatures and pH. Lectins are more active at optimum temperature and pH which various among lectins of different sources. Hemagglutination of lectins is checked at different temperatures and pH based on these factors the optimum conditions required by the lectins are discovered.

3. ANTIMICROBIAL PROPERTIES

Plant lectins mediate their microbicidal activity by triggering host immune responses that lead to the release of many cytokines which in turn activates the effector mechanism and the phagocytic activity of macrophages is enhanced during microbial infections. Recent studies have been researching the role of lectins in microbiology since lectins represent valuable tools to study the interaction between the carbohydrates present in eukaryotic cells and pathogens for uncovering infectious disease development [17]. Many pathogens initiate adhesion followed by infection using cell surface carbohydrates as either receptors or ligands [18].

Lectins interact with N-acetylglucosamine, N-acetylmuramic acid (MurNAc) and tetrapeptides linked to MurNAc present in the cell wall of Gram-positive bacteria or to lipopolysaccharide present in the cell walls of Gram-negative bacteria [19].

Lectins interact with the fungal cell wall, which is composed of chitin, glucans, and other polymers [20]. Lectins that bind to chitin can impair the synthesis and deposition of chitin in the cell wall as well as prevent hyphal development and spore germination [21] [22].

Lectin concentration and purification is crucial for the increased specificity of hemagglutination.

For examples, the lectin of *Euphorbia helioscopia* (EHL) belonging to the family Euphorbiaceae showed antibacterial activity against the pure cultures of *Klebsiella pneumonia, Escherichia coli,* and *Pseudomonas aeruginosa* where Ceftriaxone was used as the reference antibiotic. EHL was more resistant to *Klebsiella pnuemoniae* with a diameter of 16mm being the zone of inhibition followed by *Escherichia coli* and *Pseudomonas aeruginosa* showing 12mm and 9mm respectively [23]. The remarkable antibacterial property of this lectin can be significantly important for clinical microbiology and therapeutic applications.

Whereas from the family Moringaceae the lectins isolated from *Moringa oleifera* leaves namely SLL1, SLL2, and SLL3 showed antibacterial activities against pure strains of *E coli, Sh dysenteria,* and *St aureus*. The zones of inhibition exhibited by these lectins were measured in mm. Standard antibiotics such as doxycyclin, tetracycline, ampicillin, and erythromycin were used as the positive controls, and empty discs as the negative control. The zone of inhibition shown by SLL-1 was maximum comparatively, whereas SLL-3 was the least efficient. The research also reported that the lectins could inhibit the growth of various Gram-positive and Gram-negative bacteria at the concentration of 30µg/15µl. The Diameter of zones of inhibition of SLL-1 against *E. coli, Sh. Dysenteriae* and *St. aureus* were 13, 17, and 31 respectively; for SLL-2 and SLL-3, the zones were 11, 14, 24, and 10, 13, 20 respectively [24].

The PpyLL (*Phthirusa pyrifolia* leaf lectin) belonging to Loranthaceae exhibited antibacterial activity against some bacteria and the antibacterial property was investigated by the disc diffusion method. Gram-positive strains were *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (UFPEDA9),

Streptococcus faecalis (ATCC 6057), and *Bacillus subtilis* (UFPEDA16), and Gram-negative strains were *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 29665). Bacteria were grown in Nutrient Broth (NB). chloramphenicol (100 mg) was used as the positive control for Gram-negative bacteria and vancomycin (30 mg) for Gram-positive bacteria, and discs with 0.15 mol/L NaCl (15 mL) were used as the negative control. Zones of inhibition around the discs were visible. Antibacterial activity against *S. epidermidis* (10.25 ± 0.5mm), *S. faecalis* (12.45 ± 0.5mm), *B. subtilis* (10.75 ± 0.5mm), and *Klebsiella pneumonia* (11.50 ± 1.3mm) were exhibited by PpyLL [25]. The study showed that PpyLL was more efficient in inhibiting Gram-positive than Gram-negative species which could be due to the high levels of peptidoglycan present on Gram-positive bacterial cell wall.

On comparing the inhibitory effect of PpyLL with EHL both at the same concentration (15µg/disc), EHL was much more efficient than PpyLL in inhibiting *Klebsiella pneumoniae* and PpyLL had no effect on *Pseudomonas aeruginosa* but was inhibited by EHL showing 9mm inhibitory zone.

This study discovered that though PpyLL was not bactericidal to most tested bacteria, it caused agglutination of cells at the bottom of the assay tube after 6 hr which was clearly visible. This inferred that the lectin had the ability to recognize and agglutinate the carbohydrates present on bacterial cells surface and could promote immobilization and inhibition of growth or could even destroy the bacteria. The lectin-bacterial cells interaction could be due to covalent or non-covalent aggregation which was based on the molecular weight of the subunits. This study also focused on the importance of agglutination of PpyLL to bacterial cells as this property may increase the efficacy of the antibiotic in treatments when lectin is used in combination with it as the antibiotic concentration could be lowered due to the clustering of cells caused by lectin.

Antifungal activity of PpyLL was checked against *Aspergillus niger* (URM2813), *A. flavus* (URM2814), *A. fumigatus* (URM2815), *Rhizopus arrhizus* (URM2816), *Paecilomyces variotti* (URM2818), *Fusarium moniliforme* (URM2463), *F. lateritium* (URM2665), *Candida albicans* (UFPEDA1007), *C. burnenses* (UFPEDA4674), *C. tropicalis* (URM1150), *C. parapsilosis* (URM3624), *Saccharomyces cerevisae* (UFPEDA5107), and *Rhizoctonia solani* (URM 2820) [25]. Fungi were grown at 28°C on potato dextrose agar plates. Cercobin (10 mg) was used as the positive control, and 0.15 mol/L NaCl was used as the negative control. The antifungal activity was observed as an inhibition line forming around the discs. PpyLL did not inhibit the growth of most fungi tested but inhibited the growth of *F. lateritium* and *R. solani* [25].

A lectin isolated from *Calliandra surinamensis* leaf pinnulae which belongs to Fabaceae family was named CasuL and its antibacterial activity was checked against *Escherichia coli* ATCC-25922, *Staphylococcus aureus* (standard strain ATCC-6538 and oxacillin-resistant strain UFPEDA-670), and *Staphylococcus saprophyticcus* UFPEDA-833 [26]. The microorganisms were cultured in Mueller Hinton Broth (MHB) at 37°C for bacteria and Sabouraud Dextrose Broth (SDB). All the lectin concentrations tested were unable to inhibit the growth of the bacteria in more than 50%, then the MIC and MBC values were not determined. *E. coli* was resistant to the lectin treatment at all concentrations tested. On the other hand, CasuL inhibited the growth of *S. saprophyticcus* and the oxacillin-resistant isolate of *S. aureus*, although it was not higher than 30%. The best results were obtained for the non-resistant *S. aureus* isolate, whose growth was significantly reduced ($p < 0.05$) at all concentrations with inhibition values ranging from 38% to 49%. The *S.saprophyticcus* isolate was resistant to tetracycline which was used as a reference drug. Antifungal activity of CasuL was assessed against four *Candida* species but only *C. krusei* was sensitive to this lectin [26].

A plant from Anacardiaceae namely *Schinus terebinthifolius* leaf lectin (SteLL) was checked for its antibacterial and antifungal activities. Gram-positive (*Staph. aureus* WDCM 00032) and Gram-negative (*E. coli* WDCM 00013, *Klebsiella pneumoniae* ATCC 29665, *Ps. aeruginosa* WDCM 00025, *Proteus mirabilis* WDCM 00023, and *Salmonella enteritidis* MM 6247) strains were used to check its antibacterial activity [27]. Bacteria were cultured in nutrient broth (NB). The leaf extract had an inhibitory effect on *E. coli, Pr. Mirabilis,* and *Staph. aureus* and it showed no effect on *Kl. pneumoniae*, *Ps. Aeruginosa,* and *Salm. enteritidis.* Whereas the SteLL was active against all tested bacteria. For antifungal activity, the fungi were cultured in Sabouraud dextrose. *C. albicans* growth was inhibited by the leaf extract with a MIC (minimum inhibitory concentration) of 12.75 µg/ml of protein and SteLL with a MIC of 6.5 µg/ml. Minimal fungicide concentration (MFC) was defined as the lowest protein concentration showing no fungal growth [27].

4. APPLICATIONS

Lectins are found abundantly in various plant tissues with different biological properties. These molecules are isolated and analyzed for various activities that are applicable in the field of medicine.

This review mainly focuses on the antimicrobial activities of a few plant lectins. The applications of lectins are vast and are mainly used in medicine. They possess various other applications such as anti-insect activity, antitumor activity, antiviral activity, antimitogenic activity [28], antiparasitic activity, and antibiofilm.

Other applications of lectins are in signal transduction, innate immunity, cytotoxicity, activation of lymphocytes, phagocytosis, leucocytes extravasation, complement activation.

5. CONCLUSIONS

Antimicrobial infections caused by bacteria, fungus and viruses are increasing rapidly. Extensive use of antibiotics have resulted in the development of resistance to antibiotics by micro-organisms. As the resistance exhibited by the micro-organisms elevate, the concentration of antibiotics to be used against them will be increased which can be done only up to a certain limit as it may elicit unnecessary side effects to humans. Hence the alternative way is to discover natural antimicrobial sources that can be a substitute to commercially available drugs or that can increase the efficiency of a drug so that the concentration of the drug can be reduced. Lectins bind to carbohydrates or glycoconjugates present on bacteria, fungi, and viruses. This lectin-carbohydrate interaction leads to the disruption of the cell wall of bacteria and fungus which therefore leads to the death of the organism. Hence lectins with antimicrobial and antiparasitic activity can be used to improve the efficacy of antibiotic therapy. The lectin molecules comprising of various medicinal properties can be investigated and effectively used for the treatment of most microbial infections in a cost-effective manner.

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