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Biological potential of alkaline lignins: A brief review

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Abstract. Lignins are polyphenolic macromolecules found in the cell walls of plants. They are considered promising sources of material for bioproducts due to their abundance, renewability, and multiple biological activities. The purpose of this review is to present the vast biological potential of alkaline lignins, highlighting their applicability in various areas such as medicine, biotechnology, and sustainable industry. The antioxidant, immunomodulatory, antimicrobial, antitumor, leishmanicidal, trypanocidal, and antimalarial activities of lignins are presented, emphasizing the impact of the chemical structure of these macromolecules on their biological functions. Although the exact mechanism of these activities still needs to be fully elucidated, the ability of lignins to interact with diverse biological targets opens up opportunities for several future scientific investigations and demonstrates their potential for use as a bioproduct. This work highlights the

potential use of alkaline lignins as candidates for the development of new sustainable products and materials. It also emphasizes the necessity for research focused on elucidating the mechanisms underlying its biological activity. Advances in this field could significantly transform the utilization of industrial by-products into valuable bioproducts, facilitating the shift towards an economy reliant on renewable resources and fostering harmonious coexistence with natural ecosystems.
Keywords: Alkaline lignins, bioproducts, biological activities

Introduction

Lignins are natural amorphous and polyphenolic macromolecules with complex three-dimensional structures found in the cell walls of plants, associated with cellulose and various polysaccharides (Mennani et al., 2024). Lignins, following cellulose, are the most abundant biomacromolecules on the planet and are considered the primary source of natural phenols (Shorey et al., 2024).

These macromolecules are biosynthesized by dehydrogenative polymerization (initiated by enzymes) of the following primary precursors: trans-coniferyl alcohol, trans-sinapyl alcohol, and trans-p-coumaryl alcohol, giving rise to the phenylpropanoid units called guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H), respectively. These units are joined by different types of chemical bonds such as β-O-4, β-1, β-4, β-β, 5-5, and 4-O-5 (Khadem et al., 2024). From a functional point of view, lignins facilitate the transport of water and nutrients, provide resistance to the cell wall, and protect polysaccharides against microbial degradation (Yang et al., 2024).

Traditionally, these phenolic macromolecules are obtained as a co-product of the cellulose and paper industries in a form called black liquor (rich in lignin) (Pola et al., 2022). Globally, the pulp and paper sectors generate between 50 and 70 million tons of lignin annually, with a projected production of around 225 million tons per year by 2030 (Mennani et al., 2024; Yang et al., 2024). However, the lignin present in the liquor can be recovered through acid precipitation and utilized as a renewable raw material to produce various high-value products (Huang et al., 2024). The global market for lignin-based products was valued at US$864.67 million in 2021 and is projected to increase to US$1,179.48 million in 2030 (Straits et al., 2022).

The use of lignins as a bioproduct is conditioned by their complex and variable nature, which includes sources and production processes. These macromolecules have great versatility and can exhibit various biological properties, such as anticoagulant, antioxidant, antitumor, antiviral, and immunomodulatory activities (Khadem et al., 2024). Biological activities are directly associated with the chemical structure of lignins; thus, different lignins can exhibit varying biological activities (Zhu et al., 2023). However, it is worth noting that the mechanism of action for the biological activities described here is not fully elucidated.

The objective of this brief review was to demonstrate that lignins possess potential biological activities and can serve as raw materials for producing high-value products.

Lignins as antioxidant agents

Lignins are described as potential antioxidant agents. Cruz Filho et al. (2019) compared lignins isolated from *Opuntia cochenillifera* and *Opuntia ficus-indica*, both from the Cactaceae family found in arid regions of northeastern Brazil and commonly used as cattle feed. Their study revealed that lignins from *O. cochenilifera* exhibited higher antioxidant activity than those from *O. ficus-indica*. Specifically, only in the O. cochenilifera lignins showed an EC_{50} value of 253.9 µg/mL in the ABTS assay. Since the chemical structure of *O. cochenilifera* lignin contains more phenolic groups, it was proposed that the antioxidant capacity of lignins is associated with the presence of phenolic groups in the plant's structure.

Melo et al. (2020) evaluated the antioxidant activity of lignin from *Caesalpinia pulcherrima*, popularly known as Flamboyanzinho, a woody shrub from the Fabaceae family. It is native to Central America and is used by some people as a medicinal plant. This activity was evaluated using the phosphomolybdenum complexation method (40 ± 1.15%), DPPH radical scavenging (16.90 ± 0.18%), and ABTS (51.07 ± 1.26%), which indicated a good antioxidant potential closely linked to its chemical structure, primarily the content of phenolic hydroxyls.

The results obtained by Melo et al. (2020) were different when compared to those obtained by Santos et al. (2020), who evaluated the antioxidant activity of lignins from *Conocarpus erectus*, known as mangue-de-butão, an American shrub often found in mangroves. Its lignin exhibited low total antioxidant activity, measuring 17.92 ± 0.41% activity in 500 µg for the phosphomolybdenum complexation method, a value significantly lower than that reported by Melo et al. (2020). When compared with commercial antioxidants, such as ascorbic acid and butylated hydroxytoluene (BHT), lignin did not stand out significantly. In the DPPH test, it yielded a value higher than BHT but lower than ascorbic acid. In the ABTS assay, lignin from *Conocarpus erectus* exhibited an IC_{50} of 356.03 µg/mL, a value higher than that of BHT and ascorbic acid. Additionally, the IC_{50} of lignin from *C. pulcherrima* was found to be 251.34 µg/mL.*C. erectus* lignin also exhibited a moderate ability to inhibit lipid peroxidation, with values very similar to those of commercial antioxidants.

Arruda et al. (2021) conducted identical tests to assess the antioxidant capacity of lignins from *Crataeva tapia*, a tree from the Capparaceae family commonly referred to as Tapiá, which is prevalent throughout the Amazon region. In this study, the researchers concluded that the antioxidant activity of this lignin was linked to its concentration based on the results obtained from the phosphomolybdenum complexation and ABTS assay. For the DPPH and nitric oxide elimination assays, the results were practically the same for all concentrations. In summary, the lignins of *C. tapia* exhibited low values of antioxidant activity, similar to the results reported by Santos et al. (2020). This is likely attributed to the high content of aliphatic groups in these lignins, which have low antioxidant activity.

Silva et al. (2021) analyzed lignins from *Morinda citrifolia* and found that they exhibited low antioxidant activity compared to commercial antioxidants. Nevertheless, these lignins showed the highest results compared to previous studies, possibly due to the higher phenolic content present in this species.

Araújo et al. (2022) also demonstrated that the antioxidant action became more significant as the lignin dosage increased in their study with *Buchena viaviridiflora*. In the ABTS assay, *B. viridiflora*lignins exhibited an IC_{50} of 14.08 ± 0.5 µg/mL (lignins extracted from the branches) and 350.20 ± 1.6 µg/mL (lignins extracted from the leaves). However, they presented low total antioxidant activity due to insufficient hydrogen transfer in the molybdenum test. This result is related to the chemical structure of the compound, where the presence of conjugated double bonds exerts an antioxidant activity as significant as phenolic compounds. Silva et al. (2023) obtained different results when evaluating Caesalpinia ferrea lignins, with an IC_{50} of 559.9 ± 0.8 µg/mL in the DPPH assay and 484.1 ± 0.1 µg/mL in the ABTS assay. These results were lower compared to ascorbic acid and BHT. For the other tests, it was not possible to determine IC_{50} values and they were considered weak antioxidant agents.

Cruz Filho et al. (2023), as well as Arruda et al. (2021) and Araújo et al. (2022), also found that the antioxidant activity of lignins becomes more significant as the concentration increases. The results of the ABTS assay revealed that lignins isolated from the leaves and branches of *Protium puncculatum* and *Scleronema micranthum* exhibited promising antioxidant activity. Among them, lignins from the *S. micranthum* branch stood out with the highest antioxidant activity. In the DPPH radical scavenging assay, lignins from *S. micranthum* leaves were emphasized. Although both lignins did not exhibit significant total antioxidant activity, it was not possible to determine the IC_{50} in the phosphomolybdenum complex reduction assay. A correlation study was conducted between the antioxidant activity and the phenolic content of the lignins, revealing a weak correlation. This suggests that other functional groups also play a role in influencing their activity. The results of antioxidant activity varied, which is associated with the content of phenolic groups and conjugated bonds present in the structure of lignins (Cruz Filho et al., 2023). Table 1 summarizes the results of the study on antioxidant activity.

Immunomodulatory activity

The literature describes lignins as potential immunomodulatory agents; however, the mechanism for this activity is not yet fully elucidated, and different immunological responses can be obtained (Arruda et al., 2021). However, it is known that the prominent functional groups in the structure of lignins include aliphatic and aromatic hydroxyls, ethers, carbonyls and methoxyls. These groups can be recognized by one or more cellular receptors present on the cell surface (lignin does not cross the cell due to its molecular size), thus triggering various immune responses *in vitro*(Arruda et al., 2021; Santos et al., 2020).

Cruz Filho et al. (2019) evaluated alkaline lignins from *Opuntia cochenillifera* and *Opuntia ficus-indica* and found that the macromolecules demonstrated high cell viability (>96%) and cell proliferation. There was evidence of activation for both lignins, including increases in reactive oxygen species (ROS) and cytosolic calcium levels, as well as changes in mitochondrial membrane potential.

Furthermore, lignins induced a high production of TNF-α, IL-6, and IL-10, while reducing nitric oxide (NO) release. Therefore, these lignins have great potential as molecules with a pro-inflammatory profile.

Santos et al. (2020) found that lignin from *Conocarpus erectus* stimulated the differentiation of lymphocytes and monocytes, activated CD8+ T cells, and increased the release of nitrite and cytokines associated with both Th1 and Th2 responses, particularly those linked to a pro-inflammatory response.

Arruda et al. (2021) found that lignin from *Crataeva tapia* did not demonstrate cytotoxicity in immune cells, stimulated the production of TNF-α, IL-6, and IL-10, and did not cause significant changes in nitric oxide levels.

Araujo et al. (2022), evaluated the lignins from the branches and leaves of *Buchena viaviridiflora* and found that they significantly induced the production of several anti-inflammatory cytokines, characterizing a Th2 profile. These cytokines included IL-4, IL-10, and IL-6 (the latter being pleiotropic). The remaining cytokines were produced at basal levels, similar to the control, and therefore did not influence the immunological status in the cultures. Furthermore, the increase in nitric oxide, without inducing cell death, confirms that the evaluated lignins exert an anti-inflammatory effect *in vitro*. These findings show that lignins are promising immunomodulatory agents.

Table 1. Antioxidant activity of lignins

Lignins	TAA	DPPH	ABTS	FRAP	NO	FTC				
	IC_{50}	%0.5	IC_{50}	%0.5	IC_{50}	%0.5	%0.5	%0.5	%0.5	References
	µg/m L	mg/mL	µg/mL	mg/mL	µg/mL	mg/mL	mg/mL	mg/mL	mg/mL	
Opuntia fícus-indica	ND	4.0±0.0	ND	12.0±0.3	ND	26.8±0.1	ND	4.7±0.7	NR	Cruz Filho et al. (2019)
Opuntiacochenilifera	ND	8.5±0.0	ND	21.1±0.1	253.9	61.5±0.9	ND	5.8±0.2	NR	Cruz Filho et al. (2019)
Caesalpiniapulcherrima	.3.90	40±1.15	ND	16.90±0.18	251.34	51.07±1.26	ND	NR	NR	Melo et al. (2020)
Conocarpuserectus	ND	17.9±0.41	231.1	ND	356.03	ND	ND	NR	42.1±0.7	Melo et al. (2020)
Crataevatapia	ND	18.9±0.1	ND	19.7±0.2	430±1.9	58±0.01	ND	8.4±1.06	NR	Arruda et al. (2021)
Morinda citrifolia	ND	34.9±0.2	ND	40.83±0.1	175.34±1.3	74.35±0.1	ND	NR	NR	Arruda et al. (2021)
*Buchena viaviridiflora (*branches *)*	ND	1.4±0.01	884.5±1.4	51.29±0.1	14.08±0.5	87.06±0.15	37	NR	NR	Araújo et al. (2022)
Buchena viaviridiflora (leaves)	ND	0.9±0.01	ND	39.55±0.0	350.20±1.6	93.43±0.1	13	NR	NR	Araújo et al. (2022)
Caesalpiniaferrea	ND	> 5%	559.9±0.8	ND	484.1±0.1	ND	> 5%	NR	NR	Silva et al.(2023)
Protiumpuncculatum (branches)	ND	4.8±0.0	ND	25.35±0.1	454.37±0.9	83.84±1.37	25	NR	NR	Cruz Filho et al. (2023)
Protiumpuncculatum (leaves)	ND	5.4±0.01	252.72±0.3	78.12±0.2	419.83±1.0	93.81±0.0	32	NR	NR	Cruz Filho et al. (2023)
Scleronema micranthum (branches)	ND	2.1±0.02	477.35±1.2	83.60±0.19	1.4±0.0	94.0±0.1	19	NR	NR	Cruz Filho et al. (2023)
Scleronema micranthum(leaves)	ND	0.8±0.03	77.65±0.4	83.72±0.5	62.5±1.0	92.04±0.5	9	NR	NR	Cruz Filho et al. (2023)

TAA: Total Antioxidant Activity; DPPH: DPPH radical scavenging; ABTS: ABTS radical scavenging; FRAP: Ferric Reducing Antioxidant Power; NO: Nitric Oxide; FTA: Ferric Thiocyanate (FTC) assay. ND: Not Determined

Antimicrobian activity

Lignins are natural antimicrobials protecting the plant against attack by different microorganisms. The literature describes different studies evaluating the *in vitro* potential of these macromolecules. Araújo et al. (2022) evaluating the antimicrobial activity of lignins from the branches and leaves of *Buchena viaviridiflora* found that these macromolecules presented antibacterial activity against *Staphylococcus aureus* UFPEDA-709, *Enterococcus faecalis* UFPEDA-69 and *Pseudomonas aeruginosa* UFPEDA-261. Furthermore, it was able to inhibit growth from the yeast *Candida albicans* 4664. Likewise, Melo et al. (2020) observed antifungal activity of lignin isolated from the leaves of *Caesalpinia pulcherrima* against yeast strains such as *Candida parapsilosis, Candida guilliermondii* and *Cryptococcus neoformans*. Cruz Filho et al. (2023), evaluating a lignin obtained from *Morinda citrifolia* leaves, verified antimicrobial activity, with a minimum inhibitory concentration (MIC) of 512 µg/mL for *Staphylococcus aureus* UFPEDA-709 (same MIC value compared to the standard antibiotic), as well as for *Candida albicans* 4664 and *Candida glabrata* UFPEDA-6393.

Antitumor activity

The antitumor activity induced by lignins is directly related to the phenolic content in the macromolecular structure. Araújo et al. (2021) evaluated alkaline lignins obtained from the branches and leaves of *Buchena viaviridiflora*. IC_{50} values were obtained for Jurkat cells (21.37; 25.76 µg/mL), MCF-7 (12.63; 24.88 µg/mL), T47D (25.46; 47.74 µg/mL), DU145 (37.56; 41.65 µg/mL), and HepG2 (>100 µg/mL). Cruz Filho et al. [16] evaluated lignin from the leaves of *Morinda citrifolia* and confirmed its antitumor activity. They obtained IC_{50} values for Jurkat cells (19.4 ± 0.2 µg/mL), MCF-7 (27.0 ± 0.1 µg/mL), T47D (32.7 ± 0.4 µg/mL), DU145 (52.3 ± 0.9 µg/mL), and HepG2 (75.6 ± 0.1 µg/mL). Silva et al. (2023) evaluated the antitumor activity of *Caesalpinia ferrea* lignin and obtained the following IC_{50} values: MCF-7 (45.1 ± 0.1 µg/mL), T-47D (50.4 ± 0.2 µg/mL), NCI-H292 (65.9 ± 0.01 µg/mL), HT-29 (78.5 ± 0.2 µg/mL), Jurkat (79.8 ± 0.0 µg/mL), DU145 (87.2 ± 1.0 µg/mL), HepG2 (88.5 ± 0.3 µg/mL), HL-60 (95.2 ± 0.1 µg/mL), and HEp-2 (98.4 ± 0.2 µg/mL). These results show that lignins are molecules with antitumor potential.

Leishmanicidal activity

The leishmanicidal activity induced by lignins has been described for different forms of the parasite, namely, promastigotes and amastigotes. Silva et al. (2021), while investigating the leishmanicidal activity of *Morinda citrifolia* lignin against the promastigote form of *Leishmania amazonensis*, obtained IC_{50} values equal to 29.56 ± 0.6 µg/mL. Furthermore, when conducting an ultrastructural analysis using electron microscopy, they confirmed changes in the morphology of the parasite. Cruz Filho et al. (2023) evaluated the lignins from the branches and leaves of *Protium puncticulatum* and *Scleronema micranthum*, obtaining IC_{50} values ranging from 20.32 to 26.31 µg/mL. They also observed structural changes in the shape of the parasite using electron microscopy.

Trypanocidal activity

In the literature, only two reports of the trypanocidal activity of lignins were found. Silva et al. [15] evaluated the trypanocidal activity of lignin from *Caesalpinia ferrea* leaves and obtained IC_{50} values of 29.34 ± 0.5 µg/mL for the Epimastigote form, 47.59 ± 0.1 µg/mL for the trypomastigote form, and 200.49 ± 0.4 µg/mL for the amastigote form. Cruz Filho et al. (2023) evaluated lignins from the branches and leaves of *Protium puncticulatum* and *Scleronema micranthum*, obtaining IC_{50} values ranging from 15.54 to 20.34 µg/mL.

Anti- Plasmodium falciparum activity

Only two studies were found that investigated anti-*Plasmodium falciparum* activity, in addition to trypanocidal activity. Araújo et al. (2022) evaluated two lignins obtained from branches and leaves of *Buchena viaviridiflora*. They observed IC_{50} values of 5592.38 ng/mL for branch lignin and 2511.44 ng/mL for leaf lignin against the *Plasmodium falciparum* 3D7 strain. Furthermore, they demonstrated activity against the Dd2 strain of *Plasmodium falciparum*, with an IC_{50} of 9,760 ng/mL (for twig lignin) and 2,440 ng/mL (for leaf lignin), respectively. Cruz Filho et al. (2023) evaluated the lignins of the branches and leaves of *Protium puncticulatum* and *Scleronema micranthum* and obtained values of Inhibition was lower than 20% against *Plasmodium falciparum* 3D7 (A) and *Plasmodium falciparum* Dd2 strains.

Conclusion

The literature presents alkaline lignins as promising biomolecules with a wide spectrum of potential biological activities, including antioxidants, immunomodulators, antimicrobials, antitumors, leishmanicidal, trypanocidal, and antimalarials. Although the mechanism of action for these activities is not completely elucidated, the chemical structure directly modulates the results obtained. The functional diversity of lignins, combined with their abundance in nature, makes them ideal candidates for the development of new products and materials. The elucidation of the biological action mechanism of alkaline lignins presents challenges and research opportunities. Advances in these domains are expected to catalyze the transformation of alkaline lignins from industrial byproducts into valuable bioproducts, encouraging the transition to a green economy that can coexist with our ecosystems.

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