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Bioactivity evaluation of four technical lignins: Alcell, Indulin-AT, Sarkanda and Curan 27-11P

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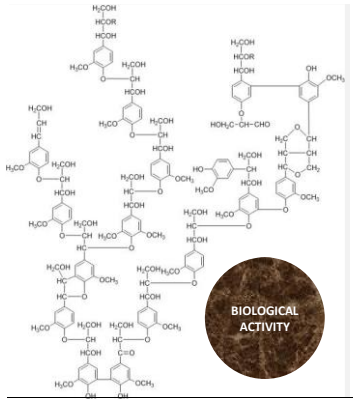

Lignin is, after cellulose, the most abundant biopolymer on earth. In what concerns bioactive properties, and due to its phenolic character, lignin is mostly studied for their antioxidant activity. In this work four technical lignins (Alcell, Indulin-AT, Sarkanda and Curan 27-11P) have been evaluated for their antioxidant activity (DPPH radical scavenging activity, reducing power and inhibition of β-carotene bleaching) and antitumor potential against MCF-7, NCI-H460, HCT-15, HeLa and HepG2 cell lines. Additionally, the toxicity for non-tumour cells (PLP2) was also evaluated. The obtained results were correlated with the chemical and structural features of the studied lignins. Based on the achieved results, lignins of GS type, i.e. lignins rich in syringyl phenol units and poor in *p*-hydroxyphenyl ones result in better antitumor potential.

Introduction

Lignin (from Latin lignum which means wood) is, after cellulose, the most abundant biopolymer on earth constituting about 15-30% of the wood and 12-20% of the annual plants. In plants lignin plays a vital role, ensuring water transportation and providing structural support by cementing cellulose fibres and fibrils. It also acts as a protection against biological attack.

Lignin arises from an enzyme-initiated dehydrogenative polymerisation of three precursors, namely *p*-coumaryl, coniferyl and sinapyl alcohol, that when incorporated into the lignin macromolecule structure originates *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) phenylpropanoid units, respectively (Figure 1). These phenylpropane units (C9 or C6-C3) are linked together covalently by several types of ether (β-O-4, α-O-4, 4-O-5) and carbon-carbon bonds (β-β, 5-5', β-5). The type of monomeric units and its relative abundance depend of lignin's botanic origin. In terms of chemical functional groups lignin structure include hydroxyl, methoxyl, carbonyl and carboxyl moieties in various amounts depending also on the botanic origin [1, 2].

In what concerns bioactive properties and due to its phenolic character, lignin is mostly studied for its antioxidant activity, envisaging its use as natural additives for functional food or in cosmetic and polymeric formulations [3]. To the best of our knowledge, the studies calling up the evaluation of its antitumor potential are scarcer being one of the most referred works the one of Skagami and co-workers [4]. In this work a pine wood lignin was reported to possess antitumor properties.

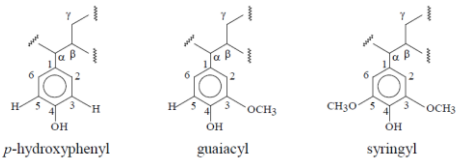

Figure 1. Lignin phenylpropane structural units.

In the present work, four technical lignins (Alcell, Indulin-AT, Sarkanda and Curan 27-11P) have been evaluated for their antioxidant activity (DPPH radical scavenging activity, reducing power and inhibition of β-carotene bleaching) and antitumor potential in MCF-7 (breast carcinoma), NCI-H460 (non-small cell lung carcinoma), HCT-15 (colon carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma). Additionally, the toxicity for non-tumour cells (PLP2) was also evaluated.

Materials and methods

Lignin samples: The technical lignins used represent three different pulp processes (Kraft, Soda and Organosolv) and various vegetal species (softwood, hardwood and non-wood). Indulin AT and Curan 27-11P (commercialized in the alkali form) are softwood lignins obtained by the Kraft pulping process and were kindly supplied by

MeadWestvaco (Glen Allen, VA) and BorregaardLignoTech (Sarpsborg, Norway), respectively. Sarkanda lignin was purchased from Granit SA (Lausanne, Switzerland). It is a non-wood lignin obtained from a soda pulping-precipitation process, patented by Granit SA. Alcell lignin of Repap Enterprises Inc. (Stamford, CT) was extracted from a mixture of hardwoods (maple, birch and poplar) by an organosolv process using aqueous ethanol.

Chemical and structural characterization: Lignin base materials were fully characterized in a previous work [5]. This characterization includes FTIR and ^{13}C-NMR for structural analysis and several complementary techniques for hydroxyl group's determination (^{13}C-NMR of acetylated samples, ^{31}P-NMR of phosphitylated lignins, and titration according to ISO 14900:2001 (E) standard).

Evaluation of antioxidant activity: Antioxidant activity was assessed by chemical and biochemical assays namely scavenging effects on 2,2-diphenyl-1-picrylhidrazyl (DPPH) radicals; reducing power by ferricyanide/Perl's Prussian blue assay; and bleaching inhibition of beta-carotene after neutralization of the linoleate-free radical and other free radicals formed in the beta-carotene-linoleate model system. The results were expressed as EC_{50} values (sample concentration responsible for 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay).

Evaluation of antitumor potential and hepatotoxicity: Antitumor potential was evaluated against a panel of human tumor cell lines, namely: MCF-7 (breast carcinoma), NCI-H460 (non-small cell lung carcinoma), HCT-15 (colon carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma), by using Sulforhodamine B assay, in accordance with the NCI (National Cancer Institute, USA) screening assays. The evaluation of hepatotoxicity was also possible by using a primary culture of porcine liver cells (non-tumor cells), following a methodology established at CIMO. The results were expressed as GI_{50} values (sample concentration responsible for 50% of net cell growth inhibition).

Results

Analysing structural characteristics, ^{13}C-NMR spectra exhibit evidences that Alcell and Sarkanda lignins belong to GS and HGS, respectively, whereas Indulin AT and Curan 27-11P lignins are built up with G type moieties. These data are in agreement with those obtained from FTIR analysis. In Alcell, the amount of syringyl phenol structures was higher than that of guaiacyl, and only a small quantity of *p*-hydroxyphenyl structures was detected. Sarkanda lignin, obtained from agricultural resources (wheat and hemp), presented a close proportion of G/S/H structures. Another important feature related to Sarkanda lignin is the evident presence of carboxylic acids. Alcell presented the high -OCH_3 content as expected from hardwood lignins. For all lignin samples it was observed that they are mainly composed of β-O-4 ether bonds together with small amounts of β-β and 5-5´carbon-carbon linkages.

Hydroxyl group determination (Table 1) revealed that Indulin AT and Curan 27-11P (softwood lignins) have the highest total hydroxyl content, 6.99 and 6.21 mmol/g, respectively. Alcell and Sarkanda lignins have approximately the same total hydroxyl content (5.26 mmol/g). The amounts of phenolic hydroxyl groups were similar for Alcell, Indulin AT and Curan 27-11P lignins. Sarkanda lignin has the lowest amount of phenolic hydroxyl groups, whereas Alcell has the lowest amount of aliphatic counterparts.

All the tested technical lignins showed bioactivity for the studied properties (antioxidant activity and antitumor potential). Table 2 presents the results achieved with the antioxidant activity, whereas Table 3 summarizes the results of antitumor potential. The Alcell lignin surpassed clearly the other samples either by its antioxidant activity, particularly in DPPH (EC_{50}=63 µg/mL) and β-carotene bleaching inhibition (EC_{50}=26 µg/mL) assays, or by the ability to inhibit tumor cell lines HCT-15 (colon carcinoma, GI_{50}=56 µg/mL), HeLa (cervical carcinoma, GI_{50}=17 µg/mL) and HepG2 (hepatocellular carcinoma, GI_{50}=46 µg/mL). It was the only sample that also showed activity against NCI-H460 (non-small cell lung carcinoma) tumor cell line. Indulin-AT revealed the highest reducing power (EC_{50}=151 µg/mL), while the greatest antitumor potential for the MCF-7 line (breast carcinoma, GI_{50}=28 µg/mL) was achieved for Sarkanda. Up to 400 µg/mL, none of the analysed lignins showed toxicity for non-tumor cells (PLP2).

Conclusions

From the analysed lignins, Alcell, a lignin extracted from a mixture of hardwoods (maple, birch and poplar) by an organosolv process using aqueous ethanol showed both the highest antioxidant activity and antitumor potential. These observations could be correlated with its guaiacyl-syringyl (GS) structure type and its high phenolic hydroxyl group's content. GS lignins are rich in syringyl phenol units and poor in *p*-hydroxyphenyl ones.

Table 1. Hydroxyl groups contents (mmol/g) for the analysed lignins (Alcell, Sarkanda, Indulin AT, Curan 27-11P).

Lignin sample	Total OH	Aliphatic OH	Phenolic OH	S-OH	G-OH	H-OH	5-condensed	Acids
Alcell	5.26	1.45	3.81	1.10	0.80	0.13	1.18	0.23
Sarkanda	5.26	2.85	2.41	1.89	0.82	0.62	0.65	0.62
Indulin AT	6.99	3.04	3.95	2.34	1.96	0.39	1.58	0.39
Curan 27-11P	6.21	2.58	3.63	2.16	2.01	0.47	1.49	0.47

Table 2. Antioxidant activity values expressed as EC_{50} values (μg/mL) for the analysed lignins. Results are presented as average±SD calculated from 3 replicas.

	Alcell	Sarkanda	Indulin AT	Curan 27-11P
DPPH scavenging activity	62.97±1.88	280.91±19.30	367.14±6.74	812.75±25.83
Reducing power	950.21±32.29	327.37±1.41	151.47±2.33	822.30±6.17
β-carotene bleaching inhibition	26.50±0.46	119.68±5.34	92.03±1.41	272.17±10.39

Table 3. Antitumor potential expressed as GI_{50} values (μg/mL) evaluated against a panel of human tumor cell lines. Results are presented as average±SD calculated from 3 replicas.

	Alcell	Sarkanda	Indulin AT	Curan 27-11P
MCF-7 (breast carcinoma)	59.44±3.98	286.32±15.16	95.60±6.40	220.83±10.93
NCI-H460 (non-small cell lung carcinoma)	197.74±15.32	>400	>400	>400
HCT-15 (colon carcinoma)	56.42±0.89	105.93±8.94	86.89±1.26	96.98±0.21
HeLa (cervical carcinoma)	16.97±3.27	85.46±1.10	57.35±2.95	82.18±5.54
HepG2 (hepatocellular carcinoma)	45.61±1.62	78.79±7.38	73.50±2.95	71.33±5.47

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