Sugarcane bagasse as source of bioactive lignin: influence of pretreatment on the antioxidant and antibacterial activities

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Introduction

Sugarcane industry generates several by-products of particular interest in the context of a circular economy, due to their potential as a source of value-added ingredients. Bagasse is one of the main by-products, consisting of a fibrous fraction obtained after juice extraction. Sugarcane bagasse is composed of cellulose, hemicellulose, and lignin, with lower amounts of extractives and ash (del Río et al., 2015). Lignin is one of the most abundant renewable resources on earth and recognized as a profitable source of high value compounds. The main functions attributed to lignin in the plant are elasticity and mechanical strength. It is a complex aromatic macromolecule formed by the dehydrogenative polymerization of three phenylpropanoid monomers coniferyl, synapyl, and p-coumaryl alcohols. The antimicrobial activity of lignin is related to plant sources and attributed to the phenolic components, particularly the side-chain structure and its functional groups (Chauhan, 2020). The antioxidant activity of lignin is mainly attributed to the scavenging action of their phenolic structures on oxygen-containing reactive free radicals. For the antioxidant activity of the lignin, free phenolic hydroxyl groups and ortho-methoxy substitution in aromatic rings are essential. The structural characteristics of lignin depend on several factors including the botanical origin, and environmental growth and extraction conditions.

Objectives

The main objective of this work was to study the potential of lignin from sugarcane bagasse as antimicrobial and antioxidant agent and assess the influence of two different pretreatments - deep eutectic solvents and organosolv – on the biological potential.

Methods

Antibacterial activity

The antibacterial activity was evaluated according to agar dilution procedure (CLSI, 2012). Lignin samples (DES lignin, organosolv lignin, and kraft lignin) were incorporated into the Mueller Hinton agar medium at different concentrations (5, 10, 15, 20 and 30 mg/mL) to be tested against representative Gram-positive and Gram-negative microorganisms, *S. aureus* DSM 799, and *E. coli* DSM 1576, respectively. The samples were tested in duplicate.

Antioxidant activity

Trolox equivalent antioxidant capacity (TEAC) assay

The antioxidant activity was assessed by the TEAC assay with the radical cation ABTS, according to the method described in literature (Gonçalves et al., 2009) with some modifications. The concentration of ABTS radical was adjusted with methanol to an initial absorbance of 0.700 (± 0.020) at 734 nm. To 200 µL of this solution of ABTS was added 15 µL of sample or Trolox (positive control) or solvent (blank) in a 96-well plate. The mixture was incubated for 5 min at room temperature in the dark, and the absorbance at 734 nm was measured in a microplate reader. The radical stock solution was freshly prepared, and all the analyses were performed in triplicate. Samples were prepared by dissolving 10 mg of each extract in 1 mL of methanol, sonicated for 30 min and filtrated through a 0.25 µm syringe filter. The percentage results of scavenging activity were calculated as % inhibition using the following equation (1):

$$\%\ Inhibition = \frac{(A_c - A_s)}{A_c} \times 100 \quad (1)$$

where, Ac is the absorbance of control and As is the absorbance of the sample. Half maximal inhibitory concentration (IC50) values were determined for all the samples.

DPPH assay

The ability of the extracts to scavenge the DPPH radical was determined by the method described in literature (Bondet et al., 1997) with minor modifications. For the DPPH assay, 175 µl of DPPH methanolic solution (600 µM) were added to 25 µl of each extract or butylated hydroxytoluene (BHT) (reference antioxidant) or solvent (blank). Samples preparation was performed as previously described for the TEAC method. After 30 min in the dark at room temperature, the inhibition of the DPPH radical was measured at an absorbance of 515 nm. All experiments were performed in triplicate. The antioxidant activity was expressed as a percentage DPPH inhibition following Equation (1). Half maximal inhibitory concentration (IC50) values were determined for all the samples.

Folin-Ciocalteu method

Total phenolic content was estimated by the Folin-Ciocalteu colorimetric method, using gallic acid as a standard phenolic compound. The absorbance was measured at 750 nm using microplate reader. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in milligrams per gram of dry extract by means of a calibration curve obtained with a standard of gallic acid.

Results and discussion

Antibacterial activity

Lignin extract from deep eutectic solvents pretreatment exhibited antibacterial activity against Gram-negative bacteria (*Escherichia coli*) with a minimum inhibitory concentration of 30 mg/mL (Table 1). Lignin extract from organosolv pretreatment was active against Gram-positive bacteria (*S. aureus*) with a minimum inhibitory of 30 mg/mL. Kraft lignin (Sigma Aldrich, CAS 8068-05-1) inhibited the growth of *S. aureus* at a concentration of 10 mg/mL

Table 1. Antibacterial activity of lignin samples and commercial cosmetic preservatives (Lexgard® Natural MB, MicroCurb™ OC and Geogard®).

Sample	*E. coli* DSM 1576		*S. aureus* DSM 799	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Kraft lignin§	NA	NA	10	30
DES lignin	30	30	NA	NA
Organosolv lignin	NA	NA	30	NA
Lexgard® Natural MB*	10	10	10	10
MicroCurb™ OC*	20	20	20	20
Geogard®*	5	10	10	15

§Sigma-Aldrich, CAS 8068-05-1, purity >95%
NA - Not active
*Values from the respective technical data sheet.

Antioxidant activity

Total phenolic content given by the Folin-Ciocalteu method was higher for the sugarcane bagasse organosolv lignin (249.9 ± 32.1 mg/g gallic acid equivalents) followed by sugarcane bagasse deep eutectic solvents lignin (165.8 ± 27.6 mg/g gallic acid equivalent) (Table 2). The antioxidant activity of the lignin extracts was evaluated by the radical 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and 2,2-diphenyl-1-picrylhydrazyl assays. Butylated hydroxytoluene was studied as reference antioxidant. For ABTS and DPPH radicals, organosolv lignin showed higher radical scavenging capacity (ABTS: 0.262 ± 0.016 mg/mL; DPPH: 0.379 ± 0.010 mg/mL) in comparison to deep eutectic solvents lignin (ABTS: 0.345 ± 0.008 mg/mL; DPPH: 0.633 ± 0.025 mg/mL).

Table 2. Antioxidant activity and total phenolic content of lignin extracts obtained from organosolv and deep eutectic solvents pretreatments.

Sample	IC 50 (mg/mL)		GAE mg/g dry extract
	TEAC	DPPH	Folin-Ciocalteu
Organosolv lignin	0.262 ± 0.016	0.379 ± 0.010	249.89 ± 32.09
DES lignin	0.345 ± 0.008	0.633 ± 0.025	165.76 ± 27.60
Kraft lignin	0.133 ± 0.002	0.188 ± 0.006	258.68 ± 5.77
BHT	0.234 ± 0.002	0.160 ± 0.002	-

Conclusions

The obtained results suggest an influence of the pretreatment on the antioxidant and antimicrobial properties of lignin extracts, corroborating the relevance of understanding lignin structural transformations during pretreatments to produce lignin with tailor-made properties.

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