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Antibacterial and hypoglycemic activity *in vitro* of polysaccharide obtained from *Periconia byssoides*

Atividade antibacteriana e hipoglicêmica *in vitro* do polissacarídeo obtido de *Periconia byssoides*

Actividad antibacteriana e hipoglucemiante in vitro del polisacárido obtenido de *Periconia byssoides*

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Abstract
Fungi are source of polysaccharides that can show biological activity. The objective of this research was obtained polysaccharides from *Periconia byssoides* and evaluate antibacterial and hypoglycemic activity *in vitro.* The number-average molecular weight and degree of polymerization were determined. The results show that the polysaccharide of *P. byssoides* has potential as hypoglycemic. Therefore, it would be interesting to conduct *in vivo* research with this polysaccharide, to know about its hypoglycemic activity.
Keywords: *P. byssoides;* Extraction; α-amylase; Antibacterial.

Resumo
Os fungos podem produzir polissacarídeos, que podem apresentar atividade biológica. O objetivo desta pesquisa foi obter polissacarídeos de *Periconia byssoides* e avaliar a atividade antibacteriana e hipoglicêmica *in vitro*. A massa molecular média e grau de polimerização foram determinados. Os resultados mostram que o polissacarídeo de *P. byssoides* tem potencial como hipoglicemiante. Portanto, seria interessante realizar pesquisas *in vivo* com esse polissacarídeo, para conhecer sua atividade hipoglicêmica.
Palavras-chave: *P. byssoides*; Extração; α-amylase; Antibacteriano.

Resumen
Los hongos pueden producir polisacáridos que pueden tener actividad biológica. El objetivo de esta investigación fue obtener polisacáridos de *Periconia byssoides* y evaluar la actividad antibacteriana e hipoglucemiante in vitro. Se determinaron la masa molecular y el grado de polimerización. Los resultados muestran que el polisacárido de *P. byssoides* tiene potencial como agente hipoglucemiante. Por tanto, sería interesante realizar una investigación in vivo con este polisacárido, para conocer su actividad hipoglucemiante.
Palabras clave: *P. byssoides*; Extracción; α-amilasa; Antibacteriano.

1. Introduction

The use of natural polysaccharides in the industry's food, pharmaceutical and cosmetics have increased considerably in recent years, being used in the form of additives, bio-absorbents, metal removal agents, bio-flocculants, delivery agents of medicaments, among other functions (De Vuyst *et al*., 2001; Welman and Maddox, 2003; Badel *et al*., 2011; Wasser, 2015) and micro-organisms, such as bacteria, fungi and algae, have shown a high capacity to synthesize and excrete polysaccharides (Wang *et* al., 2010; Badel *et al.*, 2011). From microorganisms is possible to obtain exopolysaccharides or cell wall polysaccharides (Mahapatra & Banerjee, 2013; Wang et al., 2017).

Numerous studies have shown that natural polysaccharides have a plethora of pharmacological activities, such as antitumor (Cardoso et al., 2013; Fu et al., 2015; Liu et al., 2014; Sharma et al., 2016; Yu et al., 2017), immunomodulatory (Fukuda et al., 2009; Sharma et al., 2016), anti-inflammatory (Cheng et al., 2016; Du et al., 2015) , antioxidants (Ebrahimiasl et al., 2015; Kumar et al., 2015; Raveendran et al., 2015; Giese et al., 2015; Maity et al., 2015; Wang et al., 2017; Tian et al., 2016), anticoagulant (Vasconcelos et al., 2013; Ehmann et al., 2015; Ye et al., 2012), hypoglycemic (Lazaridou et al. 2014; Sharafbafi et al. 2014), antiparasitic (Noleto et al., 2002; Barroso et al., 2007; Oliveira et al., 2012; Nogueira et al., 2014), among others.

The fungus *Periconia byssoides* was first isolated from the marine species *Aplysia kurodai* (Yamada et al., 2007). Subsequently, research with active metabolites of this fungal species identified cell adhering inhibitors (Yamada et al., 2005), that demonstrated significant inhibition on human cancer cells (Yamada et al., 2007).

Therefore, the objective of this research was obtained polysaccharides from *Periconia byssoides* and evaluate its antibacterial and hypoglycemic activity in *vitro.*

2. Methodology

2.1 Micro-organism

The fungus *Periconia byssoides* (CCMB 07.07) was obtained from the Collection of Cultures of Micro-organisms of Bahia (UEFS) and was maintained in BDA agar medium at 28 °C in an incubator (IGO 150 Cell Life - Jouan).

2.2 Cultivation conditions

The fungus *Periconia byssoides* was cultivated in BDA medium for a period of 20 days at 28°C for biomass production. After, fungal mycelium discs (~ 1 cm) were transferred to Erlenmeyer flasks containing 100 mL of liquid culture medium (malt 2%, peptone 1% and glucose 2%). The vials were placed in agitation at 120 rpm in a rotary agitator. After 15 days, the medium was filtered and centrifuged (Centrifuge 5804R - Eppendorf, São Paulo, Brazil) at 8000 x g for 15 min at 4 °C, and the precipitate, which contained biomass *of P. byssoides*, used to extract polysaccharides (Dong et al. 2009).

2.3 Polysaccharide extraction

Forty mL of a 0.1 M sodium hydroxide (NaOH) solution was added to 2.0 g of dry biomass (20mL/g) and heated in a water bath at 100°C for 10 minutes. After the incubation time, the material was centrifuged at 9000 g for 15 min and the soluble polysaccharide in the alkaline solution was dried at 55 ºC until constant weight.

2.4 Purification of polysaccharide

A polysaccharide solution (15 mg/mL) was applied to a column of Sephacryl S-200 (Amersham Bioscience, USA) (48 x 1.2 cm) previously balanced with sodium phosphate buffer (0.05 M, pH 7.0), which was used to elude the product. Fractions of 2.5 mL were collected and analyzed for polysaccharide totals by the phenol-sulfuric acid method (Dubois et.al, 1956).

2.5 The number-average molecular weight (MWn) and degree of polymerization (DPn)

The number-average molecular weight (MWn) and degree of polymerization (DPn) were determined by the measurement of the sugar reduction value by means of the 3.5-dinitrosaliclic (DNS) method (Miller, 1959) and the total carbohydrate dosage by the sulfuric phenol-acid method (Dubois, 1956), DPn = [(total carbohydrates in µg of D-

glucose)/(reducing value in µg of maltose)] × 1.9; MWn = [(DPn) × 162] + 18 (Vettoriet al., 2012)., according to Vettori et al. (2012).

2.6 Inhibition of amylase activity

For this assay, sodium phosphate buffer solution 20 mM, pH 7.0, α-pancreatic pork amylase solution (Type VI, Sigma Aldrich) (0.5 mg/mL), starch substrate solution (Kit Amylase, Bioclin), color reagent solution, H_2O-solubilized samples), or a positive control (acarbose) were incubated before determining the residual amylase activity. (1, 3, 5 e 10 mg/mL) and positive acarbose control (0.5 mg/mL), and the concentration was adjusted to a final concentration of 1; 10; 20; 40; 80; 160 µg/mL).

The concentration capable of inhibiting 50% of enzymatic activity (IC_{50}) was calculated from the inhibition value obtained from the serial dilution of the polysaccharide, expressed in a dose-response curve (1 – 10 mg/mL).

2.7 Determination of antibacterial activity

The Minimum Inhibition Concentration (MIC) of fungal polysaccharide against *Staphylococcal aureus* ATCC 29213, *Klebsiela pneumoniae* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Proteus mirabilis* ATCC 17407 were determined by broth microdilution method [32].

After bacteria growth in nutrient broth for 24 h to 37 °C, they were diluted in nutrient broth to 10^5 CFU/mL and incubated with polysaccharides (1, 3, 6, 10 mg/mL) for 24 h to 37 ºC in microplates. After, resazurine (0.01%) was added to the suspension and the MIC was determined as the lowest concentration where there was no bacterial growth after 1 h, indicated by the blue color.

Statistical analysis

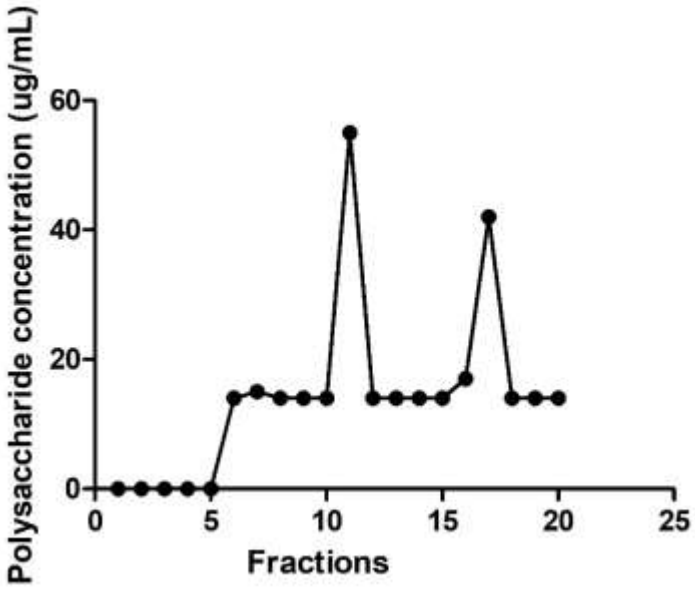
The results on amylase activity were compared with acarbose by ANOVA and Dunnett's post-test with significance level of 5% using for Program Graphpad Prism 5.0.

3. Results and Discussion

The fungus *Periconia byssoides* CCMB 07.07 showed biomass production of 2.154 ± 0.020 g/100 mL of medium. Fang and Zhong (2002) observed biomass production value of the fungus *Ganoderma lucidum* of 1.67 g/100 mL of medium. By analyzing the biomass production of the fungus *Pleurotus sajor-cashew* also through submerged fermentation, Confortin (2006) obtained a lower value than the present research (0.257 g/100 mL of medium). Lee et al. (2013) obtained values higher than the above-mentioned studies, observing biomass production equal to 2.31 g/100 mL of medium when analyzing the fermentation of the fungus *Paecilomyces japonica.*

The analysis of the content of total sugars and reducers sugars of the polysaccharides of *P. byssoides* were used to obtain the number-average molecular weight (MWn) and degree of polymerization (DPn), with values equal to 5.7 x 10^4 Da and 352, respectively. Hashemifesharaki et al. (2020) when studying the molar mass of a polysaccharide observed a slightly lower value (4.87 x 10^4 Da) than that of the polysaccharide analyzed in this study. Nuerxiati et al. (2019) obtained two polysaccharides in his research, which shows a molar mass of 25.57 and 11.64 kDa. The polysaccharide purification was conducted in a column (48 × 1.2 cm) of Sephacryl S-200 and it was possible to observe the presence of two symmetrical peaks (Figure 1).

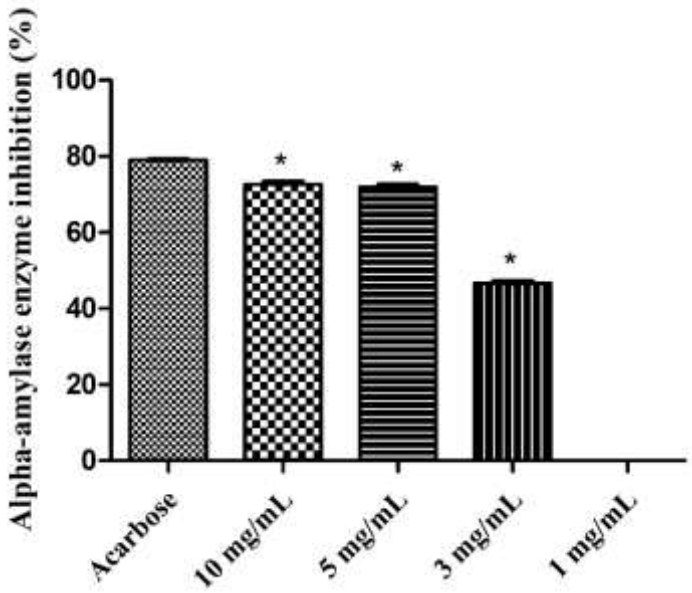
Figure 1. Elution of polysaccharide from *P. byssoide* in Sephacryl S-200 with 50 mM sodium phosphate (pH 7.0). Fractions (2.5mL).



Source: Authors.

The antibacterial and hypoglycemic activities of *P. byssoides* were analyzed with peak 1 of *P. byssoides* (fractions 5-15). The results shows that this polysaccharide exhibit good inhibition of the enzyme α-amylase (72.3%) concentration of 10 mg/mL and IC_{50} equal to 3.316 mg/mL. In addition, a significant difference was observed between all doses tested and acarbose, a positive control, as can be seen in Figure 2.

Figure 2. Inhibition of the enzyme α-amylase by the polysaccharide *of P. byssoides*.



*p<0.05, when compared with acarbose by ANOVA and Dunnet post test. The dose of Acarbose tested was 60 µg/mL. Source: Authors.

A study published in 1980 showed that valienamine, a pseudo sugar isolated from fungal cultures, is a potent inhibitor of α-glucosidase, besides being one of the components of acarbose, a potent hypoglycemic agent (LAUBE et al., 1980). In addition, more current studies have also linked polysaccharides to hypoglycemic activity. Indeed, Yuan et al. (2020) showed that polysaccharides significantly reduced fasting blood glucose levels in mice with type 2 diabetes mellitus. Additionally, Ru et al. (2020) observed that a selenized polysaccharide reduced blood glucose levels and increased insulin levels, being considered a promising hypoglycemic compound.

Wu et al. (2016) isolated polysaccharides from green tea (*Camellia sinensis*) and detected that the acid polysaccharide obtained showed an inhibiting activity of around 65% in relation to α-amylase. Wang et al. (2018) isolated three fractions of acid polysaccharides of the species *Inonotus obliquus* and observed that these fractions strongly inhibited the enzymes α-amylose and α-glycosidase.

Researches with fungal metabolites are important due the possibility can lead to new therapies for the treatment of diabetes. A non-peptide fungal metabolite (L-783,281), for example, was isolated from the endophytic fungus *Pseudomassaria* sp., collected in a forest in the Democratic Republic of Congo (Strobel* and Daisy, 2003). Oral administration of this polysaccharide in two animal models of diabetes significantly reduced serum glucose levels. In addition, these compost acts as mimetic insulin and, unlike insulin, is not destroyed in the digestive tract, and can be administered orally (ZHANG et al., 2007).

On the other hand, the polysaccharide of *P. byssoides* did not present antibacterial potential against the evaluated strains (S. *aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. coli* and *P. mirabilis*), because at the maximum tested concentration (10 mg/mL) the bacteria showed growth. Sharma et al. (2016) showed that the aqueous extract of the fungus *Lentinula edodes* showed small antibacterial activity against the bacteria *Acinetobacter* sp., *E. coli*, *Klebsiella pneumoniae*, Salmonella *typhii* and *Vibrio cholerae*.

Here, the antiproliferative activity of polysaccharide obtained from the fungus *P. byssoides* was also evaluated, however no such activity was observed in the tested concentration.

4. Conclusion

The results showed that the polysaccharide *of P. byssoides* has potential as hypoglycemic. Therefore, it would be valuable to perform *in vivo* studies with this polysaccharide aiming to confirm its hypoglycemic activity.

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