Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

In vitro cytotoxic capacity against tumor cell lines and antioxidant activity of acidic polysaccharides isolated from the Andean Patagonian fungus *Phylloporia boldo*

Verónica Albornoz, Virginia Casas-Arrojo, Fabián Figueroa, Cristian Riquelme, Víctor Hernández, Mario Rajchenberg, Carlos T. Smith, Víctor L. Campos, Roberto T. Abdala-Díaz, José Becerra, Claudia Pérez & Jaime R. Cabrera-Pardo

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2022.2158331

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Check for updates

In vitro cytotoxic capacity against tumor cell lines and antioxidant activity of acidic polysaccharides isolated from the Andean Patagonian fungus *Phylloporia boldo*

Verónica Albornoz[a,c], Virginia Casas-Arrojo[b], Fabián Figueroa[c], Cristian Riquelme[d,e], Víctor Hernández[c], Mario Rajchenberg[f], Carlos T. Smith[a], Víctor L. Campos[a], Roberto T. Abdala-Díaz[b], José Becerra[c,g], Claudia Pérez[c,g] and Jaime R. Cabrera-Pardo[h]

[a]Department of Microbiology, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile; [b]Department of Ecology, Faculty of Sciences, Universidad de Málaga, Málaga, Spain; [c]Department of Botany, Faculty of Natural and Oceano graphic Sciences, Universidad de Concepción, Concepción, Chile; [d]Programa de Doctorado en Ciencias mención Ecología y Evolución, Escuela de Graduados, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile; [e]Laboratorio de Micología, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile; [f]CIEFAP - Centro de Investigación y Extensión Forestal Andino Patagónico, Esquel, Chubut, Argentina y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Esquel, Argentina; [g]Unidad de Desarrollo Tecnológico, Universidad de Concepción, Coronel, Chile; [h]Laboratorio de Química Aplicada y Sustentable (LabQAS), Departamento de Química, Facultad de Ciencias, Universidad del Bío-Bío, Concepcion, Chile

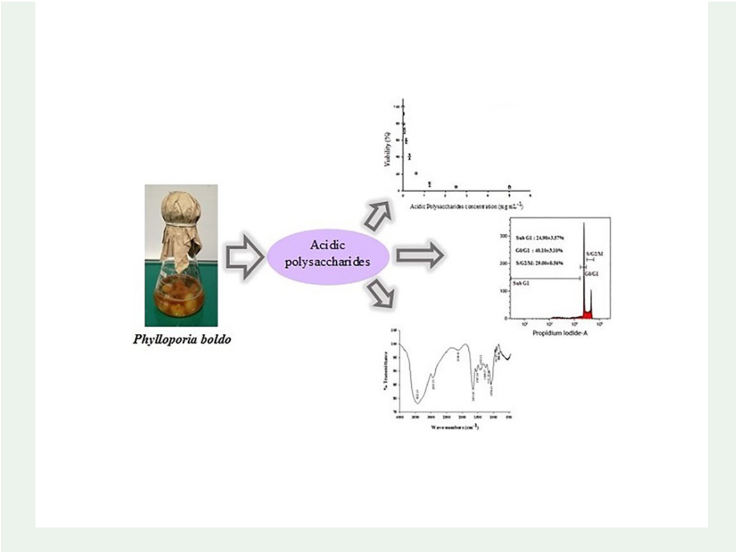
ABSTRACT

Fungal polysaccharides possess a broad biological activity, including cytotoxic and antioxidant activities. This work aimed to evaluate the cytotoxic and antioxidant activity of the acidic polysaccharides of *Phylloporia boldo* strain (named PBAP40). Cytotoxic activity of polysaccharide was evaluated determining the viability of three tumor cell lines by MTT assay. The effect of acidic polysaccharide on the cell cycle of HL-60 cell line was evaluated by flow cytometry, and the antioxidant activity was determined by DPPH and ABTS assays. PBAP40 showed cytotoxic effects in tumor cell lines. Results suggest that *P. boldo* acidic polysaccharides arrested tumor cells in the cell cycle Sub G1 phase. The acidic polysaccharides of PBAP40 strain were not cytotoxic for the non-tumor cell line. PBAP40 also showed excellent antioxidant activity. The FT-IR analysis of the acidic polysaccharides indicated the presence of glucans bearing α- and β- type glycosidic bonds.

ARTICLE HISTORY
Received 1 August 2022
Accepted 7 December 2022

KEYWORDS
Phylloporia; acidic polysaccharides; cytotoxic activity; antioxidant activity

CONTACT Víctor L. Campos vcampos@udec.cl; Claudia Pérez claudiaperez@udec.cl
Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2022.2158331



1. Introduction

Acidic fungal polysaccharides constitute a relevant group of bioactive molecules (Wang et al. 2011). These biopolymers show strong biomedical effects (Li et al. 2020). For example, the acid polysaccharide fraction extracted from *Cordyceps sinensis* displayed remarkable modulating effects on murine macrophage cell line RAW264.7, as stimulation of phagocytosis, NO production and secretion of cytokines (Chen et al. 2010; Wang et al. 2011).

Polypore fungi of the Hymenochaetaceae Donk family comprises a group known to include several forest pathogens (Rajchenberg et al. 2019) as well as species with biomedical applications (Yang et al. 2016; Wu et al. 2019). The genus *Phylloporia* Murril (1904) includes a large variety of polypore fungi with a worldwide distribution (Qin et al. 2018; Wu et al. 2019). *Phylloporia boldo* Rajchenb & Pildain (Hymenochaetaceae) has been recently described in the south of Chile (Rajchenberg et al. 2019). This fungus was found associated with the stem of the endemic Chilean species *Peumus boldus* Molina (1782) (common name 'boldo') (Monimiaceae, Laurales). Studies about bioactive compounds in *P. boldo* are very limited (Riquelme et al. 2020).

In this study, we isolated acidic polysaccharides from a strain of *P. boldo* from the Bio Bío region, Chile. Also, we examined the bioactivity of these polysaccharides by means of cytotoxicity against human tumor cell lines and the effect on their cell cycle. We also evaluated antioxidant capacity using DPPH and ABTS methods.

2. Results and discussion

2.1. Cytotoxic capacity of PBAP40 against tumor cell lines and a primary cell line

Evaluation of PBAP40 demonstrated that the viability of the three tumor cell lines (HCT-116, MCF-7 and HL-60) decreased as the acidic polysaccharide's concentration

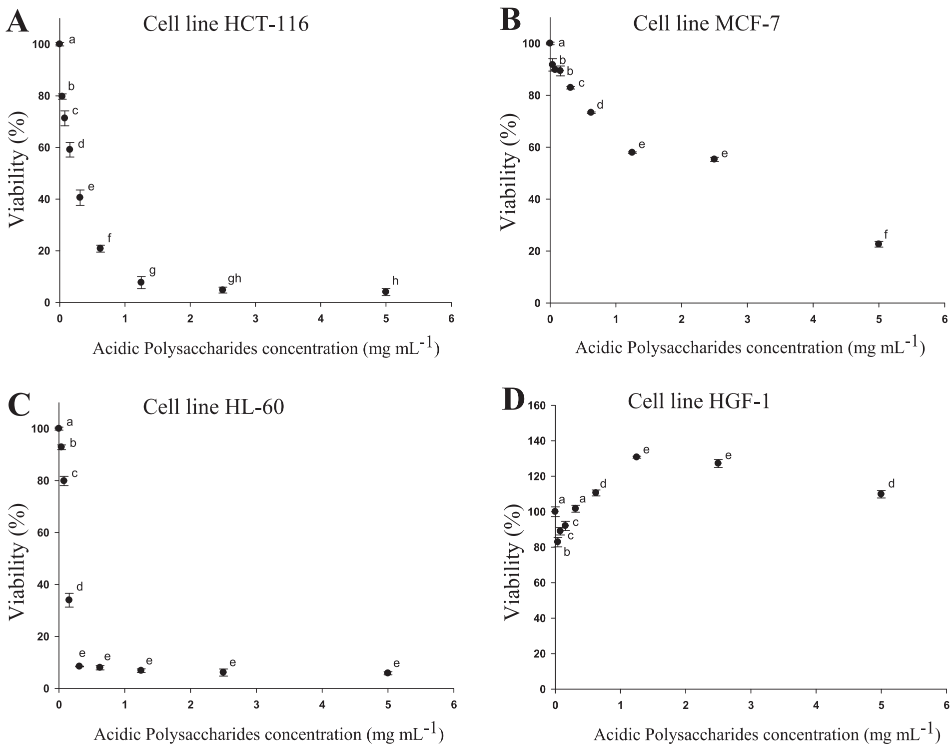


Figure 1. Viability (%) of the tumor cell lines (A-C) and the primary cell line (D) subjected to different concentrations (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 or 0.04 mg mL-1) of PBAP40; (A) cell line HCT-116; (B) cell line MCF-7; (C) cell line HL-60; (D) cell line HGF-1. Bars indicate standard deviation.

increased (Figure 1). When using high PBAP40 concentration (5 mg mL^{-1}) the viability percentages were 4.02 ± 1.37% for HCT-116 cells, 22.62 ± 1,09% for MCF-7 cells and 5.85 ± 0.58% for HL-60 cells (Figure 1A–C, respectively). The IC_{50} values for the tumor cell lines subjected to PBAP40, were 236.41 μg mL^{-1}, 2566.72 μg mL^{-1} and 127.85 μg mL^{-1} in the case of HCT-116 cells, MCF-7 cells, and HL-60 cells, respectively.

Interestingly, the cytotoxicity of the PBAP40 on the non-tumor primary HGF-1 cell line is rather mild (Figure 1D). In the presence of 0.04 mg mL^{-1} of PBAP40, the survival rate of HGF-1 cells was 86 ± 2.64%. Moreover, as the concentrations of PAPB40 increased, the viability of the non-tumor primary HGF-1 cells showed survival percentages above 100%, compared with the cells not treated with the acidic polysaccharides.

Our results show that PBAP40 significantly reduces the viability of the human tumor cell lines in a dose-dependent manner, while induces significant proliferation of the non-tumor cell line. This observation suggests a selective effect of PBAP40. Several studies have evaluated the bioactivity of crude fungal polysaccharides, reporting significant cytotoxic effect at a low concentration of polysaccharides (Liu et al. 2016, Mei et al. 2015). However, these samples are mixtures of acidic and neutral polysaccharide, hence their enhanced activity (Figueroa et al. 2020). The cytotoxic activity showed bv PBAP40 against tumor cells may be due to the presence of α and β-glucans, which have been identified using FT-IR analysis (Figure S1) (Nie et al. 2012). β-glucans are one of the main bioactive components present in fungal polysaccharides and strong anticancer activities have been attributed to them (Kozarski et al. 2011; Del Corno et al. 2020).

2.2. Effect of PBAP40 on the cell cycle of HL-60 tumor cell line

The effect of low concentration acidic polysaccharides, IC_{50} values (127.85 μg mL^{-1}) and ¼ IC_{50} values (31.96 μg mL^{-1}), on HL-60 cell line showed no significant differences when compared with cells not treated with PBAP40 (negative control (n.c.)), data not shown. The highest concentrations of PBAP40 assayed (4X IC_{50}= 511.4 μg mL^{-1}) caused a moderate increase in Sub G1 phase events percentage reaching 24.90 ± 3.57%, over the negative control (13.49 ± 0.87%) (Figure S2 A and B). Moreover, a smaller reduction of G0/G1 phase events (PBAP40 = 40.10 ± 3.1%; n.c.= 54.45 ± 1.01%) and S/G2/M phase events (PBAP40 = 29.00 ± 0.56%; n.c.= 32.06 ± 1.88%) was observed. Our results suggest that PBAP40 arrest the cell cycle of the tumor cell tested at the Sub G1 phase, inducing the death of HL-60 cells. Other studies have shown a similar effect; as an example, the percentage of HeLa cells in G0/G1 phase significantly increased from 27.11% to 48.17% when treated with polysaccharide from *Phellinus baumii* (Liu et al. 2016).

2.3. Antioxidant capacity of PBAP40

PBAP40, evaluated at a concentration of 30 mg mL^{-1}, showed antioxidant activity percentages of 24.53 ± 1.36% on DPPH and 10.20 ± 1.04% on ABTS (Table S1), which are greater than the negative control result. Also, the antioxidant activity of PBAP40 was substantially lower compared to the positive control. When the antioxidant activity of PBAP40 was contrasted with a Trolox calibration curve, it was determined that the acidic polysaccharide did not exceed the activity of 1 μg mL^{-1} Trolox (Table S1).

Studies performed with the fungi *Phellinus linteus* and *P baumii* have shown that their total polysaccharides it reaches an 86.9% antioxidant activity at a 10 mg mL^{-1} concentration and 80.29% at a 1.2 mg mL^{-1} concentration, respectively, against the free radical DPPH (Kozarski et al. 2011; Jin et al. 2016). Crude polysaccharides can be conjugated with other components, such as proteins, pigments, or polyphenols. These residues may be responsible for the radical reducing effect, causing crude polysaccharides to show a more significant antioxidant activity than after fractionation of the samples (Wang et al. 2016; Figueroa et al. 2020).

3. Experimental

The detailed description is provided in the supplementary material.

4. Conclusions

Phylloporia boldo acidic polysaccharides PBAP40 was highly effective and selective to reduce the viability of human colorectal carcinoma HCT-116, mammary adenocarcinoma MCF-7 and promyelocytic leukaemia HL-60 cell lines. Interestingly, at the concentrations tested, PBAP40 did not show cytotoxicity against non-tumor primary HGF-1 cell line. Analysis by flow cytometry revealed the effect of PBAP40 on the cell cycle – Sub G1 phase arrest. PBAP40 showed a strong antioxidant capacity by means of DPPH and ABTS assays. Further studies are required to determine the mechanisms by which these acidic polysaccharides exert their cytotoxic and antioxidant activities.

Acknowledgments

We are also grateful to the staff of the Central Services of Research Support (SCAI), Universidad de Málaga, Spain, for their assistance in the culture of tumor and non-tumor cell lines. The authors are particularly grateful to the collaboration of Mr. Daniel Cajas, Department of Botany, Faculty of Natural and Oceanographic Sciences, for the technical support and directions to culture *P. boldo* strain *in vitro*.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was partly financed by Beca de Doctorado Nacional de CONICYT Folio N°21160525. It also was supported by grants CONICYT PIA/Apoyo CCTE AFB 170007, ANID-CHILE Fondecyt regular 1190652 and MEC80180098. We also thank the Instituto Antártico Chileno INACH_16_21.

ORCID

Verónica Albornoz http://orcid.org/0000-0003-2168-4213
Fabián Figueroa http://orcid.org/0000-0001-9383-8045
Cristian Riquelme http://orcid.org/0000-0003-1652-571X
Víctor Hernández http://orcid.org/0000-0001-5092-9713
Mario Rajchenberg http://orcid.org/0000-0001-5031-5148
Víctor L. Campos http://orcid.org/0000-0003-1167-5241
Roberto T. Abdala-Díaz http://orcid.org/0000-0002-5492-8598
José Becerra http://orcid.org/0000-0003-2819-2377
Claudia Pérez http://orcid.org/0000-0002-8562-3842
Jaime R. Cabrera-Pardo http://orcid.org/0000-0001-6978-581X

References

Chen W, Zhang W, Shen W, Wang K. 2010. Effects of the acid polysaccharide fraction isolated from a cultivated *Cordyceps sinensis* on macrophages *in vitro*. Cell Immunol. 262(1):69–74.

Del Corno M, Gessani S, Conti L. 2020. Shaping the innate immune response by dietary glucans: any role in the control of cancer? Cancers. 12(155):1–17.

Figueroa FA, Abdala-Díaz RT, Hernández V, Pedreros P, Aranda M, Cabrera-Pardo JR, Pérez C, Becerra J, Urrutia R. 2020. Invasive diatom *Didymosphenia geminata* as a source of polysaccharides with antioxidant and immunomodulatory effects on macrophage cell lines. J Appl Phycol. 32(1):93–102.

Jin QL, Zhang ZF, Lv GY, Cai WM, Cheng JW, Wang JG, Fan, LF. 2016. Antioxidant and DNA damage protecting potentials of polysaccharide extracted from *Phellinus baumii* using a delignification method. Carbohydr Polym. 152:575–582.

Kozarski M, Klaus A, Niksic M, Jakovljevic D, Helsper JP, Van Griensven LJ. 2011. Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum* and *Phellinus linteus*. Food Chem. 129(4):1667–1675.

Li H, Xie W, Sun H, Cao K, Yang X. 2020. Effect of the structural characterization of the fungal polysaccharides on their immunomodulatory activity. Int J Biol Macromol. 164:3603–3610.

Liu MM, Zeng P, Li XT, Shi LG. 2016. Antitumor and immunomodulation activities of polysaccharide from *Phellinus baumii*. Int J Biol Macromol. 91:1199–1205.

Mei Y, Zhu H, Hu Q, Liu Y, Zhao S, Peng N, Liang Y. 2015. A novel polysaccharide from mycelia of cultured *Phellinus linteus* displays antitumor activity through apoptosis. Carbohydr Polym. 124:90–97.

Nie Y, Jiang H, Su Y, Zhu C, Li J, Wen F. 2012. Purification, composition analysis and antioxidant activity of different polysaccharides from the fruiting bodies of *Pholiota adiposa*. Afr J Biotechnol. 11:12885–12894.

Qin WM, Wang XW, Sawahata T, Zhou LW. 2018. *Phylloporia lonicerae* (Hymenochaetales, Basidiomycota), a new species on *Lonicera japonica* from Japan and an identification key to worldwide species of *Phylloporia*. MycoKeys. 30:17–30.

Rajchenberg M, Pildain MB, Cajas D, de Errasti A, Riquelme C, Becerra J. 2019. New poroid Hymenochaetaceae (Basidiomycota, Hymenochaetales) from Chile. Mycol Prog. 18(6):865–877.

Riquelme C, Candia B, Ruíz D, Herrera M, Becerra J, Pérez C, Rajchenberg M, Cabrera-Pardo JR. 2020. The de novo production of halogenated hydroquinone metabolites by the Andean-Patagonian white-rot fungus *Phylloporia boldo*. CREAM. 10(1):198–205.

Wang J, Hu S, Nie S, Yu Q, Xie M. 2016. Reviews on mechanisms of *in vitro* antioxidant activity of polysaccharides. Oxid Med Cell Longevity. 2016:1–13.

Wang ZM, Peng X, Lee KLD, Tang JC, Cheung PCK, Wu JY. 2011. Structural characterization and immunomodulatory property of an acidic polysaccharide from mycelial culture of *Cordyceps sinensis* fungus Cs-HK1. Food Chem. 125(2):637–643.

Wu F, Ren GJ, Wang L, Oliveira-Filho JRC, Gibertoni T, Dai YC. 2019. An updated phylogeny and diversity of *Phylloporia* (Hymenochaetales): eight new species and keys to species of the genus. Mycol Prog. 18(5):615–639.

Wu F, Zhou LW, Yang ZL, Bau T, Li TH, Dai YC. 2019. Resource diversity of Chinese macrofungi: edible, medicinal and poisonous species. Fungal Divers. 98(1):1–76.

Yang NC, Wu CC, Liu RH, Chai YC, Tseng CY. 2016. Comparing the functional components, SOD-like activities, antimutagenicity, and nutrient compositions of *Phellinus igniarius* and *Phellinus linteus* mushrooms. J Food Drug Anal. 24(2):343–349.