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Aqueous extracts of *Lentinula edodes* and *Pleurotus sajor-caju* exhibit high antioxidant capability and promising in vitro antitumor activity

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ABSTRACT

Mushroom extracts are increasingly sold as dietary supplements because of several of their properties, including the enhancement of immune function and antitumor activity. We hypothesized that soluble polar substances present in mushroom extracts may show antioxidant and anticancer properties. This report shows that Brazilian aqueous extracts of *Lentinula edodes* and *Pleurotus sajor-caju* exert inhibitory activity against the proliferation of the human tumor cell lines laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa). Cell viability was determined after using 3 different temperatures (4°C, 22°C, and 50°C) for mushroom extraction. Biochemical assays carried out in parallel indicated higher amounts of polyphenols in the *L edodes* extracts at all extraction temperatures investigated. The scavenging ability of the 2,2-diphenyl-1-picrylhydrazyl radical showed higher activity for *L edodes* extracts. Superoxide dismutase–like activity showed no statistically significant difference among the groups for the 2 tested extracts, and catalase-like activity was increased with the *L edodes* extracts at 4°C. The results for the cytotoxic activity from *P sajor-caju* extracts at 22°C revealed the half maximal inhibitory concentration values of 0.64% ± 0.02% for Hep-2 and 0.25% ± 0.02% for HeLa. A higher cytotoxic activity was found for the *L edodes* extract at 22°C, with half maximal inhibitory concentration values of 0.78% ± 0.02% for Hep-2 and 0.57% ± 0.01% for HeLa. Substantial morphological modifications in cells were confirmed by Giemsa staining after treatment with either extract, suggesting inhibition of proliferation and induction of apoptosis with increasing extract concentrations. These results indicate that the aqueous extracts of Brazilian *L edodes* and *P sajor-caju* mushrooms are potential sources of antioxidant and

Abbreviations: CAT, catalase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; Hep-2, cell line human laryngeal carcinoma; HeLa, cell line human cervical adenocarcinoma; $IC_{50(\%)}$, half maximal inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); SOD, superoxide dismutase.

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anticancer compounds. However, further investigations are needed to exploit their valuable therapeutic uses and to elucidate their modes of action.

1. Introduction

Mushrooms have long attracted great interest for use in foods and biopharmaceuticals [1]. Hot water-soluble fractions known as decoctions and essences from medicinal mushrooms, such as *Ganoderma lucidum* (Reishi), *Inonotus obliquus* (Chaga), and *Lentinula edodes* (Shiitake), have been collected and used as alternative medicines for hundreds of years in Korea, China, Japan, and eastern Russia [1]. Numerous molecules synthesized by macrofungi are known to be bioactive, including polysaccharides, glycoproteins, terpenoids, and lectins [2]. A wide variety of naturally occurring substances have been shown to protect against tumor development [3] and inflammatory processes [4]. Recent scientific evaluations of macrofungi, such as mushrooms and entomopathogenic fungi, have confirmed the efficacy of extracts from either the fruiting bodies or mycelia of these species in inhibition of the proliferation of various cancer cells lines [1,5].

Edible mushrooms have been reported to generate beneficial effects for health and in the treatment of disease through their immunomodulatory and antineoplastic properties [6,7]. The Shiitake mushroom has served as a model for investigating functional fungi properties and isolating pure compounds for pharmaceutical use [8]. Water extracts of the Shiitake fruiting body have been shown to prevent tumor growth in mice [9,10]; however, most studies have focused on the antioxidant capacity of polyphenolic compounds in *L edodes* [11,12]. A great number of polysaccharides have been isolated from basidiomycetes [13], representing homopolymers and heteropolymers primarily from β-configuration glucans. Glucans containing both α-configuration and β-configuration are less represented in basidiomycetes [2,14]. *Pleurotus* species are promising as medicinal mushrooms and exhibit hematological, antiviral, antitumor, antibacterial, hypocholesterolic, and immunomodulatory activities [15] as well as antioxidant properties [16-18]. Approximately 40 species of the genus *Pleurotus*, also known as oyster mushrooms, have been reported, including *Pleurotus florida* and *P sajor-caju*; all are commonly available, edible mushrooms, and detailed structural characterizations of their isolated polysaccharides have been reported [19]. These 2 mushrooms were selected for our study to compare their antitumor properties and to investigate the differences between their mechanisms of action. In addition to the well-known *Agaricus blazei*, *L edodes* has been extensively studied and has shown excellent biological properties. However, few studies have been performed with *P sajor-caju* to evaluate its in vitro biological activity, and all have involved nonaqueous (low temperature)–based extracts. Until now, most experimental work with mushroom extracts has been performed using hot water–based extracts or ethanol/methanol extracts [20-22].

Our work is a preliminary and pioneering study assessing the antioxidant and antitumor activity of Brazilian *L edodes* and *P sajor-caju* mushrooms. To address our hypothesis that soluble polar substances found in mushrooms may have antioxidant and anticancer properties, this article investigated the biological potential of low temperature (<50°C) aqueous extracts. The samples were tested for total polyphenol content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability, and superoxide dismutase (SOD)– and catalase (CAT)–like activities as well as the ability to inhibit the proliferation of the human tumor cell lines laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa) using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and observing in situ morphological alterations.

2. Methods and materials

2.1. Mushroom grown and extract preparation

The Brazilian *L edodes* and *P sajor-caju* mushrooms were grown in South Brazil in the region known as Serra Gaúcha. Fruiting bodies of *L edodes* and *P sajor-caju* were initially chopped and oven dried at 50°C until a dry weight was recorded, and then they were ground into powder with a knife mill. The humidity values obtained were 10.41% in *P sajor-caju* and 14.37% in *L edodes*, and the method used was that described by Burrows [23] with modifications. Extraction was carried out using distilled water at 4°C, 22°C, and 50°C in a rotational shaker for 1 hour. The extracts were than separated and filtered using filter paper, sterilized using 0.22-μm filter units in a laminar flow chamber and stored at –20°C until used, using the procedure of Zhuang et al [24] with modifications.

2.2. Phenolic content and antioxidant activity of extracts

The total phenol content of the extracts was determined using the modified Folin-Ciocalteu colorimetric method as described in Singleton and Rossi [25]. Total phenol content was determined by comparison with a catechin standard curve (0.3-10 mg% catechin; Sigma Chemical Co, São Paulo, Brazil). The total phenolic contents are expressed as %mg of the catechin equivalent.

The antioxidant activity of 6 different extracts was determined by in vitro assays. The radical scavenging activity of DPPH was measured using a method modified by Yamaguchi et al [26] in which 200 μL of the extracted solutions (1%-10% wt/vol) were added to 800 μL of Tris-HCl buffer (100 mM, pH 7.0) containing 1 mL of DPPH solution (500 μM dissolved in ethanol). Tubes were stored in the dark at room temperature for 20 minutes, and the absorbance was measured at 517 nm (model UV-1700 spectrophotometer; Shimadzu, Kyoto, Japan). The result was expressed in half maximal inhibitory concentration ($IC_{50(\%)}$) (amount of extract necessary to scavenge 50% of DPPH radical). Superoxide dismutase–like activity was determined spectrophotometrically by measuring the inhibition of the autocatalytic adrenochrome formation rate at 480 nm in a reaction medium containing 1 mmol/L adrenaline (pH

2.0) and 50 mmol/L glycine (pH 10. 2) as well as different volumes of 10% (wt/vol) solutions of the extracts. This reaction was performed at 30°C for 3 minutes [27]. The results were expressed as the $IC_{50(\%)}$ in microliters of the sample required to inhibit 50% the formation of adrenochrome [28]. Catalase-like activity was assessed using 10% (wt/vol) solutions of the extracts to determine the rate of decomposition of hydrogen peroxide at 240 nm. The results are expressed as nanomole H_2O_2 decomposed/minute [29].

2.3. *Cytotoxic assay*

The Hep-2 and HeLa cell lines were cultured in Dulbecco's Modified Eagle Medium supplemented with antibiotics and 10% fetal bovine serum [Gibco BRL; Life Technologies (Van Allen Way, Carlsbad, CA, USA)] at 5% CO_2 and 37°C. For the assessment of the cytotoxic activities of *L edodes* and *P sajor-caju* extracts, using the procedure of Alley et al [30], the cells were seeded in 96-well flat-bottom microplates at a density of approximately 7×10^4 cells/well in 10% fetal bovine serum Dulbecco's Modified Eagle Medium. After cell attachment, serial dilutions of the extracts in the culture medium were prepared, added to the cells for 1 hour, and removed, followed by incubation for 24 hours in extract-free medium.

Cell proliferation was determined by the tetrazolium salt method using MTT [31]. Briefly, 7×10^4 cells/well were cultured in 96-well plates and treated for 1 hour with increasing extract concentrations (0.05%-1.5%) at 37°C. Doxorubicin was used as a positive control. After incubation with MTT solution at room temperature for 2 hours, dimethyl sulfoxide was added, the cells were harvested, and absorption was determined at 540 nm. At least 3 independent experiments were performed for each experimental cell line, and $IC_{50(\%)}$ values (dose causing 50% cell survival) were determined as the means and SD [32].

2.4. *Morphological examination of cancer cells*

The morphology of the Hep-2 and HeLa cell lines was monitored using an inverted microscope under a conventional Giemsa staining protocol [33,34]. Changes in the cellular morphology were observed and documented after being treated for 1 hour with the aqueous extracts of *L edodes* and *P sajor-caju*, followed by cultivation for 24 hours in extract-free medium. The negative control group was treated with distilled water instead of extract for the same period.

2.5. *Statistical analyses*

The results are expressed as the means ± SD of each group. Analysis of variance followed by the Tukey post hoc test was used to test for differences among the treatment groups in triplicate. Statistical analyses were performed using SPSS 19.0 (Armonk, NY, USA). The level of significance was uniformly set at $P < .05$.

3. Results

The data for the polyphenol contents, antioxidant activities using DPPH, and the SOD-like and CAT-like activities of *L edodes* and *P sajor-caju* extracts are presented in Table 1 and indicate that the extracts of these mushrooms prepared at temperatures lower than 50°C contain polyphenol substances that are the potential sources of antioxidant activity.

The cytotoxic activity assays for the cell line Hep-2 after treatment with *P sajor-caju* extracts prepared at 4°C revealed an $IC_{50(\%)}$ of 0.23% ± 0.08%, and the mushroom extracts prepared at 22°C and 50°C showed proportionately increased values of 0.64% ± 0.02% and 1.17% ± 0.03%, respectively. When HeLa cells were treated with 4°C extracts of *P sajor-caju*, an $IC_{50(\%)}$ of 0.31% ± 0.01% was observed, which is higher than half of the maximal inhibitory concentration observed with the 22°C extracts, $IC_{50(\%)}$ of 0.25% ± 0.02%, and lower than the 50°C tested extract, $IC_{50(\%)}$ of 1.21% ± 0.01% (Table 2). The concentrations used for the HeLa and Hep-2 cells ranged from 0.05% to 1.5% (wt/vol) for the *P sajor-caju* extracts, and the data are given in Fig. 1.

Using the cell line Hep-2 after treatment with mushroom extracts derived at 4°C from *L edodes*, an $IC_{50(\%)}$ of 0.46% ± 0.08% was obtained. The extracts obtained at 22°C showed an $IC_{50(\%)}$ of 0.78% ± 0.02%, and those derived 50°C showed $IC_{50(\%)}$ of 1.03% ± 0.04%. The HeLa cell treatment using *L edodes* extracts at 4°C showed an $IC_{50(\%)}$ of 0.74% ± 0.02%, a higher value than that from the extract obtained at 22°C, which showed an $IC_{50(\%)}$ of 0.57% ± 0.01%. However, the $IC_{50(\%)}$ for *L edodes* extracts at 4°C was lower than half the maximal inhibitory concentration rate observed for the extracts derived at 50°C, with an $IC_{50(\%)}$ of 0.91% ± 0.07%. The concentrations used for the HeLa and Hep-2 cells ranged from 0.05% to 1% (wt/vol) for the *L edodes* extracts, and variances are depicted in Fig. 2.

The effect on tumor cell survival after mushroom extract treatment varied not only according to the temperature used for extraction but also according to the extract concentration, as shown in Fig. 1 (*P sajor-caju*) and Fig. 2 (*L edodes*). The changes in cell morphology following treatment with both mushroom extracts included a series of cellular modifications, including cell shrinkage, which suggests the induction of apoptosis as a consequence of exposure to the extract (Fig. 3). We monitored changes in the cell morphology under the microscope and observed that cell modifications were observed initially in the group treated with *P sajor-caju* extracts, 30 minutes after extract incubation. In contrast, cellular modifications in samples treated with *L edodes* extract were only detectable after 1 hour of incubation, after the cells had been cultured with free-extract medium for 24 hours. Furthermore, treatment with *L edodes* extracts caused less extreme changes (Fig. 3B and F) compared with incubation with *P sajor-caju* extracts (Fig. 3D and H). Samples treated exclusively with water are shown as the negative controls in Fig. 3A, C, E, and G and show uniform cell morphology.

4. Discussion

Most studies using mushroom extracts to test in vitro biological responses have focused on nonaqueous extraction protocols. Here, we provide experimental evidence that a water-based extraction protocol is sufficient to assess the inhibitory effects on tumor cells; this suggests the existence of active, polar constituents in mushroom extracts, which supports the hypothesis of our study. Previously, we have

Table 1 – Total polyphenol content and antioxidant activity of *P sajor-caju* and *L edodes* using different extraction temperatures

Samples		Total polyphenol content (mg% catechin)	DPPH scavenging ability ($IC_{50(\%)}$) *	SOD-like activity ($IC_{50(\%)}$) †	CAT-like activity (nmol H_2O_2/min)
P sajor-caju	4°C	35.22 ± 0.28[a]	10.38 ± 0.19[d]	4.30 ± 0.33[a]	3.19 ± 0.80[ab]
	22°C	34.85 ± 0.40[a]	9.68 ± 0.15[c]	4.38 ± 0.66[a]	3.75 ± 0.01[bc]
	50°C	35.98 ± 0.14[a]	9.01 ± 0.14[b]	6.76 ± 0.39[a]	3.56 ± 0.27[ab]
L edodes	4°C	55.26 ± 0.51[b]	3.31 ± 0.05[a]	5.54 ± 0.86[a]	6.28 ± 0.13[d]
	22°C	55.29 ± 0.47[b]	3.28 ± 0.05[a]	4.34 ± 1.44[a]	5.06 ± 0.27[cd]
	50°C	55.63 ± 0.08[b]	3.45 ± 0.06[a]	4.72 ± 0.20[a]	2.25 ± 0.01[a]

The results are presented as the means ± SD. The results represent the averages of 3 independent experiments performed in triplicate. Different letters represent different values for each assay, according to analysis of variance and post hoc Tukey tests ($P < .05$).

* Concentration (percentage) of the samples needed to scavenge 50% of the DPPH radicals.

† Microliter of the extracts needed to inhibit 50% of the formation of adrenochrome.

tested a 100°C extraction protocol using decoction and observed no significant difference in the viability of tumor cell lines after treatment for 1 hour. The absence of extract activity at high temperatures may be related to a decrease in the concentration of polysaccharides caused by internal β-glucanase activity [35].

Different extraction temperatures showed no influence on the content of total polyphenols. *L edodes* extracts, however, presented a higher concentration of these compounds than those of *P sajor-caju*. Phenolic compounds are important to provide protection against several degenerative diseases in humans, including brain dysfunction, cancer, and cardiovascular diseases [36,37]. The best described property of almost every group of polyphenols is their capacity to act as antioxidants, which can scavenge free radicals and reactive oxygen species [38,39]. In fact, higher scavenging activity for the DPPH radical was observed for the extracts of *L edodes*, which presented a higher CAT-like activity (using the 4°C and 22°C extraction methods) than those of *P sajor-caju*. With the 50°C extraction protocol, a significant decrease in CAT-like activity was observed for the extract of *L edodes*. This effect is most likely due to a chemical alteration in the active compounds present in this mushroom caused by the use of a higher temperature during extraction. The high antiproliferative activity exhibited by the *P sajor-caju* extract may be a result of its specific proteins, terpenoids, steroids, fatty acids, and phenolic compounds. This extract was also shown to possess bioactive effects that may be relevant for health homeostasis, such as immunomodulation, antihypertension, cytotoxic, antibacterial, and pro-oxidative effects [40,41]. No major difference in SOD-like activity was found between the 2 mushrooms. Superoxide dismutase and CAT enzymes have an important role in maintaining the physiologic redox equilibrium. Superoxide dismutase catalyzes the dismutation of the superoxide anion ($O_2^{\bullet-}$) in H_2O_2, and CAT catalyzes the direct decomposition of H_2O_2 to ground-state O_2 [42].

A positive correlation between substances with antioxidant activity and the inhibition of tumor cell proliferation is well established, suggesting that the antioxidant properties of extracts influence anticancer activity. Yet, the underlying mode of action still remains to be elucidated. Antioxidant activity is related directly to the reactive species involved in many aspects of carcinogenesis as well as the mechanisms of carcinogenesis, including proliferation, induction of senescence, apoptosis facilitation triggered by other agents, the suppression of apoptosis, and the initiation of DNA damage [43].

According to the cytotoxic activities, the *P sajor-caju* extracts were more effective against tumor cells compared with the extracts of *L edodes*, with the exception of the extracts prepared at 50°C. We observed a great variation in cell viability after incubation with the extracts of *P sajor-caju* and *L edodes* within the concentration range from 0.05% to 1.5%. Inhibitory effects were greater in tumor cells treated with *P sajor-caju* extracts obtained at 4°C and 22°C in both cell lines tested. In contrast, a higher concentration of *P sajor-caju* extracts prepared at 50°C was needed to achieve similar inhibition of tumor cell viability compared with the extracts prepared at lower temperatures. *P sajor-caju* aqueous extracts obtained at 50°C showed a lower cytotoxic activity than those from treatment with *L edodes* aqueous extracts. Consequently, lower concentrations of *L edodes* were needed to achieve the same 50% inhibitory concentration rate.

To further investigate the kinetics and mechanisms of action for the extracts, we monitored the changes in cell morphology by microscopic inspection during and after the treatment with the extracts obtained at 22°C. A comparison of the inhibition rate revealed that the extracts derived from *P*

Table 2 – Cytotoxic activity $IC_{50(\%)}$ of *P sajor-caju* and *L edodes* extracts using different extraction temperatures

		Cytotoxic activity $IC_{50(\%)}$ [a]		
		4°C	22°C	50°C
P sajor-caju	Hep-2	0.23 ± 0.08[a]	0.64 ± 0.02[b]	1.17 ± 0.03[c]
	HeLa	0.31 ± 0.01[a]	0.25 ± 0.02[a]	1.21 ± 0.01[c]
L edodes	Hep-2	0.46 ± 0.08[a]	0.78 ± 0.02[c]	1.03 ± 0.04[d]
	HeLa	0.74 ± 0.02[c]	0.57 ± 0.01[b]	0.91 ± 0.07[c]

The results represent the averages of 3 independent experiments performed in triplicate. The relative expression levels are presented as the *n*-fold increases compared with the control group across the same lines, and different superscript lowercase letters indicate the results that were significantly different with $P < .05$ by analysis of variance and post hoc Tukey tests. The results are presented as the means ± SD.

[a] Cytotoxic activity was assessed by MTT assay. The $IC_{50(\%)}$ values (dose causing 50% cell death) were calculated using dose–response curves for each condition.

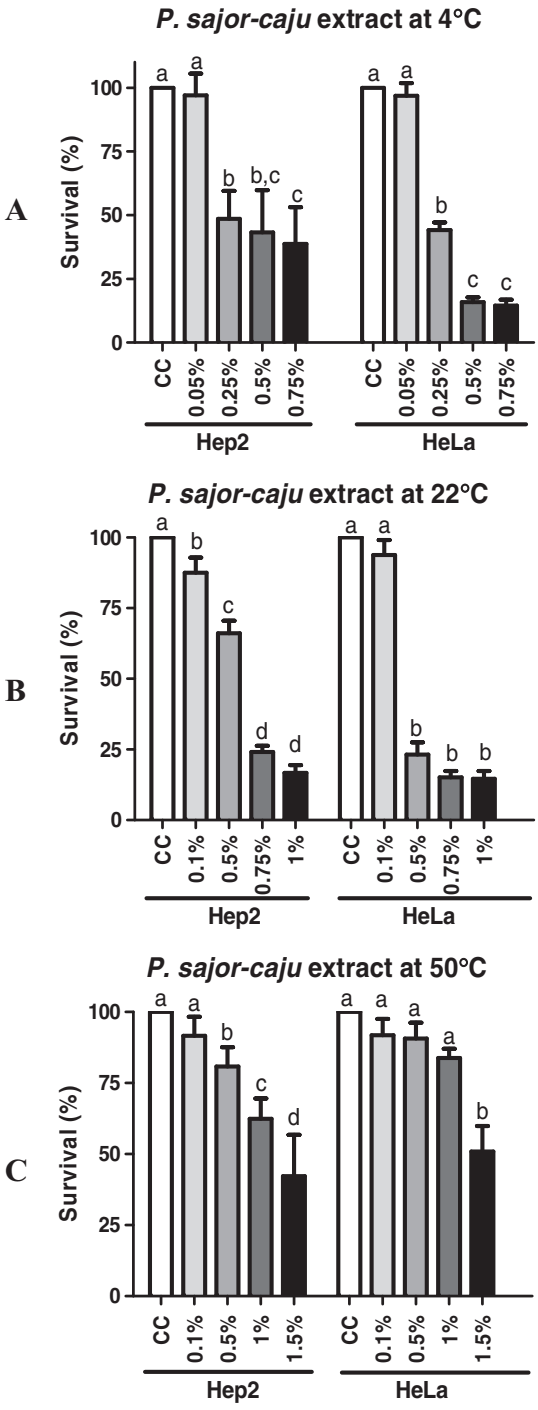

Fig. 1 – Effect of water extract from *P sajor-caju* mushroom grown in different concentrations on the of Hep-2 and HeLa cells. Values are expressed in means ± SD, and statistical analyses used Tukey test. A, Extract at 4°C; B, Extract at 22°C; C, Extract at 50°C. Different letters represent statistical significance among groups.

sajor-caju were more effective in inducing cytotoxicity in cells, thereby causing changes after 1 hour of treatment, whereas morphological modifications caused by *L edodes* were only observed 24 hours after treatment during incubation with the extract-free medium.

According to Ooi and Liu [44], morphologic analysis of cells indicates that the mushroom extract may initiate apoptotic mechanisms to trigger cell death. Tumor growth is known to be regulated by the balance between cell proliferation and apoptosis. Deregulated cell proliferation and suppressed cell death together provide the underlying basis for neoplastic

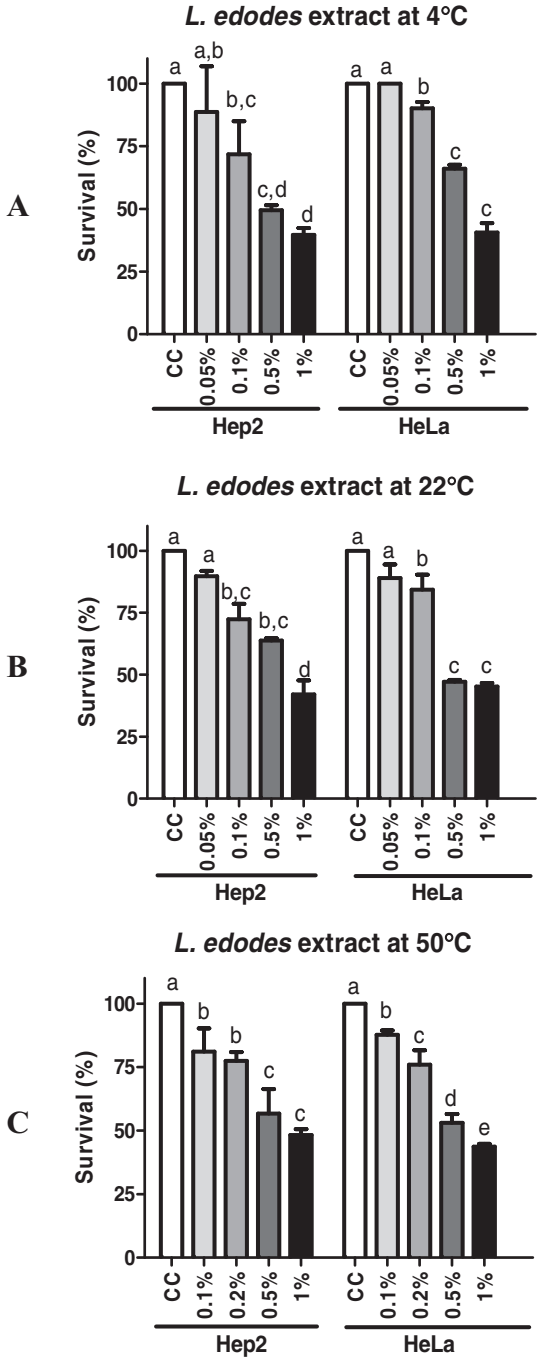

Fig. 2 – Effect of water extract from *L edodes* mushroom grown in different concentrations on the of Hep2 and HeLa cells. Values are expressed in means ± SD, and statistical analyses used Tukey test. A, Extract at 4°C. B, Extract at 22°C. C, Extract at 50°C. Different letters represent statistical significance among groups.

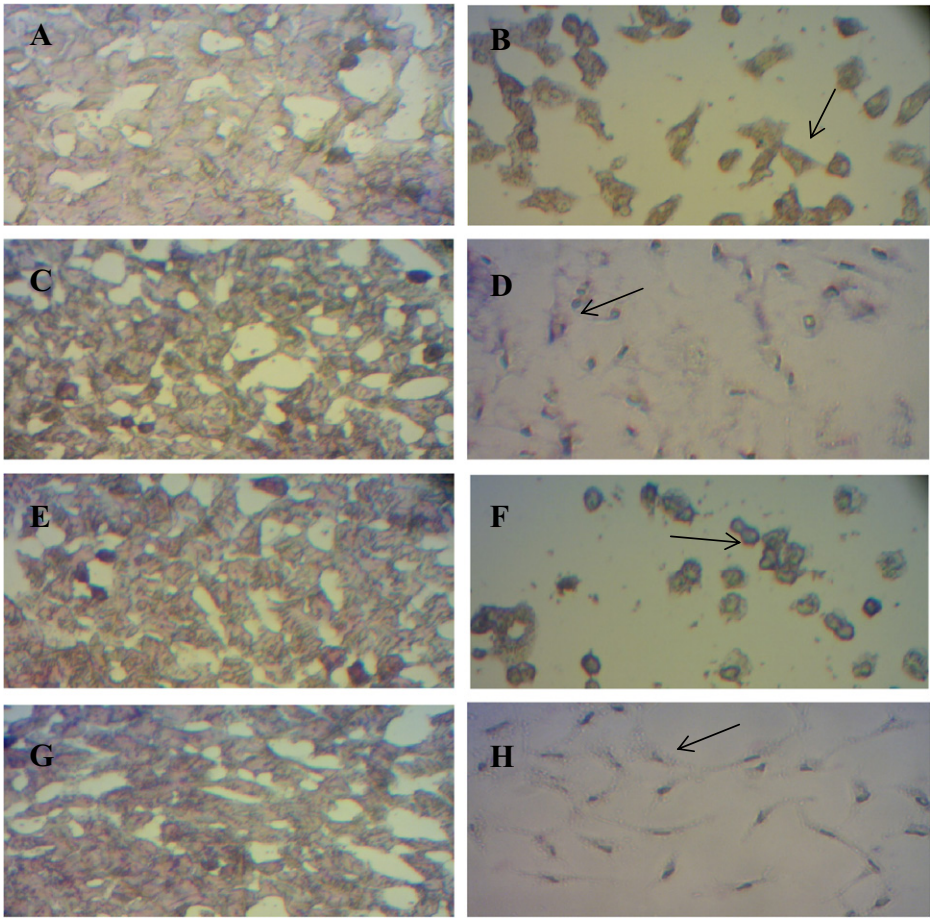

Fig. 3 – Morphological analysis of cell lines after mushroom extract treatment using the 22°C extraction protocol: Hep-2—control (A); Hep-2—$IC_{50(\%)}$ *L edodes* extract (B); Hep-2—control (C); Hep-2—$IC_{50(\%)}$ *P sajor-caju* extract (D); HeLa—control (E); HeLa—$IC_{50(\%)}$ *L edodes* extract (F); HeLa—control (G); and HeLa—$IC_{50(\%)}$ *P sajor-caju* extract (H). The arrows in "B," "D," "F," and "H" show representative cells after treatment with the extract from *L edodes* and *P sajor-caju*.

transformation and malignant progression. Consequently, 1 essential strategy for cancer therapy is to target lesions that suppress apoptosis in tumor cells [45]. It has been found that many cancer chemotherapeutic drugs exert anticancer effects on malignant cells by inducing apoptosis [5,46].

Although the underlying mode of action of the mushroom extracts on tumor cell physiology is still unclear, water-soluble heteroglycans and insoluble β-glucans have already been isolated from this species and may be involved in their antitumor activity [47,48]. So far, β-glucans are well known for their biological activity specifically related to the immune system, which differs from the mechanisms observed for conventional chemotherapeutic agents [20,49]. However, activating and reinforcing the host immune system seem to be the best strategy for inhibiting the growth of cancer cells [50-52].

According to Fang et al [21] and Wu et al [53], effective adjuvant substances from edible or medical mushrooms are capable of activating the cellular apoptotic response in cancer. One example is lectin [54], which is isolated from *Lentinula* species and potently inhibits the growth of sarcoma and hepatoma cells and prolongs the life spans of tumor-bearing mice [55,56]. Lentinan, from *L edodes*, is currently used in the clinic (ie, 0.5-1.0 mg lentinan per day, intravenous), especially in Japan and China, as an adjuvant tumor therapy for other cancer therapies such as surgical resection, radiotherapy, and chemotherapy [8,57-59]. Many interesting biological activities of lentinan have been investigated, including the activation of nonspecific inflammatory responses, such as acute phase protein production [60], vascular dilation, and hemorrhage-inducing factors in vivo [61,62] as well as the activation and generation of helper and cytotoxic T cells [63]. There are many other examples of isolated mushroom compounds with beneficial properties against cancer [8]. Maity et al [47] observed the in vitro activation of peritoneal macrophages stimulated with different concentrations of the heteroglycan isolated from *Pleurotus ostreatus*. Zhuang et al [24] discovered a protein present in *P sajor-caju* that showed antitumor effects [15,64] and antioxidant properties [22,65]. A ribonuclease isolated from *P sajor-caju* presented antimicrobial, antimitogenic, and antiproliferative activities [66]. Polysaccharides of other mushrooms that have been investigated include schizophyllan [67], active hexose-correlated compounds, maitake D-fraction [68], polysaccharide-K and polysaccharide-P [50],

scleroglucan [69] and grifolan [70], among others, and glucans always present strong antitumor effects [71].

Our results provide experimental evidence that aqueous extracts of the mushrooms *L edodes* and *P sajor-caju* grown in South Brazil are potential sources of antioxidant and anticancer compounds. We used low temperature (<50°C) water extracts and achieved striking results, in contrast to previous experiments using hot water–based extracts. Therefore, it is necessary to prepare extracts immediately before their use to guarantee the stability of the components. Further investigations, however, are needed to explore the biological properties of *L edodes* and *P sajor-caju* and to elucidate the molecular mode of action against tumor cell proliferation and the induction of apoptosis. It is known that most drugs isolated for cancer therapy are not cancer specific and, therefore, may be highly toxic to normal tissues, leading to serious adverse effects. Mushroom extracts might be considered alternative sources for adjuvant cancer therapy, as they have no adverse effects, activate the cells of the immune system, and reduce free radicals. Further studies, however, including the isolation and chemical characterization of the major compounds that contribute to the promotion of the immune system and to the inhibition of carcinogenesis, are needed and may generate new targets for therapy.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nutres.2012.11.005.

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