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Full Length Research Paper

Antibacterial and cytotoxic activity from basidiocarp extracts of the edible mushroom *Lactarius indigo* (Schw.) Fr. (Russulaceae)

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Aqueous and organic basidiocarp extracts of the edible mushroom *Lactarius indigo* were evaluated for their antibacterial and cytotoxic effects. 10, 20 and 30 mg of organic extracts were tested against diarrheagenic *Escherichia coli* strains (EIEC, EPEC, ETEC-LT and ETEC-ST), *Pseudomonas aeruginosa, Enterobacter cloacae*, *Staphylococcus aureus* and *Salmonella enterica*. 10 mg of hexane extract showed activity against ETEC-LT (18.8 mm zone of inhibition) and *P. aeruginosa* (10.5 mm). All levels of the ethyl acetate extract inhibited all the strains, with stronger activity against EIEC (19.0 mm) and *P. aeruginosa* (21.0 mm) at 30 mg. Methanol extract inhibited all bacterial growth, but *E. cloacae*. 100 µg/ml of aqueous extract showed antiproliferative activity against MCF7 cells, but not on HeLa, A549 and normal bovine mammary epithelium cells. Methanol and ethyl acetate extracts inhibited proliferation of HeLa cells (50 to 1000 ng/ml) but increased proliferation of A549 (100 ng/ml), as did methanol extract (500 ng/ml). Methanol extract did not inhibit normal rabbit serum fibroblast cells, while hexane and ethyl acetate extracts showed an inhibitory effect with 50 and 100 ng/ml, respectively, less than in proliferation of HeLa cells. These results show that *L. indigo* basidiocarps contain substances with antibacterial and cytotoxic activities.

Key words: *Lactarius indigo* extracts, antibacterial activity, cytotoxic activity.

INTRODUCTION

Fungi from the division Basidiomycota have been widely studied as an alternative source of metabolites with pharmacological properties, including anticancerigenous, antitumor, immunomodulating, antibacterial and cytotoxic activities (Wasser, 2002; Daba and Ezeronye, 2003; Fan et al., 2006; Borchers et al., 2008). Antibiotic resistance of human pathogenic bacteria has become a major worldwide public health concern (Finch, 2002; Harbarth and Samore, 2005), this is why the search for new substances with antimicrobial activity is a priority (Livermore, 2005). Antimicrobial activity has already been documented in extracts from the mycelium (Suay et al., 2000) and fruiting bodies (Zjawioney, 2004) of different wild species from Basidiomycota.

Another worldwide public health problem is cancer, given that it is estimated that approximately 25 million people suffer one of its different manifestations and 10 million new cases are annually reported (WHO, 2002); for that reason, there is an increasing demand for more effective anticancerigenous substances and therapies (Lord and Ashworth, 2010). In that regard, several studies have reported cytotoxic activity against cancer cells of organic extracts of spores (Fukuzawa et al., 2008), vegetative mycelium (Hu et al., 2002; Choi et al., 2004) and basidiocarps (Takaku et al., 2001; Hu et al., 2002) from several species of Basidiomycota. The genus *Lactarius* (Russulaceae) includes species reported as edible in different parts of the world (Boa, 2004) and reports exist of the antimicrobial activity of methanol extracts of *Lactarius deterrimus*, *Lactarius sanguifluus, Lactarius semisanguifluus, Lactarius piperatus, Lactarius*

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deliciosus and *Lactarius salmonicolor* (Dulger et al*.,* 2002; Barros et al., 2007a, b). In addition, the organic extracts of some *Lactarius* species have shown immuno-modulating, cytotoxic, antiviral and antigenotoxic activities (Krawczyk et al., 2003, 2005, 2006; Mlinaric et al., 2004).

Lactarius indigo (Schw.) Fr. is an edible mushroom that distributed from East Asia (China and Japan) (Wu and Mueller, 1997) to Northeastern and Central America (Hesler and Smith, 1979; Hutchinson, 1991; Mueller and Halling, 1995; Montoya and Bandala, 1996; Wu and Mueller, 1997). In México, *L. indigo* is associated to diverse plant communities (Montoya and Bandala, 1996) and is highly valued as food (Boa, 2004; Pérez et al*.,* 2006), being sold in local markets (Montoya et al., 2001; Martínez-Carrera et al., 2005). It is known by the Spanish common names *indigo*, *hongo azul* (blue mushroom) and the combined Spanish-Nahuatl names *tecax azul* (blue *tecax*) and *tecosan morado* (purple *tecosan*) (Montoya et al., 2001, 2003).

A single report is known about the nutritional value of *L. indigo* (León-Guzmán et al., 1997); however, there is not a single report regarding the pharmacological properties of this species. In the present work, the antibacterial and cytotoxic activities of aqueous and organic extracts of *L. indigo* are evaluated for the first time and the results are compared with those from similar reports for other species of Basidiomycota, mainly for the genus *Lactarius*.

MATERIALS AND METHODS

Basidiocarp collection

Basidiocarps of *L. indigo* were collected in the Parque Nacional Insurgente José María Morelos in the municipality of Charo, state of Michoacán, México (19° 39.918' N, 101° 00.450' W) on September 2006, and were authenticated by M.Sc. Marlene Gómez Peralta, curator of the herbarium of the Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, where a voucher specimen (KM03) was deposited. The basidiocarps were frozen at -80°C the same day of collection and freeze-dried after 24 h. The freeze-dried material was preserved in the dark at 4°C in a container with silica gel until processed.

Test microorganisms

Strains of *Escherichia c*oli EPEC, ETEC, and EIEC pathotypes used for the antibacterial activity assays were purchased from the Institute of Diagnostic and Epidemic Reference of México (InDRE, Table 1). The Children's Hospital Eva Sámano de López Mateos of Morelia, Michoacán, Mexico, donated the tested strains of *Pseudo-monas aeruginosa*, *Enterobacter cloacae*, and *Salmonella enterica* subsp. *enterica*. The tested strain of *Staphylococcus aureus* was ATCC27543.

Cell lines

Several cell lines corresponding to human cancers were used for the cytotoxicity assays: HeLa (cervicouterine cancer), MCF7 (breast cancer) and A549 (lung cancer). As controls for evaluating the effect on normal cells, bovine mammary epithelial cells (BME) were used for assays with aqueous extracts, and rabbit skin fibroblasts (RSF) for assays with organic extracts. The latter two cell lines were generated in our laboratory. All cell lines were kept in Dulbecco's modified Eagle's medium (DMEM)-F12K (1:1, Sigma, USA) supplemented with 30 mM $NaHCO_3$, 15% fetal bovine serum (Gibco, USA), 50 U/ml of penicillin and 50 U/ml of streptomycin (Gibco, USA). Cells were cultured at 37°C in an atmosphere with 5% CO_2 and 95% humidity.

Extract preparation

For obtaining the aqueous extract 100 g (dry weight) of basidiocarp mass was boiled in 700 ml of deionized water for 60 min, continuously replacing the evaporated water during the extraction time. After extraction was completed the residual biomass was eliminated by filtration through sterile gauze. The recovered filtrate was lyophilized and resuspended in deionized water at a concentration of 500 mg/ml. The resulting solution of aqueous extract was sterilized by filtering through a 0.25 µm pore size membrane (Millipore, USA) and stored at 4°C.

Organic extracts were prepared by crushing 50 g of freeze-dried basidiocarp until a fine powder was obtained; the resulting material was successively extracted with 100 ml of each hexane, ethyl acetate, and methanol during 72 h. The extracts were later centrifuged at 1500 *x* g at room temperature and filtered. The solvents were eliminated by rotoevaporation until dryness and the dry extracts were stored at 4°C. Solutions for the bioassays were prepared by resuspending the dry extracts in the same solvent used for the extraction at a concentration of 500 mg/ml.

Bioassays

Antimicrobial activity by disk diffusion method

The antimicrobial assays were performed *in vitro* using the agar-disk diffusion method (National Committee for Clinical Laboratory Standards, 1993). Petri dishes with Mueller-Hinton Agar (Oxoid, USA) were inoculated massively with a 200 µl suspension of each of the bacterial cultures and grown to their mid-log phase, after which the plate's surfaces were air-dried. Filter paper sensidisks (6 mm in diameter) were impregnated with the necessary volume of each of the extracts in order to reach the final levels of 10, 20, and 30 mg/disk. The impregnated sensidisks were air-dried before being placed on the Petri dishes with the test microorganisms. The plates were incubated for 24 h at 37°C and the inhibition areas were measured in mm using a digital caliper (precision of 0.01 mm, Model CD-6" C, Mitutoyo Corp., Japan). Controls consisted of sensidisks impregnated with the corresponding pure solvent and air-dried. The inhibition diameter of the control disk was subtracted from the inhibition diameter resulting from the application of the corresponding extracts of *L. indigo*. In some tests, control sensidisks with ampicillin (AM) and carbenicillin (CB) (BioRad, USA) were used (Table 1). All assays were performed in triplicate and the results were reported as mean ± standard deviation (SD).

Cytotoxicity assays

For cytotoxicity assays four different concentrations were used of the organic extracts and the same volume of the corresponding solvent as a control. All tested cell lines were cultivated to confluence as described above and were detached from the culture plate using PBS saline solution at pH 7.4 supplemented with antibiotics and 0.25% trypsin-EDTA (w/v, Sigma, USA) and agitated

for 10 min. Afterwards, 1 x 10^6 cells were seeded in OPTIMEM (Gibco, USA) growth medium without serum or antibiotics in 96-well microtitration plates. To these cultures the corresponding extract was added incubating the treated plates during 24 to 48 h at 37°C. After incubation, 10 µl of a 5 mg/ml solution of tetrazolium bromide 3-(4,5-dimetil-2-tiazol)-2,5-diphenil-2H-tetrazolium salt (MTT, Sigma, USA) were added and further incubated for 4 h at 37°C. Finally, 100 µl of 10% SDS were added in order to dissolve the formazan crystals and the viability of cells was determined by measuring the reduction of MTT at 595 nm in a microplate reader (Bio-Rad, USA). Each assay was carried out at least in sextuplicate.

Statistical analysis

In order to evaluate the effect of the extract concentration on the different studied strains and cell lines, the results were analyzed by one-way analysis of variance (ANOVA) and Tukey tests ($p<0.05$), using StatistiXL ver. 1.8.

RESULTS

Antibacterial activity

The controls showed that hexane alone did not cause any inhibition to the tested strains whereas the ethyl acetate and methanol caused a minimal inhibition that was subtracted as described in Materials and Methods. The hexane extract caused inhibition of *P. aeruginosa* and ETEC-LT (Table 2). For the ETEC-LT strain, 10 and 20 mg of hexane extract did not show significant differences in the inhibition zone diameter but 30 mg caused a significantly larger inhibition zone diameter than that for lower amounts. For *P. aeruginosa*, the maximum inhibition was observed at 20 mg of hexane extract, since no significant differences could be observed with 20 and 30 mg of the extract (Table 2). The compounds present in the hexane extract had a greater effect on the ETEC-LT strain than on *P. aeruginosa*. The inhibition caused by the hexane extract is comparable with that caused by the standard antibiotics AM and CB (Table 1).

The ethyl acetate extract inhibited all tested strains (Table 2). In all strains, except for ETEC-LT and *S. enterica*, an apparent dose-response effect could be observed, since the inhibition increased in relation to the applied level of ethyl acetate extract. No significant differences could be observed in *E. cloacae* and *S. aureus* when applying the two lower amounts of the extract. At 10 mg of ethyl acetate extract, no differences could be observed in the inhibition of any of the tested microorganisms. However, at 20 and 30 mg of ethyl acetate extract, significant differences were observed in the inhibition of *P. aeruginosa*, EIEC, ETC-ST, and EPEC (Table 2). At the highest level, all strains showed different susceptibilities to the ethyl acetate extract, except for *E. cloacae* and *S. aureus*. The inhibition of the different *E. coli* pathotypes caused by 30 mg of the ethyl acetate extract was comparable to that caused by AM and CB, except for strain ETEC-LT (Table 1).

The methanol extract caused inhibition in all bacteria tested. Only *E. cloacae* did not show susceptibility at 10 mg (Table 2). At this level, *S. enterica* and *P. aeruginosa* were much more susceptible than the other tested bacteria. With 20 mg of the methanol extract, the inhibitory effect was greater in the ETEC-LT and EIEC strains, whereas EPEC was less susceptible. Even though the inhibition effect caused by the methanol extract differed little from that caused by the other tested extracts, the differences were statistically significant. Using 30 mg of methanol extract, EIEC and *E. cloacae* exhibited the greatest inhibition, followed by ETEC-ST and *S. aureus* (Table 2). The increase in level of the methanol extract had different effects among strains. The strains EIEC, EPEC, *E. cloacae*, and *S. aureus* displayed an apparent dose-response effect, with an increase in the inhibition zone diameter corresponding to the increment in amount of extract. ETEC and *P. aeruginosa* increased their inhibition zone diameter as the level of methanol extract augmented from 10 to 20 mg, but their inhibition zone did not increase when the level was raised to 30 mg of the extract. Finally, *S. enterica* showed no variation in its inhibition zone diameter as the extract level increased. The different *E. coli* pathotypes showed inhibition zone diameters comparable to those caused by AM and CB, except from ETEC-LT (Table 1).

Cytotoxic activity

The aqueous extract of *L. indigo* basidiocarp had different effects on the various cell lines tested. No antiproliferative effect of the aqueous extract was observed against the A549 and MCF7 cell lines, but it significantly inhibited the proliferation of HeLa cells at a concentration of 100 µg/ml (Figure 1). In the case of primary BME cells, a significant stimulation of prolife-ration was observed at a concen-tration of 10 µg/ml and no effect was observed at any other tested concen-tration.

The organic extracts of *L. indigo* also showed contrasting effects between studied cell lines. Thus, in normal RSF cells only the hexane extract showed an inhibitory effect of proliferation relative to the control at all concentrations tested (Figure 2), although the maximum inhibition value was only of 25.63% compared to the control at the maximum concentration of 1000 ng/ml. The ethyl acetate extract showed significant inhibitory values at the concentrations of 100, 500 and 1000 ng/ml. Nevertheless, the maximum inhibition percentage was of only 20.80% relative to the control. The methanolic extract did not alter the capacity for proliferation of fibroblasts at any concentration used (Figure 2). In the case of HeLa cells, the three organic extracts had a significant inhibitory effect on proliferation at all concentrations evaluated. Using ethyl acetate extract, the maximum inhibition was observed at a concentration of 100 ng/ml with a value of 95.74%, while for the metha-

Table 1. Reference strains and clinical isolates used in the present study.

Strain	Pathotype/comment	Code	AM[a]	CB[a]
E. coli	Enterotoxigenic with heat-labile toxin (ETEC-LT)	H10407	18.0	17.0
	Enterotoxaemia with heat-stable toxin (ETEC-ST)	25611	16.0	18.0
	Enteroinvasive (EIEC)	E11	20.0	12.0
	Enteropathogenic (EPEC)	O111	15.0	12.0
S. aureus	isolated from bovine mastitis	ATCC27543	nt	nt
P. aeruginosa	clinical isolate	VGPM01	nt	nt
E. cloacae	clinical isolate	VGEM17	nt	nt
S. enterica subsp. *enterica*	clinical isolate	VGSM33	nt	nt

[a]Inhibition zone diameter (mm) caused by 10 µg of ampicillin (AM) and 100 µg of carbenicillin (CB), nt, not tested.

Table 2. Antibacterial activity of the organic extracts of *L. indigo**.

Bacteria	mg/disk of each extract								
	Hexane			Ethyl acetate			Methanol		
	10	20	30	10	20	30	10	20	30
E. coli									
EIEC	-	-	-	9.6 (0.58)^{1,a}	14.0 (0.73)^{1,b}	19.0 (0.58)^{1,c}	11.3 (0.53)^{1,a}	16.0 (0.31)^{1,b}	20.6 (0.31)^{1,c}
EPEC	-	-	-	9.3 (0.52)^{1,a}	12.0 (0.50)^{2,3,b}	15.3 (0.15)^{2,c}	11.0 (0.78)^{1,a}	12.3 (0.22)^{2,b}	14.3 (0.58)^{2,5,c}
ETEC-LT	18.8 (0.73)^{1,a}	18.6 (0.30)^{1,a}	21.0 (0.80)^{1,b}	10.5 (0.73)^{1,a}	11.3 (0.53)^{3,5,a}	12.0 (0.78)^{3,a}	11.6 (0.15)^{1,a}	17.0 (0.73)^{1,b}	17.6 (0.15)^{3,b}
ETEC-ST	-	-	-	10.0 (0.19)^{1,a}	13.0 (0.75)^{1,2,b}	17.1 (0.53)^{4,c}	10.7 (0.58)^{1,a}	15.0 (0.31)^{3,b}	16.0 (0.73)^{3,4,b}
P. aeruginosa	10.5 (0.51)^{2,a}	11.2 (0.75)^{2,ab}	12.1 (0.52)^{2,b}	9.6 (0.33)^{1,a}	17.2 (0.25)^{4,b}	21.0 (0.65)^{5,c}	13.3 (0.53)^{2,a}	15.1 (0.53)^{3,b}	15.3 (0.15)^{4,b}
E. cloacae	-	-	-	9.7 (0.36)^{1,a}	10.1 (0.75)^{5,a}	13.5 (0.17)^{6,b}	-	13.6 (0.15)^{4,a}	19.0 (0.73)^{1,b}
S. aureus	-	-	-	10.1 (0.29)^{1,a}	11.3 (0.65)^{3,5,a}	13.0 (0.73)^{3,6,b}	9.6 (0.18)^{3,a}	14.0 (0.78)^{3,4,b}	16.3 (0.29)^{3,c}
S. enterica	-	-	-	9.7 (0.75)^{1,a}	11.0 (0.58)^{3,5,a}	11.0 (0.59)^{7,a}	13.2 (0.38)^{2,a}	13.7 (0.17)^{4,a}	13.6 (0.15)^{5,a}

*The mean radius of the inhibition zone of three independent experiments is shown in mm (±SD). Values in the same row with the same superscript letter have non-significant differences between them. Values in the same column with the same superscript number have non-significant differences between them. For both rows and columns, the significance test was evaluated at $p < 0.05$. This significance analysis was made only for results within the same kind of extract for all levels tested.
-, no inhibition zone.

nolic extract the percentage inhibition value at the same concentration was of 67.61%. The hexane extract caused maximum inhibitory activity (76.51%) at 500 ng/ml. MCF7 cells showed a significant stimulation of proliferation at different concentrations of hexane and methanol extracts (Figure 2), the response was higher for the hexane extract at a concentration of 100 ng/ml with a value of 98.08%.

DISCUSSION

The present work shows that organic extracts from *L. indigo* are active against *E. coli* diarrheagenic strains and other bacteria that are pathogenic to humans. This is the first study in which basidiocarps of the genus *Lactarius* are tested against different *E. coli* diarrheagenic strains. Some of the tested strains showed significant differences in their responses to the extracts. The ETEC-LT pathotype was susceptible

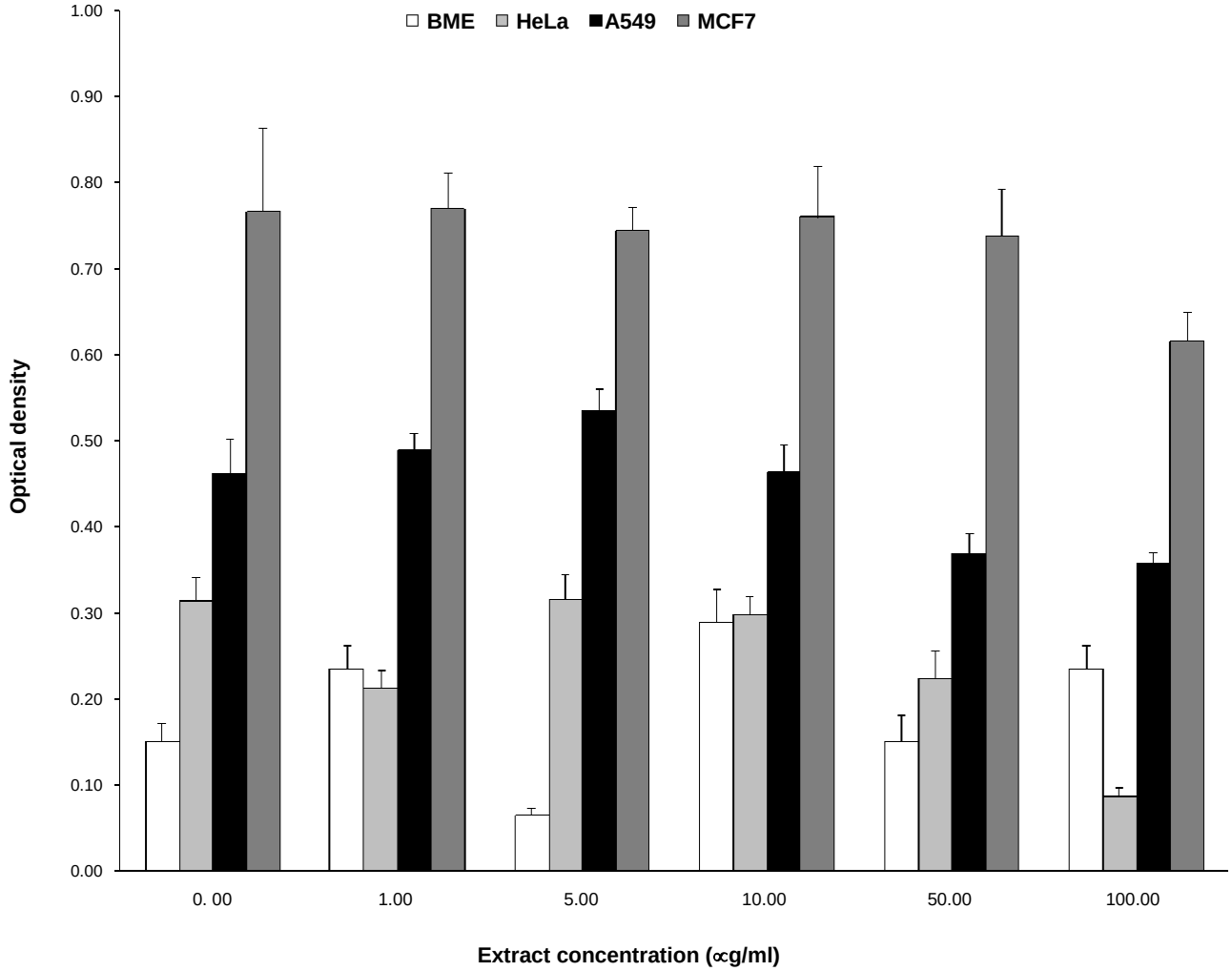

Figure 1. Effect of aqueous extract of *L. indigo* basidiocarp on proliferation of the four cell types evaluated. Bars represent the mean of sextuplicates with its relative standard error. Asterisks indicate extract doses having significant differences relative to the control (deionized water).

to the hexane extract whereas the EIEC pathotype was more susceptible to the ethyl acetate and methanol extracts. Our results agree with those reported by Dulger et al. (2002), who documented the activity against *E. coli* of methanol extracts of different species of *Lactarius*, although they used a non-pathogenic strain. There are some reports about the effect of methanol extracts of *Lactarius deliciosus* on *E. coli*, with very contrasting results. On one side, extracts of *L. deliciosus* collected in Portugal had no activity against non-diarrheagenic *E. coli* (Barros et al., 2007a, b); on the other extreme, basidiocarp extracts of the same species collected in Turkey gave positive inhibitory results against a different strain of non-diarrheagenic *E. coli* (Dulger et al., 2002). Methanolic extracts of *Lactarius piperatus* inhibited growth of *E. coli*, but its activity was dependent on the maturity of the basidiocarp used to make the extracts (Barros et al., 2007b). These results may be due to differences in the susceptibilities of the different strains tested, although differences in the chemical composition of the basidiocarps and in the extraction methods cannot be discarded. In relation to the other microorganisms tested, the three extracts showed strong activity against *P. aeruginosa* and only the methanol extract exhibited activity against *E. cloacae* and *S. aureus*. These results agree with other reports stating that *P. aeruginosa* and *S. aureus* can be inhibited by methanol extracts of basidiocarps of species in the genus *Lactarius* (Dulger et al*.,* 2002; Barros et al., 2007a, b). However, the inhibition activity of the methanol extract of *L. indigo* on *S. aureus* that was observed in the present work seems to be greater than the activity previously reported for methanol extracts of other species in the same genus (Dulger et al., 2002). No previous reports were found about the inhibition of *E. cloacae* using extracts of species of the genus *Lactarius*.

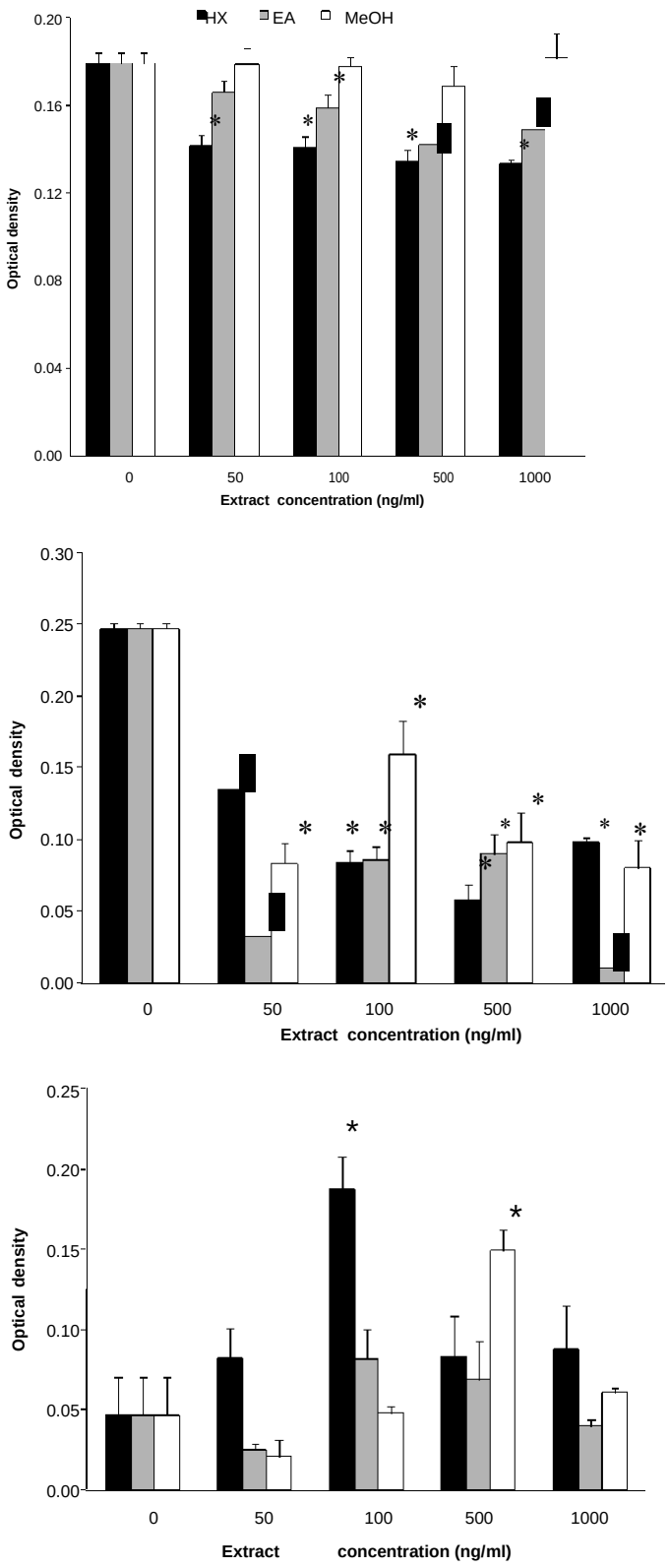

Figure 2. Effect of hexane (HX), ethyl acetate (EA) and methanolic (MeOH) extracts of *L. indigo* basidiocarp on the proliferation of normal RSF (upper panel), HeLa (middle panel) and MCF7 (bottom panel) cell lines. Bars represent the mean of sextuplicates with its relative standard error. Asterisks indicate extract doses with significant differences relative to the control (pure solvent).

The inhibition caused by the ethyl acetate and methanol extracts was observed in practically all tested strains. Therefore, it can be hypothesized that the basidiocarps from *L. indigo* contain a wide spectrum of antibacterial compounds. It has been previously established that compounds with antibacterial activity extracted from species in the genus *Lactarius* are mainly sesquiterpenes (Anke et al., 1989; Vidari et al., 1995). Further studies are now needed to elucidate the chemical structure of the antibacterial compounds extracted from basidiocarps of *L. indigo*.

In the present work the aqueous extract of *L. indigo* significantly inhibited the proliferation of HeLa cells and showed a significant stimulatory effect on the proliferation of primary BME cells. These results contrast with reports for other species of Basidiomycota, although at significantly higher concentrations of aqueous extract than those used in the present study. For example, the aqueous extracts of *Coprinellus* sp., *Flammulina velutipes* and *Coprinus comatus* were capable of inhibi-ting the proliferation *in* vitro of MCF7 cells, with IC_{50} values of 120, 150 and 450 µg/ml, respectively (Gu and Leonard, 2006). Consequently, we cannot discard the possibility that the aqueous extract of *L. indigo* may present antiproliferative activity against the other cell lines evaluated in the present work at higher concentrations than those used by us, a possibility that needs to be evaluated in future works. On the other side, the fact that low concentrations of the aqueous extract had a significant stimulatory effect on the proliferation of normal BME cells is indicative of the possibility that the chemical compounds in the aqueous extract of *L. indigo*, which inhibit the proliferation of HeLa cells may be specific against certain types of cancerous cells. This fact is relevant given that not all aqueous extracts of Basidiomycota present a specific activity against cancer cells; for example, the vegetative mycelia of *Funalia trogii* and of *Trametes* (*Coriolus*) *vers-icolor* contain water-soluble metabolites that inhibit the proliferation of both HeLa and normal fibroblasts (Ünyayar et al., 2006). One possibility is that the aqueous extract of *L. indigo* contains active polysaccharides that are specific against cancer cell lines. One polysaccharide has been isolated from the aqueous extract of *Pleuotus ostreatus* that has an antiproliferative effect against HeLa cells at concentrations of 100, 200 and 400 µM, but that has no significant inhibitory effect on normal human embryonic kidney cells 293T (Tong et al., 2009). Recently, a polysaccharide enriched with selenium was obtained from an aqueous extract of vegetative mycelium of *Ganoderma lucidum* that displays a high antiproliferative activity *in vitro* against HeLa and MCF7 cells with IC_{50} values of 0.17 and 0.14 µM, respectively (Shang et al., 2009). The determination and characterization of anticarcinogenic polysaccharides in aqueous extracts of *L. indigo* is certainly an essential task to be carried out.

The organic extracts of the basidiocarp of *L. indigo* did not show cytotoxic activity against the MCF7 cell line but

were capable for inhibiting the proliferation of HeLa cells. While ethyl acetate and methanol extracts showed a significant inhibitory effect of proliferation on normal RSF, this was relatively low. Regarding MCF7 cells, the results of our study contrast with those of several previous reports about the effect of organic extracts of Basidiomycota. Thus, methanolic extracts of *Pleurotus ostreatus* significantly inhibit the proliferation *in vitro* of MCF7 cells at concentrations above 120 ∝g/ml (Jedinak and Sliva, 2008). Such extracts induce the arrest of the cell cycle at the G0/G1 transition by means of induction of the expression of p53 and p21 proteins, which inhibit the cyclinkinase complexes (CDK). Analyses have been made of the antiproliferative effects of the organic extracts of the Basidiomycota fungus *Naematoloma fasciculare* as well as those of the unsaturated aliphatic acids, ergosterol and ergosterol peroxide, isolated from such extracts (Yan et al., 2009). The pure chemical compounds showed an antiproliferative effect against MCF7 cells that is from 10 to 100 times larger than that of the extracts; while for the former the IC_{50} values oscillated between 0.16 and 1.54 µg/ml, in the latter case those values varied from 13.80 to over 30.0 µg/ml (Yan et al., 2009). Triterpenoids isolated by means of extraction with ethyl acetate of basidiocarp of *Leucopaxillus gentianeus* showed a high antiproliferative activity against MCF7 cells, some of these compounds having IC_{50} values lower than 300 µM (Clericuzio et al., 2004).

In contrast with the case of the MCF7 cells, our results with HeLa cells agree with a number of literature reports. Thus, the inhibition of proliferation of HeLa cells by ethanolic extracts of spores of *G. lucidum* has been reported (Fukuzawa et al., 2008), an effect that was attributed to long chain fatty acids; however, there is a recent report of triterpenoids of the same species having high cytotoxic potential against HeLa cells (Cheng et al., 2010).

An interesting observation made during the present work is the stimulation of the proliferation of the MCF7 cell line, an activity that has been previously reported for aqueous extracts of *Agaricus blazei* in the presence of the estrogenic compound nonylophenol (Talorete et al., 2002). Such proliferative activity has been associated with induction of the expression of the c-Jun protein by the extract, which is synthesized by one of the genes from the proto-oncogene family *c-jun*, characterized by being induced by mitogenic agents (Talorete et al., 2002). Because the MCF7 cell line is characterized by presenting estrogen receptors, it is possible that the organic extracts of *L. indigo* may contain a substance that stimuli-tes those receptors, something to be analyzed in the future.

Conclusion

Both aqueous and organic extracts of *L. indigo* basidiocarp posses pharmacological activity; in some cases, due to inhibition of the proliferation capacity of pathogenic bacteria, and in others, that of carcinogenic cells, without significantly affecting normal cells. Our results show that the basidiocarp of the edible *L. indigo* is a source of pharmacological substances having diverse therapeutic applications, which makes it necessary to perform further studies in that regard by isolating and characterizing the molecules responsible for the observed activities.

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