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RESEARCH ARTICLE

Effect of ethanolic extracts from *Agave potatorum* Zucc. leaves in the mycelial growth of *Pleurotus* spp.

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ABSTRACT

Description or the subject. *Agave potatorum* Zucc. leaves make up the highest proportion of non-valued residue of the agave-mezcal process. Interest in their study lies in the presence of secondary metabolites with potential biological activity. **Objectives.** To evaluate the effect of a crude (CE) and fractionated extract of *Agave* leaves on mycelial growth of different strains of the genus *Pleurotus.* **Method:** The CE, obtained by maceration of *A. potatorum* leaves, was fractionated giving rise to ethyl acetate (AcOEt) and aqueous (Aq) fractions, in which a phytochemical screening was assessed. The effect *in vitro* of the CE and the AcOEt fraction on mycelial growth rate of *Pleurotus* sp. (12 days at 25 °C) was evaluated, whereas the CE and the Aq fraction were assessed for *P. pulmonarius* and *P. djamor*.**Results:** The CE and the AcOEt fraction at 1 000 ppm increased the mycelium growth rate of *Pleurotus* sp. by 61 and 36 %, respectively, relative to the control. CE induced greater growth, significantly different from the control at 1 000 ppm, only on *P. djamor*, while the Aq fraction at 1 000 ppm was not significantly different from the control; and at 5 000 ppm growth in both strains was inhibited. Regardless of the concentration or the fraction assessed, mycelia showed higher density and more aerial growth than the control. **Conclusions:** The *A. potatorum* leaf extract contains secondary metabolites capable of accelerating radial growth of *Pleurotus* strains, which may lead to revaluing the leaves of this *Agave*.

Keywords. *Agave* residues; Coumarin volatiles; Tannins; *Pleurotus pulmonarius*; *Pleurotus djamor*; Secondary metabolites

INTRODUCTION

The genus *Agave* is highly important economically and ecologically for Mexico (Eguiarte et al., 2000), where exists. more than 20 varieties of agave which are used principally to produce mezcal (García-Mendoza et al., 2007). One of these *Agave* species highly appreciated because of its high content of volatile aromatic compounds (Vera et al., 2009), is tobalá maguey (*Agave potatorum*). In general, the industries that produce the agave beverages, mezcal and tequila, discard as residue the leaves, which represents more than 50 % from jima of the mature *Agave* plant, and they are generally not used at all (Nava-Cruz et al., 2014).

On the other hand, oyster mushrooms (*Pleurotus* spp.) (Guzmán, 1997) is the second most cultivated and consumed in the world (Royse and Sánchez, 2017), they are rich in proteins, essential amino acids, polysaccharides, essential fatty acids, dietary fiber, minerals, and some vitamins (Khan and Tania, 2012), and some species are of great interest for growers because of their nutraceutical and bioactive properties (Salmones, 2017).

In general, *Pleurotus* spp. are easily cultivated and can grow in a wide variety of lignocellulosic substrates. *Pleurotus pulmonarius* (*P. florida* Eger s. auct.) (*P. ostreatus* var. *florida* Eger) cultivation has had rapid development in Mexico and other tropical countries using foreign strains on diverse lignocellulosic residues (Gaitán-Hernández et al., 2009; Mata et al., 2013). Also, *Pleurotus djamor* (Rumph. ex Fr.) Boedijn is abundant in Mexican warm regions where it grows on rotting trunks of diverse tree species (Salmones et al., 2004). Despite the rapid development of oyster mushroom cultivation, it is necessary to study the response

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of *Pleurotus* strains to stimulation by additives that favor faster mycelial growth and potential basidiome production in shorter periods.

It has been documented that both yield and chemical composition of the mushrooms are affected by the substrate where they grow (Khan and Tania, 2012). To improve or increase a particular compound in mushrooms, different supplemented substrates have been evaluated. Vlasenko and Kuznetsova (2018) supplemented the substrate with sunflower and maize oils; they determined that both oils at different concentrations increase the content of volatiles in different basidiomes of *P. ostreatus*. Cai et al. (1993) studied the effect exerted by different phenolic monomers and tannins on the mycelial development of *Lentinula edodes, Pleurotus sajor-caju* and *Volvariella volvacea*. They found that *P. sajor caju* responded favorably to different concentrations of phenolic monomers, increasing mycelial growth up to 74 %, about the control, while tannins caused growth to decrease. In contrast, with *L. edodes*, the presence of phenols decreased mycelial diameter, and the presence of tannins increased by up to 42 % (0.-02 % gallic acid), with respect to the control. Also, different agro-industrial residues have been evaluated as growth substrate and to enrich basidiomes of different species of *Pleurotus*, such as coffee residues (Dias et al., 2003; da Silva et al., 2012), wheat and barley straw, maize stover, sugar beet pulp, sawdust, and wheat and rice bran (Jeznabadi et al., 2017). With the same goal, bagasse from different *Agaves* has been studied: *A. tequilana* (Guzmán-Dávalos et al., 1987), *A. cupreata* (Bernabé-González et al., 2004) and *A. salmiana* spp. *crassispina* and *A. tequilana* Weber var. Azul (Heredia-Solis et al., 2016).

Besides some authors have reported the presence of specific secondary metabolites as triterpenes, steroids, tannins, phenolic compounds, volatile coumarins and cardiotonic glycosides (Ahumada-Santos et al., 2013; Nava-Cruz et al., 2014, Soto-Castro et al., 2021) in leaves of *Agave* plants, and there is evidence of their potential biological activity (López-Romero et al., 2018).

With the aim of proposing alternative uses for *Agave* leaves as a supplement that accelerates the growth of edible mushrooms, in this study, an ethanolic extract of *A. potatorum* leaves was obtained and fractionated, and its effect on mycelial growth of different strains of *Pleurotus* is shown.

MATERIALS AND METHODS

Biological material

Leaves from 6-year-old *Agave potatorum* Zucc. plants collected in Villa Sola de Vega, Oaxaca, Mexico, were used, and the following mushroom strains: *Pleurotus* sp. conserved in the CIIDIR Oaxaca, and *Pleurotus pulmonaris* and *Pleurotus djamor*, which are deposited in the Fungal Strain Collection at the Institute of Ecology (INECOL, Veracruz, Xalapa, México) and registered as IE-115 and IE-202, respectively. The strains under study were kept on a PDA medium (potato, dextrose, agar/Bioxon® USA) at 25 °C.

Preparation of crude extract (CE) from *Agave potatorum* Zucc. leaves

Fresh *A. potatorum* Zucc. leaves were washed with abundant running water and cut into strips. The crude ethanolic extract (CE) was obtained by macerating leaves using absolute ethanol as solvent (Sigma-Aldrich) in a 1:2 (leaves:solvent) ratio for 72 h. The extract was then filtered by gravity and concentrated under vacuum in a rotatory evaporator (Buchi® R-100) at 42 °C. The surplus was placed in a Petri dish and left for 24 h under air drag. It was then placed in a drier with anhydrous silica until the water was completely removed (constant weight) in absence of light. In Fig. 1 can see an illustration of the general methodology. The process was carried out in triplicate. Yield of the extract was determined with equation 1.

$$\%\ yield = \frac{g\ dried\ extract}{g\ fresh\ leaves} * 100 \quad (1)$$

Fractionating by ethanol extract polarities

Ten grams of concentrated *A. potatorum* leaf extract was diluted in 2 mL of distilled water. This dilution was maintained in magnetic shaking for 2 h with 10 mL of chloroform ($CHCl_3$, Sigma Aldrich). Later, the organic phase was separated in a separation funnel, dried with anhydrous sodium sulphate (Na_2SO_4, Fermont) and concentrated in the rotatory evaporator. Following the same methodology, the aqueous remnant was extracted with ethyl acetate (AcOEt, Sigma Aldrich), then with acetonitrile (CH_3CN, Sigma-Aldrich), and finally precipitated with acetone (Sigma Aldrich). The aqueous remnant was dried on anhydrous silica gel. The extraction yields for each solvent were determined with equation 1, in which grams of fresh leaves was substituted by 10 g of extract.

Preparation of aqueous (Aq fraction) and ethyl acetate (AcOEt) fractions

Fifty grams of concentrated *Agave* leaf extract was diluted in 10 mL distilled water, then 150 mL AcOEt was added. The mixture was covered and kept under magnetic shaking for 24 h. Later, the organic and aqueous phases were separated with a separation funnel. The AcOEt fraction was dried with anhydrous Na_2SO_4 and concentrated in

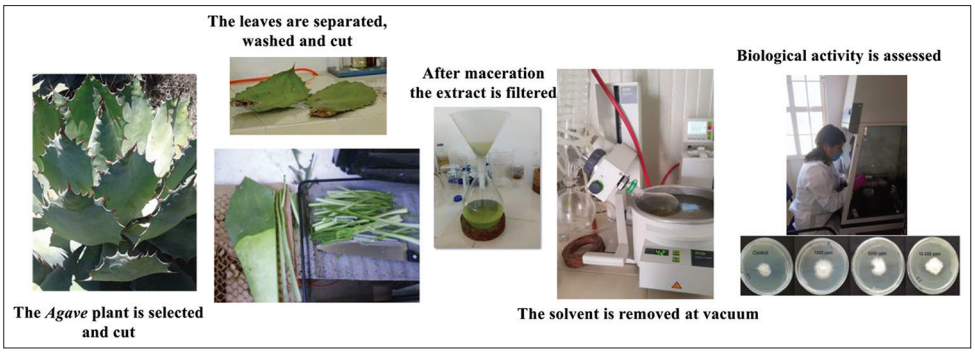

Fig 1. General methodology to assess the biological activity of ethanolic extract of *A. potatorum* leaves.

the rotatory evaporator at 40 °C, resulting in the fraction denominated "AcOEt fraction". The remnant aqueous fraction (Aq fraction) was placed in a drier with anhydrous silica gel, unexposed to light until reaching constant weight.

Characterization of the crude extract and fractionate

Determination of direct and total reducing sugars

The direct and total reducing sugars were measured following the NOM-F-312-S-1978, from 0.400 g of CE and reducing the reactive amounts to one-fifth. Each was replicated three times independently and in triplicate. The result was expressed as the average ± standard deviation.

Phytochemical screening in the crude extract and fractionate

Phytochemical screening was assessed in thin layer chromatography using the conventional reveals. Dissolutions of $FeCl_3$ (Fermont) in methanol-water, 0.5 % fast blue (Sigma Aldrich) in water, 10% $CoCl_2$ (Fermont) in water and a DPPH (Sigma Aldrich) dissolution, were used as reveal reagents of tannin, phenolic compounds, coumarins and antioxidant potential, respectively (Harborne, 1973; Domínguez, 1973). The crude extract and the obtained fractions were dissolved and applied over a silica plate in small circles and left to dry, so that each plate contained all the fractions and the crude extract. Potential antioxidants were considered positive when the DPPH dissolution sprayed over the TLC, originally purple, turned brown in the area with the sample. The foam test was carried out to identify the presence of saponins.

Elution in thin layer chromatography (TLC)

The obtained fractions and the crude extract were characterized by TLC, for which 20 mg of each fraction was dissolved in 1 mL ethanol and later deposited an aliquot on a chromatographic plate (Merk, TLC silica gel 60 F_{254}) in circles. On these plates, different elution media were tested: Hexane (Sigma Aldrich), Hexane:AcOEt (8:2 and 5:5), AcOEt, and AcOEt:Methanol (1:1). Once eluted, the plates were visualized with UV light at 254 and 365 nm.

Evaluation of the biological effectiveness of the crude extract and fractions of *Agave potatorum* Zucc. leaves on *Pleurotus spp*. mycelial growth

The infiltrated agar technique with PDA as the culture medium was used. This methodology consisted of preparing the medium following the indications of the manufacturer (Bioxon® USA) and sterilizing for 20 min at 121 °C. The agar was cooled to approximately 40 °C and, still in liquid form, the adequate volume of extract from a mother dissolution (5 g CE or Aq fraction gauged to 25 mL with sterilized water) was added, shaken to homogenize and gauged to 250 mL with sterile agar. Then, 25 mL of agar was poured into each Petri dish. When the agar solidified, each Petri dish was inoculated with agar plug colonized (4 mm Ø) with mycelia from each strain pre-cultured in PDA. Samples were incubated at 25 °C for 12 days in darkness. For the AcOEt fraction, the mother solution was prepared with 1.0 g of fraction and 200 µL Tween 80 as emulsifier and gauged to 10 mL. For each concentration, 80 mL of medium was prepared with 8 mL per dish. Of each trial, ten replicates and a control in PDA were prepared.

To evaluate the effect of the extracts, regardless of the strain and concentration, mycelium radial growth of the strains was registered every 72 h. Mycelial growth was estimated based upon two measurements (Cartesian plane) of the culture mycelial diameter. The mycelium growth rate (*Kr*) was calculated by fitting the growth function $y = kr\,x + c$ (where y is the distance, x is the time and c is the constant factor) and expressed in mm per day (mm d^{-1}). The assay was performed with ten triplicates (Gaitán-Hernández and Salmones, 2015).

Biological effectiveness of the crude extract and AcOEt fraction on growth of the *Pleurotus* sp. strain

Three concentrations (1 000, 5 000 and 10 000 ppm) of the crude extract were evaluated; 1.25, 6.25, and 12.5 mL, respectively, of the mother solution were used. For the AcOEt fraction, the evaluated concentrations were 500, 1 000, 2 500 and 5 000 ppm, for which 0.40, 0.80, 2.00, and 4.00 mL, respectively, of the mother solution were used.

Ten replicates were prepared. The volumes of the mother solution used were mixed by manual shaking with the agar, as described in the previous section, and mycelial growth was monitored.

Biological effectiveness of the crude extract and aqueous fraction on growth of the *Pleurotus pulmonarius* and *Pleurotus djamor* strains

Two concentrations (1 000 and 5 000 ppm) of the crude extract and the aqueous fraction were evaluated, and 1.25 and 6.25 mL, respectively, of the mother solution were used. The volumes of the mother solution were mixed with agar by manual shaking and poured into Petri dishes, following the methodology for *Pleurotus* sp. Separately, the dishes were inoculated with *P. pulmonarius* and *P. djamor* mycelia, and mycelial growth was estimated. Ten Petri dishes were evaluated per treatment.

Statistical analysis

A completely random design with a factorial arrangement was applied to mycelial growth of *Pleurotus* sp. in function of crude extract concentration (4 x 4), to mycelial growth of *Pleurotus* sp. in function of the AcOEt fraction concentration (4 x 5), to mycelial growth of *Pleurotus pulmonarius* and *P. djamor* in function of the CE and the Aq fraction concentration (4 x 5 x 2), and to growth rate of *P. pulmonarius* y *P. djamor* in function of the CE, and the Aq fractions concentration (2 x 3 x 2). The factors were the incubation days, concentrations, fractions, and strains, and the response variables were the mycelial growth and growth rate. An analysis of variance (ANOVA) was conducted for all values and comparison of means according to Duncan's test ($p < 0.05$). For the concentrations of CE, Aq, and AcOEt fractions, ten replications were evaluated, and the results were shown as the mean ± standard deviation. All the data were analyzed using the statistical software Statistica (v.10.0).

RESULTS

Extraction, characterization, and fractionating of crude ethanol extract of *Agave potatorum* Zucc.

Maceration in ethanol of fresh *A. potatorum* Zucc. leaves had a yield of 3.6 ± 1.2%. The crude ethanol extract had a content of total and direct reducing sugars of 6.03 ± 0.36 % and 2.97 ± 0.06 %, respectively. Moreover, the presence of tannins, phenolic compounds and volatile coumarins was confirmed. Together, they have antioxidant potential corroborated by DPPH as the revealing reagent.

The crude extract was eluted in different media with polarity indexes from 0 to 4.75 (Hexane to AcOEt:Methanol 1:1), and clear separation of the compounds was achieved by eluting with Hex:AcOEt and AcOEt. With AcOEt, three spots of compounds were identified on the TLC plate (Table 1), seen under UV light at 365 nm with retention factors (*rf*) of 1, 0.5 and 0. An increase in polarity (AcOEt:Methanol 1:1) did not achieve better separation.

Based on the above, four medium-polarity solvents (chloroform, ethyl acetate, acetonitrile, and acetone) were evaluated to fractionate the crude extract successively. The use of the four solvents led to four fractions and an aqueous remnant. For each fraction, yield was determined, and assessed the presence of tannins, phenolic compounds, coumarins, saponins, and antioxidant potential (Table 1).

The highest extraction yield with organic solvents was obtained with acetone, followed by AcOEt (Table 1). Both fractions were positive for presence of tannins, phenolic compounds and coumarins and, consequently, both have antioxidant potential. However, TLC determined that the most interesting fraction for evaluating its activity was the AcOEt fraction, which showed a higher diversity of lower polarity compounds (several red marks with 365 nm light), while acetone was perceived as similar (elution profile) to the aqueous fraction (Table 1).

Biological effectiveness of crude extract and fractionate on mycelial growth of *Pleurotus* strains

With the aim of proposing new uses for the *Agave* leaves (residue of the process of jima) and accelerating growth of edible mushrooms (*Pleurotus* spp.) on a substrate that can enhance its productive potential as a food, we determined the *in vitro* effect of the CE on three different *Pleurotus* strains. Moreover, with the objective of delimiting the origin of the compounds that may be exerting a positive effect on mycelial growth of the *Pleurotus* strains, the effect of the AcOEt fraction was evaluated on the *Pleurotus* sp. strain, and the effect of the Aq fraction on growth of *P. pulmonarius* and *P. djamor*. In this way, we inferred the effect of the other fraction.

Biological effectiveness of the crude extract on mycelial growth of the Pleurotus sp. strain

The mycelial growth of *Pleurotus* sp. in function of the concentration of the CE after 3, 6, 9, and 12 days of incubation at 25 °C is shown in Table 2. According to the statistical analysis, the crude ethanol extract of *A. potatorum* leaves at 1 000 and 5 000 ppm has a positive effect on mycelium radial growth of *Pleurotus* sp. The concentration of 1 000 ppm promoted more radial growth as of day 6, significantly different from the control and the rest of the concentrations. At the concentration of 5 000 ppm, the difference was more evident as of day 9, while that of 10 000 ppm was consistently equal to the control.

Table 1: Extraction yield with different solvents and presence of secondary metabolites in crude extract.

Solvent (Polarity index)	Percentage of extraction	Tannins ($FeCl_3$)	Phenolic compound (fast blue)	Coumarins ($CoCl_2$)	DPPH	TLC eluted with AcOEt* rf=0 rf=1
AcOEt (4.4)	1.576	+	+	+	+	
$CHCl_3$ (4.1)	0.076	+	+	-	+	
CH_3CN (5.0)	0.180	+	+	-	+	
Acetone (5.1)	3.763	+	+	+	+	
Aqueos (9.0)	94.405	+	+	+	+	

*The TLC plate was turned so that each elution track coincided with the extraction solvent. Saponins were not detected in any fraction, only in the aqueous fraction the foam lasts longer, but is not definitive

Table 2: Mycelial growth (mm) of *Pleurotus* sp. during 12 days of incubation at 25°C, in function of crude extract concentration

Concentration (ppm)	Day 3	Day 6	Day 9	Day 12
Control	5.50 ± 0.52[a]	15.01 ± 0.46[b]	23.30 ± 0.42[d]	32.46 ± 1.09[e]
1000	7.00 ± 1.04[a]	18.89 ± 3.99[c]	38.35 ± 5.71[f]	52.48 ± 6.25[g]
5000	7.33 ± 0.98[a]	16.15 ± 2.38[bc]	29.37 ± 3.63[e]	40.11 ± 5.10[f]
10000	6.50 ± 1.31[a]	16.90 ± 3.06[bc]	24.95 ± 5.83[d]	30.92 ± 8.57[e]

Values are means ± standard deviation of ten replicates. Values that do not share at least one letter are significantly different ($p < 0.05$, Duncan)

Statistical analysis of growth rate (*Kr*) determined that *Pleurotus* sp. growth responded better to the 1 000 ppm concentration (70 % higher than the control), significantly different from the control and the rest of the concentrations (Fig. 2).

After 7 days of incubation, mycelial growth of *Pleurotus* sp. was denser and irregular, as the extract concentration increased (Fig. 3).

Biological effectiveness of AcOEt fraction on Pleurotus sp. mycelial growth

According to radial growth of *Pleurotus* sp., the AcOEt fraction induced growth inversely proportional to fraction concentration. Also, at a given concentration, there is a linear trend in mycelial growth from day 0 to day 12, and more radial growth was produced at 500 ppm and 1 000 ppm, with diameters of 31.63 and 30.78 mm, respectively (Table 3). In general, the statistical analysis indicated that there were significant differences among concentrations, but not between 500 and 1 000 ppm, and mycelial growth was statistically different among evaluation days.

After 12 days of growth, the average mycelium diameter to the control, and under the effect of the AcOEt fraction, and the CE at 1 000 ppm, was of 32.46 ± 1.09 mm, 44.28 ± 0.30, and 52.48 ± 6.25, respectively.

As with the CE, with a higher concentration of the AcOEt fraction, daily growth rate is lower; at 5 000 ppm, it is lower even than the control (Fig. 4).

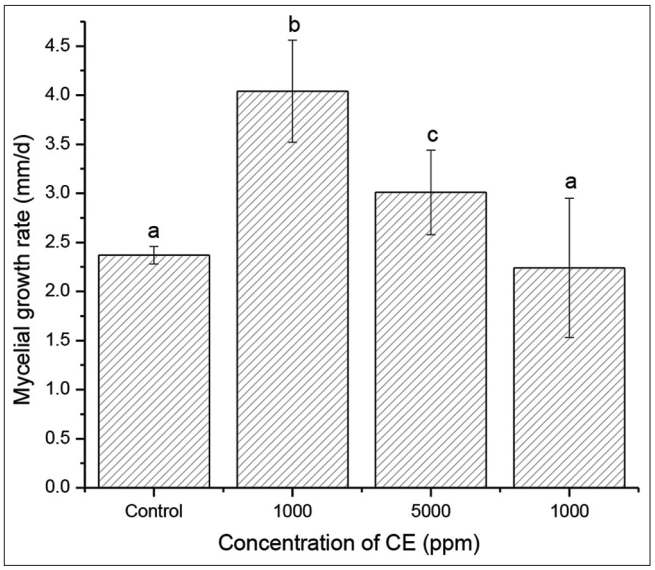

Fig 2. Growth rate (mm d^{-1}) of *Pleurotus* sp. during 12 days of incubation at 25 °C, in function of crude extract concentration. Values are means ± standard deviation of ten replicates. Bars that do not share at least one letter indicate significant differences ($p < 0.05$, Duncan).

Biological effectiveness of the crude extract and aqueous fraction on mycelial growth of P. pulmonarius and P. djamor strains

The results of testing the effect of concentrations of CE and Aq fraction on *P. pulmonarius* and *P. djamor* mycelial growth on different days of evaluation are shown in Tables 4 and 5, respectively. It should be mentioned that for these strains, only the concentrations 1 000 and 5 000 ppm were evaluated since the effect of the10 000 ppm concentration was not different from the control for the *Pleurotus* sp. strain.

For *P. pulmonarius* the mycelial growth was significantly different between evaluation days. It was also observed that the 1 000 ppm concentration favored growth of this species but was not significantly different from the control. The average daily growth rate clearly shows that the Aq fraction at 5 000 ppm produces a decrease in mycelial growth (5.04 mm d^{-1}), compared with the 5 000 ppm concentration of the CE (7.45 mm d^{-1}) (Fig. 5).

For *P. djamor*, on day 12 the extracts were significantly different from the control, and the CE at 1 000 ppm was

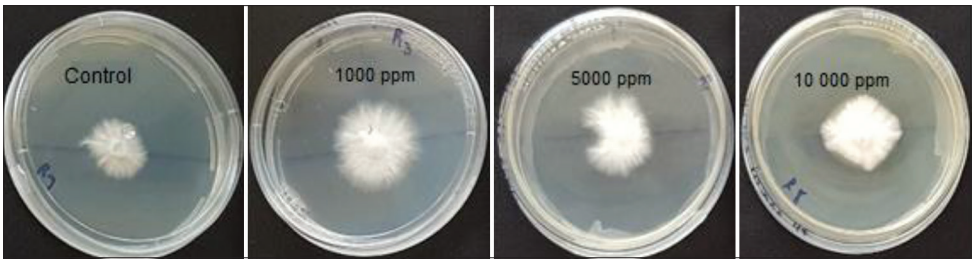

Fig 3. Behavior of *Pleurotus* sp. mycelia at 7 days of incubation at 25 °C, in function of the concentration of *A. potatorum* crude extract.

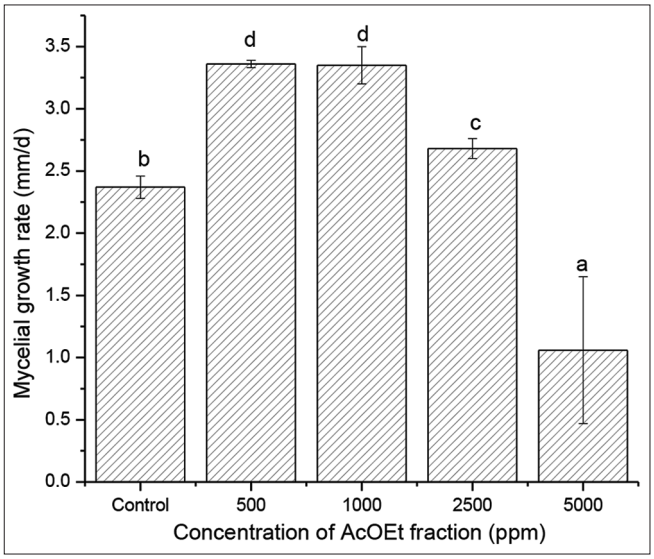

Fig 4. Growth rate (mm d^{-1}) of *Pleurotus* sp. during 12 days of incubation at 25 °C, in function of the AcOEt fraction concentration. Values are means ± standard deviation of ten replicates. Bars that do not share at least one letter indicate significant differences ($p < 0.05$, Duncan).

better (Table 5). There were also significant differences among the three tested concentrations of the Aq fraction. *P. djamor* had better development with the concentration of 1 000 ppm.

In general, the statistical analysis of the daily growth rate indicated that behavior of the *P. pulmonarius* strain was significantly different from the *P. djamor* strain (Fig. 5), although differences between the two types of extract used were not observed; the concentration of 5 000 ppm was less efficient for mycelial growth of the two strains. It is worth mentioning that, like *Pleurotus* sp., mycelial growth of *P. pulmonarius* and *P. djamor* in the culture medium with extract was denser than in the control and tended to show more aerial grow than creeping.

DISCUSSION

In this study, the extraction yield from *A. potatorum* leaves was lower than that reported by Cerda de los Santos (2011), who obtained a yield of 5.6 % using acetone to extract bioactive compounds from *A. atrovirens* Karw leaves. This

Table 3: Mycelial growth (mm) of *Pleurotus* sp. during 12 days of incubation at 25°C, in function of the AcOEt fraction concentration.

Concentration (ppm)	Day 3	Day 6	Day 9	Day 12
Control	5.50 ± 0.52^{a}	15.02 ± 0.46^{de}	23.30 ± 0.42^{g}	32.47 ± 1.09^{j}
500	17.05 ± 0.52^{e}	26.82 ± 1.55^{h}	38.38 ± 1.05^{l}	44.28 ± 0.30^{m}
1000	16.38 ± 0.52^{e}	26.21 ± 1.15^{h}	36.40 ± 1.32^{k}	44.16 ± 1.78^{m}
2500	13.22 ± 1.54^{cd}	19.78 ± 0.87^{f}	29.00 ± 0.80^{i}	36.11 ± 0.98^{k}
5000	6.90 ± 1.97^{ab}	8.58 ± 2.93^{b}	12.39 ± 6.49^{c}	16.72 ± 7.07^{e}

Values are means ± standard deviation of ten replicates. Values that do not share at least one letter indicate significant differences ($p < 0.05$, Duncan).

Table 4: Mycelial growth (mm) of *Pleurotus pulmonarius* during 12 days of incubation at 25 °C, in function of the CE and the Aq fraction concentration.

	Crude Extract (CE)			
Concentration (ppm)	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	16.47 ± 2.40^{a}	45.0 ± 6.06^{cd}	81.42 ± 9.8^{h}	90.0 ± 0.0^{i}
1000	19.31 ± 2.14^{a}	48.94 ± 4.79^{d}	75.46 ± 10.79^{gh}	90.0 ± 0.0^{i}
5000	18.30 ± 1.33^{a}	31.50 ± 16.04^{b}	71.91 ± 3.39^{fg}	90.0 ± 0.0^{i}
	Aqueous Fraction (Aq)			
1000	16.38 ± 1.33^{a}	37.90 ± 3.32^{bc}	67.39 ± 5.19^{ef}	90.0 ± 0.0^{i}
5000	16.70 ± 1.96^{a}	33.78 ± 6.12^{b}	48.92 ± 12.0^{d}	62.64 ± 6.41^{e}

Values are means ± standard deviation of ten replicates. Values that do not share at least one letter in both extracts are significantly different ($p < 0.05$, Duncan)

opens the possibility that we can test other solvents to increase yield in the case of *A. potatorum*.

In agreement with the elution profiles, it was concluded that the compounds present in the CE have medium and high polarity. Therefore, only medium polar solvents were assessed to fractionate the CE, and based on the highest yield and separation of compounds, AcOEt and water (Aq fraction) were selected to fractionate the crude extract at a higher scale and begin the study of the effect on mycelial growth of mushrooms of the genus *Pleurotus*.

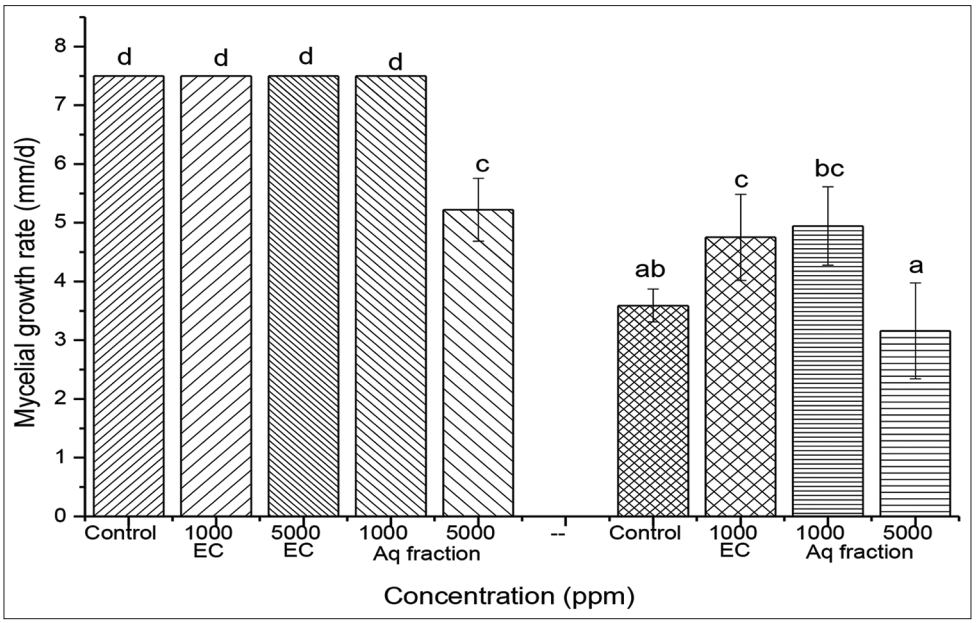

Fig 5. Growth rate (mm d^{-1}) of *P. pulmonarius* y *P. djamor* during 12 days of incubation at 25 °C, in function of the CE, and the Aq fractions concentration - Values are means ± standard deviation of ten replicates. Bars that do not share at least one letter are significantly different ($p < 0.05$, Duncan).

Table 5: Mycelial growth (mm) of *Pleurotus djamor* during 12 days of incubation at 25°C, in function of the CE and the Aq fraction concentration.

	Crude Extract (CE)			
Concentration (ppm)	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	10.34 ± 1.41^{a}	24.50 ± 4.07^{b}	35.98 ± 3.35^{de}	43.07 ± 2.99^{fg}
1000	12.12 ± 3.52^{a}	28.32 ± 3.13^{bc}	45.89 ± 6.07^{g}	57.30 ± 7.87^{h}
5000	n.d.	n.d	n.d.	n.d.
	Aqueous Fraction (Aq)			
1000	10.625 ± 1.92^{a}	24.65 ± 3.55^{b}	41.58 ± 5.54^{efg}	55.88 ± 11.09^{h}
5000	8.86 ± 1.47^{a}	23.20 ± 8.19^{b}	32.37 ± 7.89^{cd}	37.90 ± 9.79^{def}

Values are means ± standard deviation of ten replicates. Values that do not share at least one letter are significantly different ($p<0.05$, Duncan), n.d.= no data

According to biological effectiveness of the CE and fractionate extract on mycelial growth of *Pleurotus* strains during the time, was determined that *Pleurotus* sp. growth responded better to the 1 000 ppm concentration (Fig. 2). This would be reflected in the use of the extract at low concentrations to obtain the best effect and better use of the residue. It is worth mentioning that the concentration also showed an effect on morphological characteristics of the mycelia, like that reported by Suárez (2010) for *P. pulmonarius* and *P. ostreatus*: dense abundant mycelia of irregular growth. In this study we observed a denser and irregular growth in *Pleurotus* sp. as the extract concentration increased (Fig. 3). An explanation could be that the secondary metabolites produced by *Agave potatorum* could have stimulated the mycelial growth.

The effect of the AcOEt fraction on the mycelial growth of *Pleurotus* sp., induced growth inversely proportional to fraction concentration. However, abundantly more aerial mycelium growth is observed with concentrations of 2 500 and 5 000 ppm, while in the case of concentrations of 500 and 1 000 ppm, radial growth was more symmetrical and flush with the agar, unlike the irregular growth observed with the crude extract. The higher mycelial growth of *Pleurotus* sp., at 500 ppm and 1 000 ppm with the AcOEt fraction (Table 3), suggest that for the AcOEt fraction there is a lower optimal concentration that would induce a higher growth rate of the mushroom.

A comparison among the control, the AcOEt fraction, and the CE, showed that the AcOEt fraction induces 36 % more growth than the control, but this is a lesser effect than that of the CE (61 % larger diameter). Considering that the AcOEt fraction represents only 1.6% of the crude extract, the data indicate that in the aqueous fraction there are also secondary metabolites present, possibly tannins and phenolic compounds, with the capacity of inducing an accelerated mycelial growth of *Pleurotus* sp. at low concentrations.

For the *P. pulmonarius* strain, over which only the effect of the CE and the Aq fraction were evaluated, we observed that the effects of these were significantly different, with higher mycelium growth with the CE. It was also observed a decrease in mycelial growth at 5 000 ppm of Aq fraction, compared with the same concentration of the CE (Fig. 5). Thus, it can be inferred that the AcOEt fraction contains

metabolites with the potential to accelerate the growth of this strain, so that when we evaluate the CE, the negative effect of some metabolites is compensated by the positive effect of others, and no significant acceleration in mycelial growth is produced.

In terms of the average daily growth rate of *Pleurotus djamor*, only the CE concentration of 1 000 ppm showed a significantly higher rate than the control (Fig. 5). This concentration promoted an increase of 22 %, while the increase with the Aq fraction at the same concentration was 19 %, which was not statistically different from the control. For this reason, it can be inferred that the AcOEt fraction would have very poor effect on mycelial growth.

The results obtained here, provide evidence that, although the three strains are of the genus *Pleurotus*, their metabolism is quite different, responding differentially to the same secondary metabolites. It would thus be of interest to determine what types of phenolic compounds, tannins and coumarins are present in the extract and in each of the fractions since, although the evaluated fractions tested positive for phenolic compounds, tannins and volatile coumarins, we should not lose sight of the fact that the phytochemical tests do not differentiate structures, but only identify general groups. Clearly, the metabolites present in the AcOEt fraction, even though they are phenolic, are structurally different from those that remain in the Aq fraction. The *Pleurotus* strains studied here may have behavior like that reported by Cai et al. (1993) for *P. ostreatus*, whose strain responds favorably to phenolic compounds, while the tannins induce a reduction in growth. Moreover, it was expected that beyond accelerating mycelial growth, the compounds present in the substrate can influence quality of the mushrooms produced (Gaitán-Hernández et al., 2017), however this will be the next step.

CONCLUSIONS

The ethanolic extract of *A. potatorum*, as well as the Aq and the AcOEt fractions, contains phenolic compounds, tannins and volatile coumarins and, therefore, possess antioxidant capacity. Based on mycelial growth of the different *Pleurotus* strains studied, it is concluded that the crude extract, and the AcOEt and the Aq fractions contain secondary metabolites capable of accelerating mycelial growth at concentrations of 1 000 ppm or lower. In *Pleurotus* sp. Strain, CE (1 000 ppm) and AcOEt fraction (500 ppm) promoted faster mycelial growth, significantly different from the control. *P. djamor* responded similarly to CE and the Aq fraction showing an increase in the growing rate at 1000 ppm, while *P. pulmonarius* showed no difference with the control, neither with CE nor with Aq fraction. Both, the crude extract, and the fractions, induced morphological changes of mycelium of the three studied strains, promoting denser mycelia. For this reason, we conclude that determining the biomass to evaluate the effect of the extract on mycelium growth would be a more adequate technique.

Finally, it is necessary to determine the chemical nature of the compounds contained in *Agave potatorum* leaf extract to revaluate as a by-product what today is considered agro-industrial waste.

Author contributions

Delia Soto Castro. Conceived the main research idea, evaluated the effect of the ethyl acetate fraction on the mycelial growth of *Pleurotus* sp. Carried out the analysis of the results and wrote of the manuscript in equal participation with R. Gaitán-Hernández. **Patricia Araceli Santiago García.** Participated in data acquisition of the effect of the ethyl acetate fraction on the mycelial growth of *Pleurotus* sp. **Alfonso Vásquez López.** Participated in the experimental design to evaluate the effect of crude extract on *Pleurotus* sp. and collaborated on the manuscript revision. **Sánchez Heraz Florisela** and **Yanet Vargas Mendoza.** Carried out the experimental part and the data acquisition. **Rigoberto Gaitán-Hernández.** Designed the methodology to evaluate the effect of the extract and aqueous fraction on the mycelial growth of *P. pulmonarius* and *P. djamour*, carried out the statistical analysis of all results, and in equal participation with D. Soto-Castro wrote the manuscript.

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