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Antifungal Activity of *Datura stramonium* L. Extractives against Xylophagous Fungi

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Abstract: Some plants have great resistance against herbivores, invertebrates, insects, bacteria, and fungi. This resistance is mostly present in plants containing alkaloids, which are the substances responsible for giving them defensive properties. The genus *Datura* contains tropane alkaloids and all plants from this genus have defensive properties. In this work, we report the toxic effect against fungi of *Datura stramonium* extracts, obtained by the Petri dish method. The extraction solvents were water, ethanol, 2-propanol, n-butanol, propanone, butanone, 3-methyl-2-pentanone, dichloromethane, xylene, and toluene. The test fungi were *Trametes versicolor* (L. ex. Fr) Pilát and *Rhodonia placenta* (Fr.) Niemelä, K.H.Larss. & Schigel. It was found that water, butanone, and toluene extracts promoted mycelial growth, xylene extracts neither inhibited nor promoted mycelial growth, while the other extracts slightly inhibited the growth of these fungi.

Keywords: xylophagous fungi; extracts; natural preservatives; Petri dish method

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1. Introduction

Nowadays, the worldwide demographic explosion means that the demand for forest resources is increasing daily. The devastation of the flora, along with the growing increase of indiscriminate use of wood worldwide, the scarcity of raw material, the cost of marketing, and especially, the awareness of the risks of toxicity for man and the environment, have stimulated the search for less toxic natural preservatives [1–3].

Wood was probably the first material used for structural purposes. It is abundant in nature and can be used as sawn or round wood. Its manufacture requires much less energy consumption than other materials, such as steel, which allows for significant savings. It is also a very tenacious material with great resistance to bending. However, wood bends, shrinks, and swells due to moisture loss or absorption [4]. Because of its organic nature, various biological agents can rot and destroy it under certain conditions, making protection against these agents indispensable. Fungi are the main wood degrading agents; they cause great economic losses and considerably decrease the service life of wood [5–8].

The main biological degradation of wood occurs through the action of fungi known as xylophages [5,9]. In the wood industry, the losses due to this are substantial. In the cellulose and paper industry wood degradation affects the quality of the final product, paper [10].

Wood rot has been classified into two main groups: "brown rot" and "white rot"; in the former, the fungus degrades only cellulose and hemicelluloses, while in the latter, it degrades both holocellulose and lignin. Soft rot fungi are simple beings that live parasitically

inside the wood. From it, they feed on the secondary cellulose, especially when conditions of high humidity occur; in this process, it loses density and resistance [11–14].

Some woods have natural resistance to fungal attack, while others may be susceptible, so preservation treatments are required for additional protection; otherwise, the wood pieces would have to be replaced often, resulting in replacement costs. Chemical treatments are widely used and require the immersion of the wood or the injection of pesticide oils, metallic salts, or organic compounds into the wood [15–18].

Datura stramonium belongs to Kingdom: *Plantae*, Division: *Magnoliophyta*, Class: *Magnoliopsida*, Order: *Solanales*, Family: *Solanaceae*, Genus: *Datura*, Species: *Datura stramonium* [19,20]. Some of its synonyms are apple *D. stramonium*, tapa [21], and toloache [22]. The toloache is of American origin but is now can be found throughout the world, including the warmer regions of North, Central, and South America, Europe, Asia, and Africa [23]. It has been used for a variety of purposes such as hallucinogens, for total relaxation, among others, since before the arrival of the Spaniards to the continent [24]. The cultivation of toloache is legal and can be traded freely; in fact, it is relatively easy to find in Mexican markets. Its cost is moderate and the conditions for handling it are not demanding [25]. Preparations used in herbal practice are high risk and should be carefully monitored in their dosage and administration, especially orally. It was also used for the treatment of ulcers, injuries, inflammation, sciatica, hematomas and swelling, rheumatism, gout, asthma, bronchitis, and toothache [26,27]. In Mexico, several species of *Datura* have similar properties and a reputation for being toxic [28,29]. *D. stramonium* showed protective anti-inflammatory effects [30], as well as antidiabetic, antidyslipidemic, antioxidant [31], antimicrobial [32], antiepileptic, antiasthmatic, analgesics, and insecticidal properties [16,20,33]. *D. stramonium* has been reported to contain: carbohydrates [34,35], alkaloids [36–39], saponins, tannins [35,40], steroids, flavonoids, phenols, and glycosides [40]. Phytochemical analysis showed that the aqueous and ethanolic extract of *D. stramonium* stem bark contained alkaloids, saponins, tannins, steroids, flavonoids, phenols, and glycosides [41]. Many amino acids were isolated from the seeds, in particular alanine, glutamate, phenylalanine, and tyrosine [42]. The tropane alkaloids were the important anticholinergic alkaloids isolated from *D. stramonium* [43]. The main toxic alkaloids of *D. stramonium* are the tropane alkaloids, which are the atropines (dl-hyoscyamine) and scopolamine (l-hyoscine) [44,45].

In this context, this work contributes to evaluating the antifungal activity of several extractable compounds from *D. stramonium* L. (toloache) against xylophagous fungi.

2. Materials and Methods

2.1. Materials

Adult *D. stramonium* L. plants were obtained from the local market. First, it was washed with water at 60 °C in order to remove superficial impurities, then it was dried at room temperature and ground to increase its superficial area to facilitate the extraction. Bidistilled water, ethanol, propanone, isopropyl alcohol, and dichloromethane were purchased from Golden Bell. Butanone (99%) and p-xylene were obtained from J. T. Backer. 3-methylpenta-2-one was bought from Chempure. 1-butanol was purchased from Merck and toluene from Spectrum. Malt extract agar was obtained from BD Difco.

2.2. Methods

For the evaluation of the antifungal activity of the extracts of *D. stramonium* L., a general scheme of the methodology followed in the present investigation is presented in Figure 1.

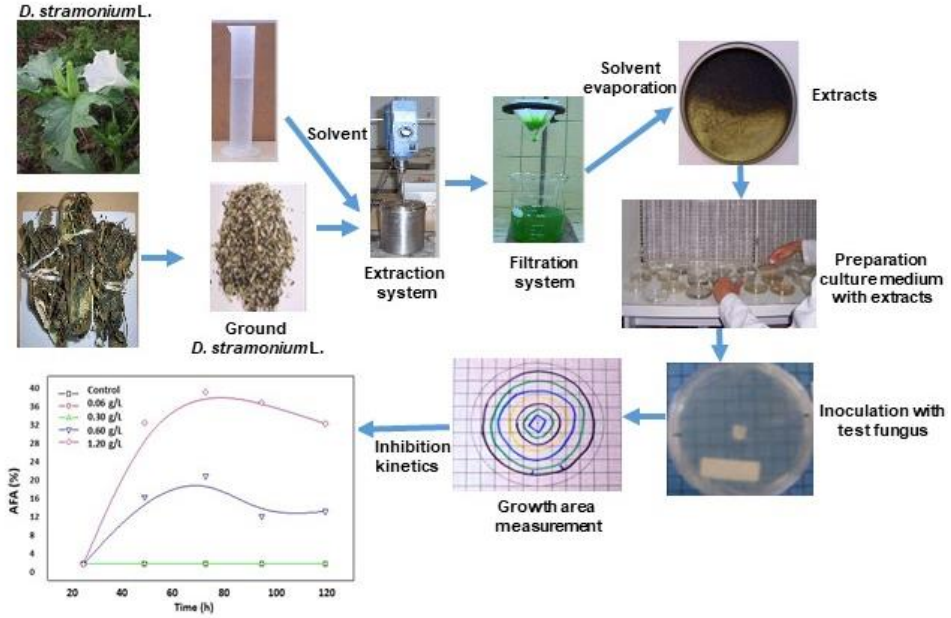


Figure 1. Outline of the followed methodology.

2.3. Obtaining Extracts

For each extraction, 50 g of *D. stramonium* L. in powder form was used. For the extraction, a batch system with continuous agitation of 670 rpm, for 6 h, a 1/8 hydro module, at 25 °C was used. The solution obtained from the extraction was then filtered with filter paper. The concentrated solutions were left to dry in a hood at room temperature until total removal of the solvent.

2.4. Test Fungi

The bioassay was conducted using the white-rot fungus *Trametes versicolor* (L. ex Fr.) Pilát (CSNL01760) and the brown rot fungus *Rhodonia placenta* (Fr.) Niemelä, K.H.Larss. & Schigel (IEXpp00). The culture medium for the fungal growth test was prepared using 33.6 g/L malt-agar extracts and sterilized at 15 lb/in^2 for 15 min. These strains belong to the strain collection of the Wood Properties and Uses Laboratory of the Department of Wood, Cellulose and Paper, University of Guadalajara, Mexico. The fungal strain *T. versicolor* was donated by the Faculty of Forest Sciences, Autonomous University of Nuevo León, Monterrey, Mexico. The strain of the fungus *R. placenta* strain was donated by the Institute of Ecology of Xalapa, Veracruz, Mexico. Fungal strains were maintained in agar test tubes kept refrigerated at 5 °C. The strain was kept viable by periodic reinoculation. The strains were reinoculated from one of the test tubes, a first reinoculation was performed in a Petri dish, and from these a second reinoculation. From these second reinoculated Petri dishes, the mycelium for the Petri dishes of the bioassay was obtained. The inoculated Petri dishes were incubated in a laboratory incubator at 28 °C.

2.5. Bioassay

To perform the bioassay, acetone/hexane/water solutions (54%/44%/2%) were prepared to dissolve the non-water-soluble extracts and incorporate them into the culture medium [1]. In order to evaluate the antifungal effects, the "Petri dish" method was used [46–48]. This method consists in placing the necessary amount of solution containing the extracts into the culture medium (malt agar). The culture medium was sterilized for 15 min at 15 lb/in^2. The extracts were not sterilized to avoid chemical alteration. Once the culture medium was cooled, the culture medium and the solutions containing the extracts at different concentrations (Table 1) were mixed with rotating movements in a 50 mL Erlenmeyer flask. Then, the mixing was poured into Petri dishes and allowed to gel. They were then inoculated using 0.5 cm^2 of the mycelium of the test fungus under sterile

conditions in a laminar flow hood. They were incubated during the test time, 6 days for *T. versicolor* and 9 days for *R. placenta*, and control growth surface was covered in a VWR incubator, at 28 ± 2 °C, in darkness. The areas of growth of the test fungi were measured both in Petri dishes with extract and in Petri dishes containing only the culture medium (blank) or culture medium and solvent solution (control), at 48 h intervals. The antifungal activity (AFA) was calculated according to the following formula [47]:

$$\text{AFA (\%)} = [(\text{Growth control} - \text{Growth treatment})/\text{Growth control}] \times 100 \quad (1)$$

Table 1. Solvent used, extraction yield, and antifungal activity for extracts.

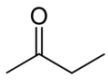
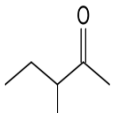
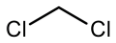
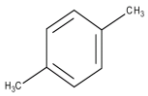
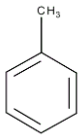
Solvent	Formula	Yields (%)	Concentration (mg/mL)	Antifungal Activity (%) *T. versicolor*			Antifungal Activity (%) *R. placenta*		
Water		6.01 ± 0.04	0	0	-	-	0	-	-
			0.1	−27.5	a	P	−35.7	a	P
			0.5	−31.5	b	P	−44	b	P
			1.0	−44	c	P	−41.2	ac	P
			2.0	−44	cd	P	−43.8	cd	P
Ethanol		3.04 ± 0.02	0	0	-	-	0	-	-
			0.06	9.3	a	NT	0	a	NT
			0.3	13.8	ab	NT	14.8	b	NT
			0.6	15.5	bc	NT	17.2	bc	NT
			1.2	26.3	d	LT	20.3	cd	NT
Propan-2-ol		1.30 ± 0.01	0	0	-	-	0	-	-
			0.06	0	a	NT	25.4	a	LT
			0.3	14.1	b	NT	26.3	ab	LT
			0.6	21.8	bc	NT	27.9	abc	LT
			1.2	25.4	cd	LT	29.7	abcd	LT
			2.5	44.8		LT	40.5	e	LT
			5.0	51		MT	80.6	f	T
Butan-1-ol		1.01 ± 0.01	0	0	-	-	0	-	-
			0.06	0	a	NT	0	a	NT
			0.3	0	ab	NT	0	ab	NT
			0.6	12.1	c	NT	0	abc	NT
			1.2	20.5	d	NT	20.6	d	NT
Propanone		1.16 ± 0.01	0	0	-	-	0	-	-
			0.06	0	a	NT	16.3	a	NT
			0.3	0	ab	NT	23.1	b	NT
			0.6	9.5	c	NT	31.3	c	LT
			1.2	25.2	d	LT	42	d	LT
			5.0	-	-		51	e	MT
Butanone		1.25 ± 0.01	0	0	-	-	0	-	-
			0.05	16.2	a	NT	0	a	NT
			0.25	30.2	b	LT	0	ab	NT
			0.5	41.5	c	LT	0	abc	NT
			1.0	70.9	d	MT	−25.2	d	P
3-methilpentan-2-ona		1.08 ± 0.01	0	0	-	-	0	-	-
			0.06	10.2	a	NT	0	a	NT
			0.3	11.1	b	NT	0	ab	NT
			0.6	12	bc	NT	13.8	c	NT
			1.2	15.5	d	NT	22.4	d	NT

Table 1. *Cont.*

Solvent	Formula	Yields (%)	Concentration (mg/mL)	Antifungal Activity (%) *T. versicolor*			*R. placenta*		
Methylene chloride		1.09 ± 0.01	0	0	-	-	0	-	-
			0.06	0	a	NT	9.5	a	NT
			0.3	9.5	b	NT	22.9	b	NT
			0.6	10.6	bc	NT	24.6	bc	NT
			1.2	32	d	LT	29.2	d	LT
1,4-dimethyl-1,3,5-cyclohexatriene (paraxylene)		1.02 ± 0.01	0	0	-	-	0	-	-
			0.06	0	a	NT	0	a	NT
			0.3	0	ab	NT	0	ab	NT
			0.6	0	abc	NT	0	abc	NT
			1.2	0	abcd	NT	0	abcd	NT
Methylbenzene (toluene)		1.26 ± 0.01	0	0	-	-	0	-	-
			0.06	15.9	a	NT	−29.7	a	P
			0.3	24.6	b	NT	−29.7	ab	P
			0.6	25.4	bc	LT	−28.4	abc	P
			1.2	36	d	LT	−15.2	d	P
			2.5	55.3	e	MT	-	-	-

Different letters (a–e) are significant differences at the 0.05 level. Toxicity was classified according to AFA value: P stands for "growth promoter" and refers to AFA values less than 0 (negative). 0–25% as non-toxic (NT), 26–50% as little toxic (LT), 51–75% as moderately toxic (MT), and >75% as toxic (T) [49].

2.6. Determination of Minimal Inhibitory Concentration 50 (MIC_{50}) and Minimum Inhibitory Concentration (MIC)

The MIC_{50} is the concentration in mg/mL required to inhibit 50% of the mycelial growth of the fungi. Values of MIC_{50} were calculated from the percentage of antifungal activity obtained at each concentration. The minimal inhibitory concentration (MIC) was calculated by applying a simple regression using the Statgraphics program (Centurion XVI, version 16.2.04, Virginia, VA, USA).

3. Results

3.1. Yields of Extracts

Table 1 shows the results of the yields of extracts with each solvent. In general, the obtained yield percentages of extracts are within the ranges reported cited in the literature, which is less than 8% for non-timber materials. The predominant color of the extracts was green for all except the water extracts, which had a dark orange color and a powdery texture. In terms of consistency, all except the water solution had a viscous consistency.

3.2. Antifungal Activity (AFA)

For both test fungi, Table 1 shows the results of the antifungal activity (AFA) of the extracts, as well as the toxicity classification of the extracts. The results of the water extracts for the fungus *T. versicolor* are shown in Figure 2, and for *R. placenta* in Figure 3. Water extracts helped the growth of both fungi and, the higher the concentration of the extracts, the higher the growth, which is why in Table 1 negative AFA values are reported. Water extracts promoted fungal growth because substances that were extracted were most likely sugars, which serve as food for the fungi.

Ethanol extracts showed inhibition for both fungi, observing that the higher the concentration, the greater the inhibition (Figures 2 and 3). For *T. versicolor* it was observed that at the highest concentration evaluated (1.2 mg/mL), AFA was classified as LT while at lower concentrations it was considered NT. For *R. placenta*, all evaluated concentrations were classified as NT.

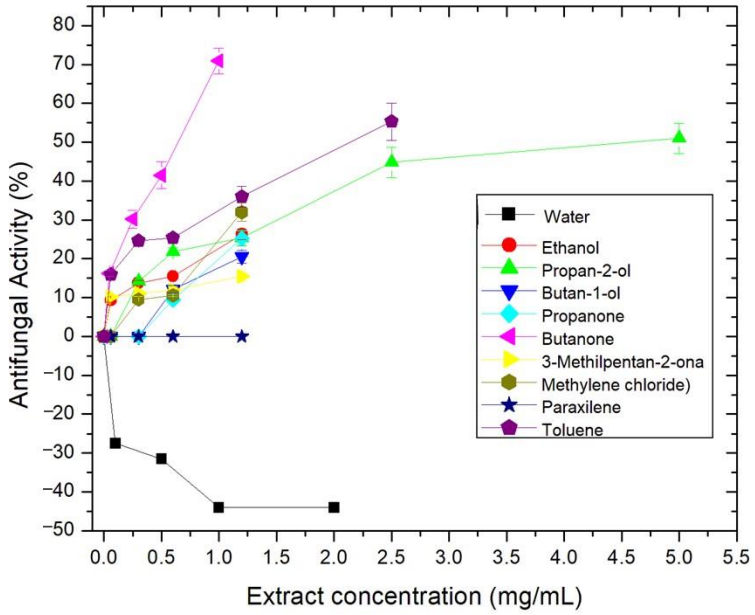


Figure 2. Antifungal activity of the extracts against the fungus *T. versicolor*.

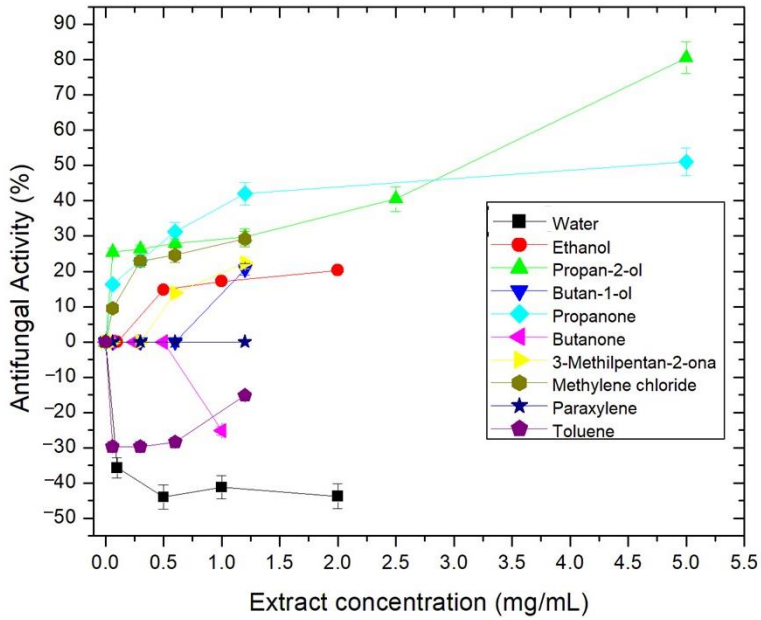


Figure 3. Antifungal activity of the extracts against the fungus *R. placenta*.

Propane-2 of extracts showed that the higher the concentration of the extracts, the higher the AFA for both fungi. The extracts showed greater inhibition for *R. placenta* than for *T. versicolor*. The extracts obtained with butan-1-ol showed, for the fungus *T. versicolor*, that the first two concentrations (0.06 mg/mL and 0.30 mg/mL) were not able to inhibit the fungus so that it grew as if it were in its usual cultivation medium, and in the following two concentrations (0.60 mg/mL and 1.2 mg/mL) the fungus was inhibited. The inhibition effect increased with increasing concentration (Figure 2). For *R. placenta* (Figure 3), it was observed that the concentration of 1.2 mg/mL was able to inhibit the fungus. The other three concentrations showed growth equal to the target. The activity of the extracts against both fungi was classified as NT.

Moreover, it was observed that propanone extracts inhibited the growth of both fungi, showing that the higher the concentration of extracts in the medium, the lower the mycelial growth. The trends of AFA against *T. versicolor* and *R. placenta* can be seen

in Figures 2 and 3. For *T. versicolor*, the AFA of the extracts was classified as NT at the 0.06 mg/mL, 0.30 mg/mL, and 0.60 mg/mL concentrations, and as LT at the concentration of 1.2 mg/mL. For *R. placenta*, all concentrations evaluated were considered NT.

Butanone extracts showed mycelial inhibition for *T. versicolor* at all concentrations evaluated. In Figure 2, it can be observed that with increasing concentration of the extract, the inhibitory effect is greater. The extracts were classified as NT, LT, LT, and MT at concentrations of 0.05 mg/mL, 0.25 mg/mL, 0.50 mg/mL, and 1.0 mg/mL, respectively. For *R. placenta* at the first three concentrations, growth similar to that of the control was observed, while at the concentration of 1.0 mg/mL, a mycelial growth-promoting effect was observed.

The AFA of the extract obtained with 3-methyl pentane-2-one showed that it inhibits the growth of the fungus *T. versicolor*. The tendency was to increase the AFA by increasing the concentration of the extract, being that of 1.2 g/L the one showing the highest % AFA value, as shown in Figure 2. In the case of the fungus *R. placenta*, Table 1 shows the values for the first two concentrations (0.06 mg/mL and 0.30 mg/mL) that the growth of the fungus in the Petri dishes was equal to the blank. The next two concentrations inhibit the fungus, and the trend of these data is shown in Figure 3. This extract was more toxic to *R. placenta* than to *T. versicolor*. In general, it was observed that the extracts with 3 methyl,-2 pentanone were classified as NT for both fungi in the four concentrations evaluated.

The compounds extracted by dichloromethane showed inhibition against *T. versicolor*, the highest AFA was at the concentration of 1.2 mg/mL. The AFA values are shown in Table 1, and it is observed that by increasing the concentration, the toxicity increased. This trend is observed in Figure 2. The fungus *R. placenta* was slightly affected in its growth by the solution used in the concentration of 1.2 mg/mL. It can be seen in Figure 3 that the higher the concentration of extracts, the greater the inhibition. For both fungi, statistically, the concentrations of 0.30 and 0.60 mg/mL did not show significant differences between them, while the other concentrations did. The effect of the extracts against *T. versicolor* were classified as NT, PT, PT, and MT at the concentrations of 0.06 mg/mL, 0.30 mg/mL, 0.60 mg/mL, and 1.20 mg/mL, respectively. For *R. placenta*, all concentrations evaluated were considered as PT.

The compounds present in the toluene extracts inhibited the mycelial growth of *T. versicolor*, and this effect increased with increasing extract concentration. The concentrations of 0.06 mg/mL and 0.30 mg/mL were classified as NT, the concentrations of 0.60 mg/mL and 1.2 mg/mL were classified as LT and the concentration of 2.5 mg/mL as MT. For *R. placenta*, all the concentrations evaluated showed a mycelial growth-promoting effect.

3.3. Minimal Inhibitory Concentration 50 (MIC_{50}) and Minimum Inhibitory Concentration (MIC)

The MCI_{50} and MIC were calculated (see Tables 2 and 3) using the biological models of simple regression, adjusted, and obtained with the inhibition percentages, taken at the endpoint of the fungal growth kinetics. Figure 4 shows the biological models plotted for the tested extracts that inhibited mycelial growth for both fungi.

Table 2. Adjusted model by simple regression, MIC_{50}, and required for inhibition of *T. versicolor* growth.

Extracts Solvent	Adjusted Regression Model	*p*-Value Model	R^2	MIC_{50} (mg mL^{-1})	MIC (mg mL^{-1})
Ethanol	AFA(%) = sqrt (10.4693 + 535.428 × C)	0.0038	0.9784	4.651	18.66
Propan-2-ol	AFA(%) = −0.786175 + 25.0934 × sqrt C	0.0001	0.9798	3.874	15.64
Butan-1-ol	AFA(%) = sqrt (−42.899 + 361.641 × C)	0.0066	0.9688	7.032	27.78
Propanone	AFA(%) = sqrt (−25.338 + 449.926 × C^2)	0.0006	0.9939	2.37	4.725
Butanone	AFA(%) = sqrt (332.376 + 4761.72 × C^2)	0.0007	0.9927	0.675	1.425
3-methilpentan-2-ona	AFA(%) = sqrt [20.3534 + 191.434 × sqrt (C)]	0.0105	0.9572	167.5	2720
Methylene chloride	AFA(%) = sqrt (−22.994 + 708.481 × C^2)	0.0016	0.9879	1.888	3.762
Methylbenzene	AFA(%) = sqrt (77.5182 + 1157.11 × C)	0.0001	0.9916	2.094	8.576

Table 3. Adjusted model by simple regression, MIC_{50}, and required for inhibition of *R. placenta* growth.

Extracts Solvent	Adjusted Regression Model	*p*-Value Model	R^2	MIC_{50} (mg mL^{-1})	MIC (mg mL^{-1})
Ethanol	AFA(%) = sqrt [−34.875 + 320.387 × sqrt (C)]	0.0047	0.9753	62.6	981.1
Propan-2-ol	AFA(%) = sqrt (460.576 + 238.656 × C^2)	0.0165	0.9923	2.924	6.323
Butan-1-ol	AFA(%) = sqrt (−617,135 + 339.318 × C)	0.0513	0.8761	7.55	29.657
Propanone	AFA(%) = sqrt [29.8105 + 1217.68 × sqrt (C)]	0.0007	0.9779	4.117	67.045
3-methilpentan-2-ona	AFA(%) = sqrt (4.37133 + 354.005 × C^2)	0.0018	0.9870	2.656	5.315
Methylene chloride	AFA(%) = sqrt [−22.1977 + 820.0009 × sqrt (C)]	0.0029	0.9820	9.468	149.4

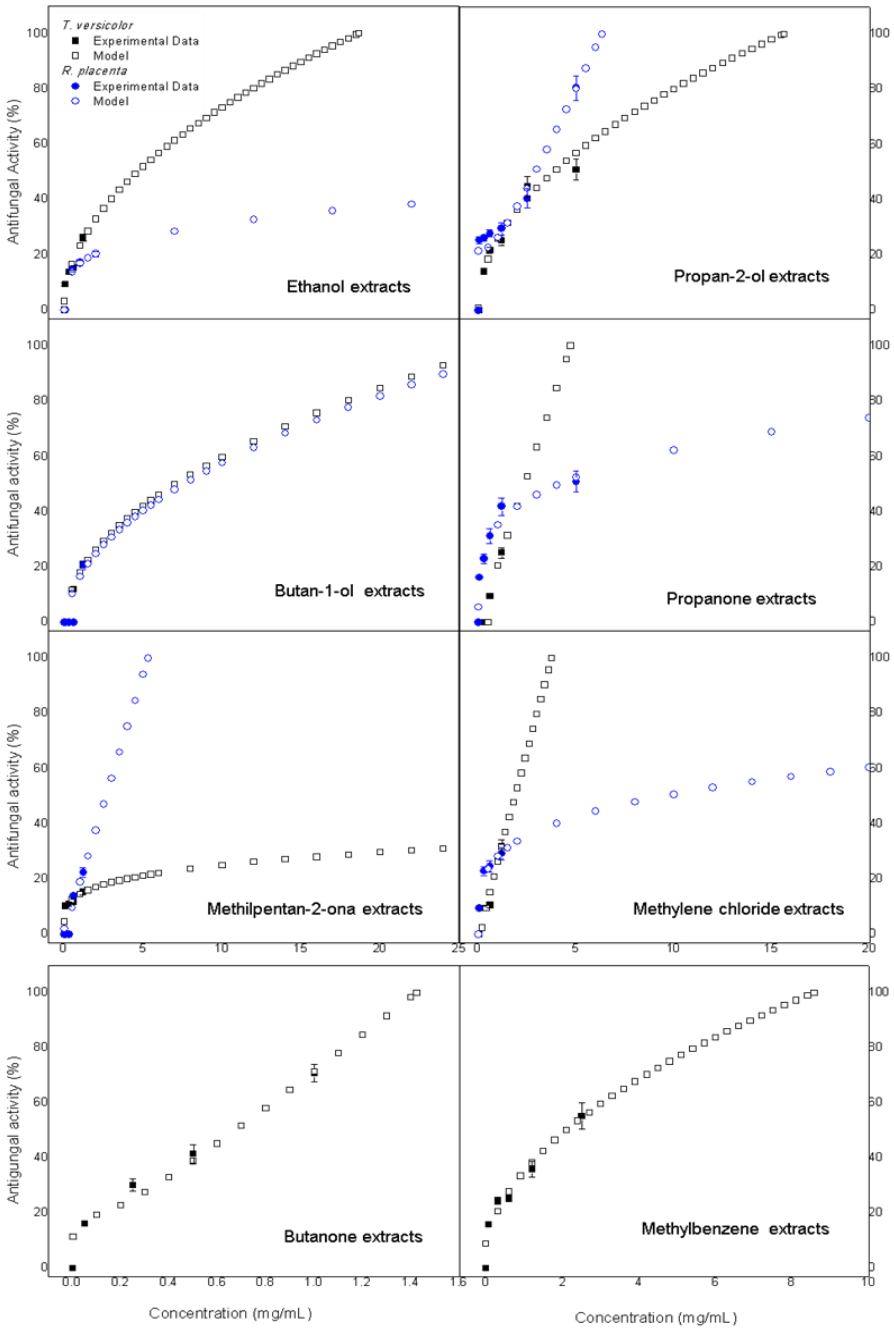


Figure 4. Biological model by simple regression for tested extracts.

The extracts with the lowest MICs, for the fungus *T. versicolor*, were those extracted from butanone, dichloromethane, and propanone. While for *R. placenta* they were 3-methilpentan-2-one, propan-2-ol, and butan-1-ol.

4. Discussions

The yield of the extraction depends on the temperature, the type of solvent, and whether the extraction is sequential when performed with various solvents. Therefore, the yields obtained in this work are similar amounts reported by other authors. Flores-Villegas et al. (2019) reported extraction yields of 9.7%, 5.7%, and 7.1%, in methanol extraction of the root, stem, and leaf of *D. stramonium*, respectively [50]. Rai et al. (2013), reported that the seeds of *Datura metel* plant contained fat (14.72%), carbohydrate (51.22%), protein (20.73%), moisture (4.63%), ash content (5.14%), total sugar (5.63%), reducing sugar (2.65%), crude fiber (17.35%), and trace elements (mg/100 gm): calcium 174.0, phosphorus 690.0, potassium 0.50, sodium 0.085, iron 16.8, zinc 2.63, copper 6.9, and magnesium 390.0 [51].

Wood as a renewable natural resource has played an important role in the global economy, especially in the construction industry. Under conditions favorable to the growth of wood-degrading organisms, the most commonly used species of wood may deteriorate. Non-durable wood products can be protected from attack by xylophagous fungi, insects, bacteria, and marine borers by treating them with natural biocidal extracts.

The biocidal properties of secondary plant metabolites are of great interest in many fields, such as pharmacology, the food industry, and wood preservatives. It is a growing trend that compounds that have natural antifungal activity are used to replace traditional preservatives due to their side effects. The test fungi are fungi recommended by the standards for the determination of the natural durability of wood, and in recent years they have been widely studied due to their great capacity to degrade wood [52–58].

The "Petri dish" method was used, which is a non-standardized biological method, but widely recommended and used as a simple and relatively fast quantitative procedure to measure the toxic effect of extractives on rot fungi, consisting of adding solutions of *D. stramonium* extracts to the cultivation medium. Bravo and Lomelí (1992) reported that ethanol extracts of Barcino and Nogal wood showed inhibitory effects against the fungi *Lentinus lepideus* Fr., causing dark or brown rot and *Laetiporus sulphureus* Murr., causing white rot [59]. Beltrán et al. (1997) reported a promoter effect of ethanolic extracts of corn against the fungi *Pleorotus ostreatus*, *Lentinus sajor-caju*, *Lentinula edodes*, *Agaricus bisporus*, and *Agaricus campestris* [60]. Torres et al. (2004), evaluated the toxic effect against fungi of ethanolic extracts of corncob and blue agave leaves on the fungus *T. versicolor*. They observed that the agave extracts, at concentrations lower than 1.0 g/L, presented greater AFA than the corncob extracts, but at the 1.0 g/L concentration, AFA was similar for both extracts [61]. Shagal et al. (2012) reported that the aqueous and ethanolic extracts of *D. stramonium* stem bark contained alkaloids, saponins, tannins, steroids, flavonoids, phenols, and glycosides [41]. Perez-Najera et al. (2013), reported that glycoside flavonoids and aglycones, which are more polar, are extracted with alcohols or alcohol–water mixtures [62]. Altameme et al. (2015), through GC-MS analysis of alkaloid leaves ethanolic extract of *D. stramonium*, revealed the existence of ethyl iso-allocholate, D-asycarpidan-1-methanol, acetate (ester), 3-(1,5-dimethyl-hexyl)3a,10,10,12btetramethyl1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b-tetradec-ahydro-benzo [4,5] cyclohept,2,7-diphenyl-1,6-dioxopyridazino [4,5:2,3] pyrrolo [4,5-d] pyridazine, 3,8,8-trime thoxy-3-piperidyl-2,2-benaphthalene-1,1,4,4-tetrone, [5β] pregnane3, 20β-diol,14α,18α-[4-methyl,3-oxo-[1-oxa-4-azabutane-1,4-diyl], diacetate, 1-monolinoleoylglycerol trimethylsi-lyl ether, and 17-[1,5-dimethylhexyl]-10,13-dimethyl3sstyrylhexadecahydrocyclopenta[a] phenathren-2-one. Alkaloids extracted from leaves of *D. stramonium* were assayed for in vitro antibacterial activity against *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* by using the diffusion method in agar [63]. Carpa et al. (2017) reported the presence of atropine and scopolamine in ethanol extracts of *D. stramonium.* The plant extracts were tested on Gram-negative bacteria *Escherichia coli* and on Gram-positive bacteria *S. aureus*. Both tested strains showed resistance but for *E. coli*

a higher inhibition was observed in all samples containing *Datura* extract [64]. Girmay 2015 reported that with propanone, substances such as flavonoids, cholesterol, tannins, glycosides, alkaloids, phenols, samonins, proteins, carbohydrates, and terpenoids can be extracted [65]. Bravo and Lomelí (1992) reported that propanone extracts of Barsino and Walnut wood showed good results in the inhibition of the fungi *L. lepideus* Fr. and *Laetiporus sulphureus* Murr [59]. With dichloromethane as a solvent for extraction, isoflavones, flavanones, flavones, and methylated flavonols can be extracted [62]. Obomanu et al. (2018) reported that using dichloromethane as a solvent, alkaloids, flavonoids, saponins, and tannins can be extracted from *D. stramonium* flowers. These substances may be responsible for the inhibitory function of these extracts [66]. Paraxylene failed to extract components of *D. stramonium* that can inhibit or promote the growth of the test fungi. The values reported for both fungi were 0% AFA at all concentrations tested. Beltrán et al. (1997) reported that toluene extracts from corn stover contain inhibitory substances for the growth of the fungi *P. ostreatus*, *L. sajor-caju*, *L. edodes*, *A. bisporus*, and *A. campestris* [60].

For the inhibition of *T. versicolor*, the extracts of *D. stramonium* that presented the greatest antifungal potential were those of butanone, propan-2-ol, and methylbenzene. For the inhibition of *R. placenta*, the extracts that presented the highest antifungal potency were those of propan-2-ol and propanone. These are the extracts that could be economically profitable for exploitation as biocides.

The minimum inhibitory concentration (MIC) has been defined by the Clinical and Laboratory Standards Institute (CLSI) as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism [67]. The MIC is generally considered to be the most widely adopted laboratory measure of the relative in vitro activity of an antimicrobial agent against an organism: a lower MIC value indicates that a smaller amount of the preservative is required to inhibit the growth of an organism [68]. Traditionally, MIC values are determined by agar dilution and broth dilution. Yen et al. (2008) reported MIC 25 and 30 µg/mL for β-γ-thujaplicin and thujaplicin compounds extracted with ethanol heartwood from *Calocedrus macrolepis* var. Formosa, to inhibit *T. versicolor* [69]. Theapparat et al. (2015) reported MICs against *T. versicolor* of 12.5 mg/mL, 12.0 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 6.25 mg/mL from pyrolean acids of *Leucaena leucocephala*, *Azadirachta indica*, *Eucalyptus camaldulensis*, *Dentrocalamus asper*, and *Hevea brasilensis*, respectively [70]. Lomeli et al. (2016) reported MICs against *T. versicolor* of 1.584 mg/mL, 1.723 mg/mL, 1.243 mg/mL, and 1.861 mg/mL from *P. strobus*, *P. douglaciana*, *P. caribaea*, and *P. leophylla*, respectively [48].

The half maximal inhibitory concentration (MIC_{50}) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. MIC_{50} is a quantitative measure that indicates how much of a particular inhibitory substance (e.g., drug) is needed to inhibit, in vitro, a given biological process or biological component by 50%. Mansor and Ali (1992) reported MIC_{50} values of 0.640 mg/mL of pyrolytic rubberwood tar oil against *Coriolus versicolor* [71]. In another study, Jantan et al. (1994) showed that *Capsicum pubescens* leaf oil had the most effective MIC_{50} among seven *Cinnamomum* species at 0.060 mg/mL for *C. versicolor* [72]. Cheng et al. (2005) reported MCI_{50} values of 91 mg/mL, 139 mg/mL, and greater than 500 mg/mL for heartwood, sapwood, and bark of *Cryptomeria japonica*, respectively, showing that bark extracts have the lowest AFA [73]. Wang et al. (2011) reported that extracts with hexane, ethyl acetate, and methanol, from the heartwood of *Cunninghamia lanceolata*, require 0.47, 0.64, and 0.84 g/L, respectively, to inhibit by 50% the growth of the fungus *T. versicolor* [74]. Lomelí et al. (2016) reported that, to inhibit 50% growth of the fungus *T. versicolor*, the necessary concentrations were 0.071 mg/mL, 0069 mg/mL, 0.279 mg/mL, and 0.096 mg/mL for bark extracts of *Pinus strobus*, *Pinus douglasiana*, *Pinus caribaea*, and *Pinus leiophyla*, respectively [48].

The effect of extracts with butanone, methylene chloride, propane, and methylbenzene against the fungus *T. versicolor* and extracts with 3-methylpentan-2-one and propan-2-ol against the fungus *R. placenta* show that low amounts are required to achieve total inhibition. Therefore, in the future, it will be possible to proceed with impregnation tests

on wood susceptible to degradation by these fungi and really test its protection capacity on wooden blocks.

5. Conclusions

The highest extraction yields were obtained with water and ethanol (6.01% and 3.04%, respectively). While for the other solvents yields varied from 1.01% to 1.30%.

The compounds extracted with water showed similar results for the two xylophagous test fungi. They showed to be growth promoters, favoring the mycelial development of the fungi *T. versicolor* and *R. placenta*.

Ethanol extracts at the concentrations tested showed low toxicity to both fungi. The extract obtained with propan-2-ol generally inhibited the two test fungi. It was observed that the higher the concentration, the greater the inhibition, being more effective the inhibition of *R. placenta*. In the concentration of 5.0 mg/mL, the antifungal activity was 80.6%, being classified as toxic. The extract obtained with butan-1-ol, at lower concentrations of 0.30 mg/mL and 0.60 mg/mL for *T. versicolor* and *R. placenta*, respectively, showed no effect. These were classified as non-toxic (NT). The extract with propanone generally inhibited both fungi. The AFA of the extracts proved to be more aggressive for *R. placenta*. The extract with butanone showed to be the best inhibitor of *T. versicolor* growth, while for *R. placenta* it showed a promoter effect. The 3-methyl-2, pentanone extract, at the concentrations evaluated, showed low toxicity to both fungi. The biological activity of the dichloromethane extracts showed that the higher the concentration, the greater the degree of inhibition against both fungi. The xylene solvent was not able to extract components of *D. stramonium* that inhibited or promoted the growth of the xylophagous fungi. The compounds extracted with toluene inhibited *T. versicolor*, with an AFA that increased with the concentration. In the case of *R. placenta*, toluene extracts promoted growth.

The biological models of the extracts that showed an inhibitory effect on mycelial growth showed a good fit to the experimental data.

The extracts with the lowest MICs for the fungus *T. versicolor* were butanone, dichloromethane, and propanone, while for *R. placenta* they were 3-methylpentan-2-one, propan-2-ol, and butan-1-ol.

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