 doi:10.20944/preprints202011.0167.v1

Article

Establishment of a Cell Suspension Culture of *Eysenhardtia platycarpa*: Phytochemical Screening of extracts and Evaluation of Antifungal Activity.

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Abstract: *Eysenhardtia platycarpa* (Fabaceae) is a medicinal plant used in México and it lacks biotechnological studies for its use. The aim of this work was to establish a cell suspension cultures (CSC) of *E. platycarpa*, determine the phytochemical profile, and evaluate its antifungal activity. Friable callus and CSC were established with 2 mg/L 1-naphthaleneacetic acid plus 0.1 mg/L kinetin. The highest total phenolics of CSC was 15.6 mg GAE/g dry weight and the total flavonoids content ranged from 56.2 to 104.1 µg QE/g dry weight. CG–MS analysis showed that the dichloromethane extracts of CSC, sapwood and heartwood have a high amount of hexadecanoic acid (22.3 – 35.3 %) and steroids (13.5 – 14.7%). Heartwood and sapwood defatted hexane extracts have the highest amount of stigmasterol (≈ 23.4%) and *β*-sitosterol (≈ 43%), and leaf extracts presented *β*-amyrin (16.3%). Methanolic leaves extracts showed mostly sugars and some polyols, mainly D-pinitol (74.3%). Dichloromethane and fatty hexane extracts of CSC exhibited the percentages inhibition higher for *Sclerotium cepivorum* with 71.5 and 62.0%, respectively. The maximum inhibition for *Rhizoctonia solani* was with fatty hexane extracts of the sapwood (51.4%). Our study suggest that CSC extracts could be used as a possible complementary alternative to synthetic fungicides.

Keywords: plant *in vitro* culture; plant extracts; gas chromatography; hexadecanoic acid, antifungal activity

1. Introduction

For centuries, plants have been an important source of natural products for humans, and they have been used as flavorings, condiments, treating health disorders and preventing diseases, including epidemics [1]. Products plants has formed the basis for many of useful pharmaceuticals and agrichemicals, and through their rational use, plants can be a potential alternative for obtaining extracts or bioactive compounds to control several diseases in both human and agricultural [2,3]. However, many of these extracts or compounds are isolated from wild plants, but its collection generally has a negative impact on the environment [4]. Furthermore, the lack of agricultural soils is increasingly limited and, in the future, the production of plants in the field will not be feasible. Although some plants can be farmed, many of them need several years to be harvested, in addition, the yields of bioactive compounds and biological activity of farmed or wild plants are low when compared with cultured plant cell [5,6]. Plant cell culture is a biotechnological tool that has the potential to accelerate the production of natural products in a controlled environment, in addition,

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cell culture provides a renewable source of natural products, since plant cell culture can be produced and harvested at all times of the year [3,4]. In fact, some plants extract or compounds such as ajmalicine, anthraquinones, berberine, caffeic acid, ginsenoside, nicotine, rosmarinic acid, shikonin have been produced using the plant cell, tissues, and organ culture [5].

In this regard, *Eysenhardtia platycarpa* (Fabaceae), is a plant with a wide variety of uses such as manufacture of utensils, furniture, as well in traditional Mexican medicine [7,8,9]. Among the few existing investigations for *E. platycarpa*, all of them have used the wild plant as a source of pharmacological studies [10]. For instance, flavonoids with cytotoxic and antibacterial activity were isolated from methanolic extracts of branches and leaves of *E. platycarpa* [11]. The *in vivo* anti-inflammatory activity of flavones isolated from the leaves has also been reported [12]. However, no effort has been made to carry out studies aimed at sustainable use of this species. In a biotechnological study developed for *Eysenhardtia polystachya* (a closely related species), the antifungal activity of cell suspension culture extracts was reported against *R. solani* and *S. cepivorum* [13]. To our knowledge, however, there are no reports using biotechnological studies of *E. platycarpa*. Therefore, it is necessary to look for biotechnological techniques that allow to obtain bioactive extracts while preserving natural diversity and the environment.

The main aim of this study was to establish a cell suspension culture from *E. platycarpa* internodal segments and characterize their growth. We also characterized the CSC and intact plant extracts by gas chromatography-mass spectrometry. Further, the biological activity of the extracts against the phytopathogenic fungi *Rhizoctonia solani* and *Sclorotium cepivorum* was explored.

2. Results and Discussion

2.1. Plantlets Obtainment and Callus Induction

In vitro culture of *Eysenhardtia platycarpa* are shown in Figure 1. Seeds had 98% germination under *in vitro* conditions after 10 days of culture. The *in vitro* plants grew easily without plant growth regulators after being transferred to 1 L jars. (Figure 1a). In another Fabaceae species such as *Prosopis laevigata*, the *in vitro* germination can occur from 3 to 7 days in mechanically scarified seeds [14,15].

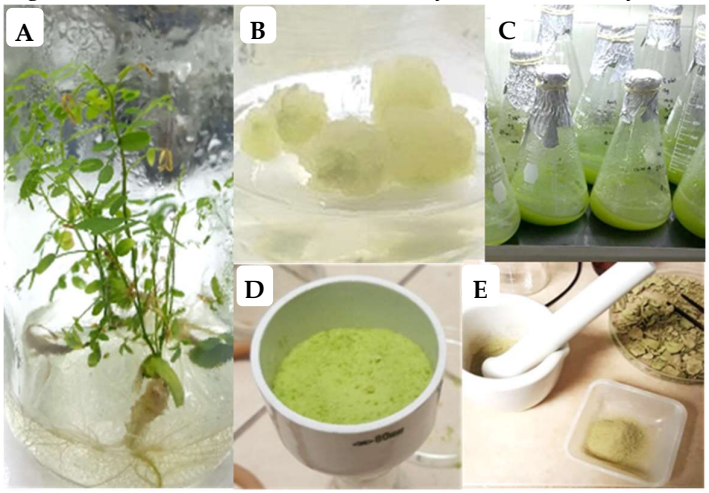

Figure 1. *In vitro* cultures of *Eysenhardtia platycarpa*. (**a**) Plantlets grown in MS culture medium without plant growth regulators; (**b**) Callus production at 15 days of culture; (**c**) Cell suspension cultures with 2 mg/L NAA and 0.5 mg/L KIN; (**d**) Fresh biomass harvested at 14 days of culture; (**e**) Dried biomass used for obtaining extracts.

Regarding callus induction, a suitable response of callus formation was exhibited on internodal segments at 15 days of culture (Figure 1b). All treatments had percentages of callus induction greater than 50.0%, regardless of the type of auxins NAA and 2,4-D or cytokinin KIN (Table 1).

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Table 1. Percentage of internodal explants of *Eysenhardtia platycarpa* inducing callus in MS culture medium at 30 days of culture

PGRs (mg/L)		Callus induction (%)	PGRs (mg/L)		Callus induction (%)
2,4-D	KIN		NAA	KIN	
0.0	0.0	50.0 ± 0.0 b	0.0	0.0	75.0 ± 8.8 ab
0.0	0.1	66.7 ± 7.2 ab	0.0	0.1	58.3 ± 19.1 b
0.0	1.0	93.8 ± 6.3 a	0.0	1.0	100.0 ± 0.0 a
0.0	2.0	75.0 ± 10.2 ab	0.0	2.0	100.0 ± 0.0 a
0.1	0.0	81.3 ± 12.0 ab	0.1	0.0	75.0 ± 10.2 ab
0.1	0.1	81.3 ± 12.0 ab	0.1	0.1	93.8 ± 6.3 a
0.1	1.0	93.8 ± 6.3 a	0.1	1.0	93.8 ± 6.3 a
0.1	2.0	87.5 ± 12.5 ab	0.1	2.0	100.0 ± 0.0 a
1.0	0.0	75.0 ± 10.2 ab	1.0	0.0	93.8 ± 6.3 a
1.0	0.1	100.0 ± 0.0 a	1.0	0.1	100.0 ± 0.0 a
1.0	1.0	83.3 ± 14.4 ab	1.0	1.0	100.0 ± 0.0 a
1.0	2.0	100.0 ± 0.0 a	1.0	2.0	100.0 ± 0.0 a
2.0	0.0	83.3 ± 14.4 ab	2.0	0.0	100.0 ± 0.0 a
2.0	0.1	100.0 ± 0.0 a	2.0	0.1	100.0 ± 0.0 a
2.0	1.0	91.7 ± 7.2 a	2.0	1.0	100.0 ± 0.0 a
2.0	2.0	100.0 ± 0.0 a	2.0	2.0	100.0 ± 6.3 a

PGRs: Plant growth regulators; 2,4-D: 2,4–dichlorophenoxyacetic acid; NAA: naphthaleneacetic acid; KIN: kinetin. Values represent mean ± standard error of 4 replicates per treatment in two repeated experiments. Means followed by the same letter in column are not significantly different (p = 0.05) using Tukey's multiple range test.

The control (PGRs-free) showed callus formation in 50% of segments, but it exhibited scarce growth, showed a brown color, and later died. It is possible that *E. platycarpa* contains amounts of auxin in their leaves and stems, which, may have caused that even in the control treatment the segments formed callus. In fact, it has been reported that indoleacetic acid (IAA) occurs naturally in plants, mainly in young leaves or seeds [16,17]. Moreover, it is known that levels of naturally occurring auxin in explant tissues depend on the mother plant from which the explants were taken [18].

In internodal segments of *E. platycarpa*, all evaluated treatments showed a positive effect on callus formation; 62.0% of the treatments with NAA and KIN formed callus on 100% of the segments, mainly with NAA (1.0 or 2.0 mg/L), regardless KIN concentration. Callus of this treatment were clear greenish and more friable in appearance than the other treatments; moreover, callus had a homogeneous growth during all subcultures. The other treatments exhibited smaller and semi-compact callus, and most of them did not show growth. In the case of treatments with 2,4-D plus KIN, it was observed that as the concentration of 2,4-D increased, there was a trend in increasing the percentage of callus induction and this occurred generally in presence of KIN (Table 1). Similarly, treatments with 2,4-D (1.0 or 2.0 mg/L) combined with KIN (0.1 or 2.0 mg/L) had 100% of explants with callus. The remaining treatments, including control, showed calluses with percentages between 50.0 and 94.0%. Calluses of the best treatments with both auxins, i.e., NAA (2.0 mg/L) with KIN (0.1 mg/L) or 2,4-D (2.0 mg/L) with KIN (0.1 mg/L) were subcultured periodically for 6 months to increase callus production.

In a study reported for *E. polystachya* was found that callus induction was variable, according to the PGRs, i.e., percentages of callus between 65.6 and 98.4% in leaf explants were obtained with picloram (PIC) plus KIN, and from 64.1 to 100% with NAA plus KIN [13]. Studies carried out in *P. laevigata*, callus from cotyledons, hypocotyls and roots explant were obtained with 2,4-D plus 6-

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benzylaminopurine (BAP) or KIN, in percentages of 28.0 to 100% [15]. This indicates that combining both, auxins and cytokinins play an indispensable role in inducing and increasing the percentage of calluses [19].

2.2. Cell Suspension Cultures

2.2.1. Growth Kinetics and Sucrose Consumption

Callus induced with NAA (2.0 mg L) and KIN (0.1 mg/L) or 2,4-D (2.0 mg L) and KIN (0.1 mg L) were the best treatments showing friable characteristics and they were used to initiate the establishment of the cell suspension cultures (CSC) of *E. platycarpa* with the same plant growth regulators; however, during one month of culture, cells cultured in MS liquid medium with 2,4-D (2.0 mg/L) and KIN (0.1 mg/L) had poor growth; therefore, this treatment was discarded from the experiment. In contrast, cell cultured with NAA (2.0 mg/L) and KIN (0.1 mg L) exhibited growth and an abundant accumulation of biomass was observed from day 10 of growth kinetics (Figures 1c–e,2).

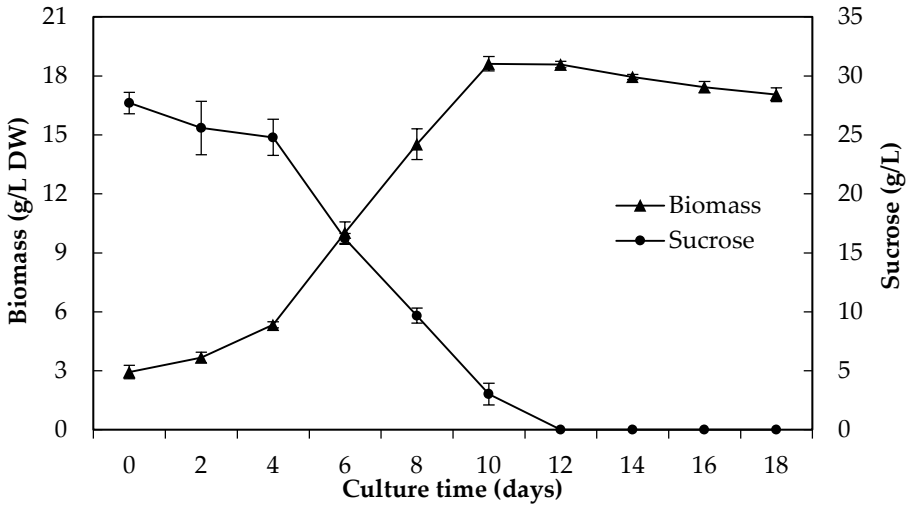

Figure 2. Growth kinetics and consumption of sucrose of a cell suspension culture of *Eysenhardtia platycarpa* for 18 days of culture. Error bars represent means ± standard error of three replicates measurements.

Another species such as *P. laevigata*, the treatment with 2,4-D (1.5 mg/L) and KIN (1.0 mg/L) was the most suitable for establishing the CSC [15]. It is known that response to callus induction treatments on semisolid culture medium, are not always the ones that best adapt to the liquid culture medium. For instance, *P. laevigata* leaf explants presented 100% of callus with trichlorophenoxyacetic acid (2,4,5-T, 1.28 mg/L) with BAP (1.13 mg/L) or 2,4,5-T (1.28 mg/L) with KIN (1.08 mg/L); however, only callus containing KIN were the most suitable for establishing the CSC [14]. This indicates that even among closely related species, the genotype is an influential factor in the response of cell cultures; moreover, the PGRs activity varies depending on the presence of transporter or receptor biosynthesized proteins in the explants, affecting *in vitro* culture development [20].

The growth kinetics of *E. platycarpa* was maintained for 18 days, during which it exhibited typical growth (Figure 2). The lag phase lasted 4 days, the exponential phase was 6 days. The stationary phase was observed between 10 and 12 days; then, the senescence phase was gradually observed. In addition, the maximum accumulation of biomass dry weight (18.62 g/L DW) occurred at 10 days. The specific growth rate (μ) was 0.206, the doubling time (td) was 3.36 days, and the growth rate was 5.35, obtaining a yield of 0.621 g dry biomass/g sucrose.

In another study conducted in *E. polystachya*, the maximum accumulation of dry biomass was 14 g/L at 10 days of culture [13]. Biomass yield was lower than that reported in the present study for *E platycarpa* at the same time. Studies conducted by Maldonado-Magaña et al. [15] in *P. laevigata* also found a dry biomass yield of 15.6 g/L at 21 days of culture. During the adaptation phase of *E.*

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platycarpa, the amount of sugars remained unchanged, however, in the exponential growth phase there was a greater demand for sugars and, consequently, a higher production of biomass. From day 12, the sugars in the culture medium almost completely consumed and coincided with the stationary phase and the senescence phase. This behavior is also reported in other species such as *C. brasiliense* cell suspension cultures [21].

2.2.2. Total Phenolics and Flavonoids Content

It has been reported that phenolics (TPH), flavonoids (TFL) and other compounds of plant extracts are effective against phytopathogenic fungi [22,23]; therefore, it is important to quantify these groups of compounds in the extracts. The TPH and TFL content from *E. platycarpa* CSC had low variation during culture growth (Figure 3).

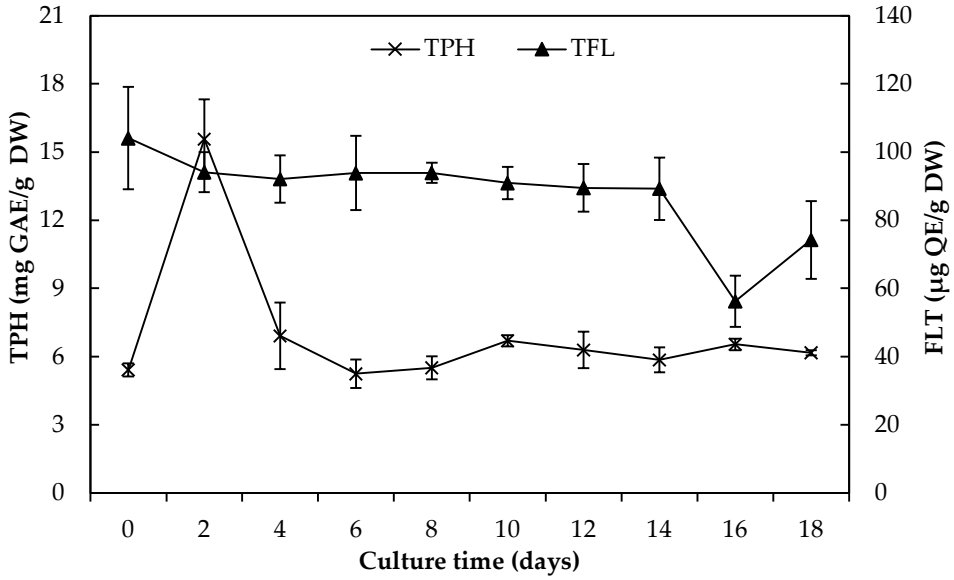

Figure 3. Production of total phenolics (TPH) and total flavonoids content (TFL) of a cell suspension culture of *Eysenhardtia platycarpa* for 18 days of culture. Error bars represent means ± standard error of three replicates measurements.

During the adaptation phase of the culture, there was a notable increase in TPH (15.6 mg GAE/g DW) and then it remained constant, showing amounts between 5.2±0.63 and 6.2 mg GAE/g DW. Similar studies reported by Giri et al. [24] found a concentration of 10.17 mg GAE/g DW at 28 days of culture of *Habenaria edgeworthii*. In this work, the TPH content of *E. platycarpa* leaves (11.77 mg GAE/g DW), was lower than cell suspension cultures on day 2; however, during this stage biomass production is low.

Regarding the TFL content, this also remained constant between day 2 and 14, except the day zero was 104.1 µg QE/g DW. Near to the end of the senescence phase (day 16), the TFL decreased at 56.2 µg QE/g DW (Figure 3). On the other hand, low concentrations of TFL were also found in wild plant leaves (88.2 µg QE/g DW). Studies conducted in *Saussurea medusa* (Maxim) cell suspension cultures the total flavonoid production was 607.8 mg/L after 15 days of culture [25]. In other species such as *Clinacanthus nutans*, large amount of total polyphenols, phenolic acids and flavonoids contents were found on in vitro cultures compared with a conventional cultures [6].

2.3. Yield of Extracts of Cell Suspension Culture and Intact Plant

In general, the methanolic and dichloromethane extracts exhibited the highest dry weight yields (Table 2). This coincides with that reported in related species such as *E. polystachya*, in which the highest yields were obtained with methanol and dichloromethane [26]. In *Tectona grandis* yields of 2.9, 2.3 and 3.6% were reported, through a sequential extraction with hexane, ethyl acetate and

 doi:10.20944/preprints202011.0167.v1

methanol, respectively [27]. The methanolic extracts made of the heartwood of other Fabaceae (*Caesalpinia platyloba* and *Lysiloma latisquum*), also report the best yields of extracts using these solvents [28]. For *E. platycarpa*, the yield of the methanolic extract of the heartwood was 36.9% and 30.08% for leaf, being the highest yields (Table 2). In another study conducted for *C. sappan* L., methanolic extracts of heartwood and leaf showed higher yields with 17.60 and 17.05%, respectively [29].

Table 2. Yield of different solvent extraction of intact plant and cell suspension culture of *Eysenhardtia platycarpa*.

Extract	**Yield of extract (%)**			
	Sapwood	**Heartwood**	**Leaf**	**Cell suspension culture**
Fatty hexane	4.0	6.7	8.0	0.1
Defatted hexane	5.0	8.6	10.1	0.5
Dichloromethane	15.0	22.8	20.0	2.4
Methanolic	21.1	36.9	30.8	23.8
Total	45.1	75.0	68.9	26.8

2.4. Compounds Identified by GC–MS

Fabaceae family produce a high diversity of bioactive compounds as defense against bacterial and fungi [30]. In the literature, few studies have been reported on determination of intact plant compounds of *E. platycarpa*; however, there are no studies on the phytochemical profile of its cell culture extracts.

By using the gas chromatography–mass spectrometry (GC–MS) analysis, the phytochemical profile of the hexane, dichloromethane and methanolic extracts of the cell suspension cultures, sapwood, heartwood and leaves of the intact plant of *E. platycarpa* were determined.

Chromatograms with main compounds identified by GC–MS are in Figures S1–S16 and their mass spectra are in Figures S17–S29.

 doi:10.20944/preprints202011.0167.v1

1 **Table 3.** Chemical constituents of the hexane extracts of cell suspension culture (CSC) and plant of *Eysenhardtia platycarpa* identified by GC–MS.

Compound name	Chemical formula	RT (min)	Abundance (%)							
			Fatty hexane extract				Defatted hexane extract			
			Sapwood	Heartwood	Leaf	CSC	Sapwood	Heartwood	Leaf	CSC
Alkanes										
Heptacosane	$C_{27}H_{56}$	38.38	—	—	2.48	—	—	—	—	—
Nonacosane	$C_{29}H_{60}$	42.98	—	—	6.37	—	—	—	—	—
Octacosane	$C_{28}H_{58}$	47.26	—	—	1.32	—	—	—	—	—
Aromatic compounds										
Isophthalic acid	$C_8H_6O_4$	13.90	1.13	—	—	—	—	—	—	—
Terephthalic acid	$C_8H_6O_4$	14.72	1.13	—	—	—	—	—	—	—
Dibutyl phthalate	$C_{16}H_{22}O_4$	19.14	—	5.92	0.65	3.93	2.47	3.90	1.02	2.57
1,2-dihydroxyanthraquinone	$C_{14}H_8O_4$	31.87	—	—	—	—	—	—	0.56	—
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	34.69	—	—	—	—	—	—	—	0.78
4,5-dihydroxyanthraquinone-2-carboxylic acid	$C_{15}H_8O_6$	41.37	—	—	0.43	—	—	—	—	—
Derivatives from saturated alkanoic acids										
2-hydroxyheptanoic acid	$C_7H_{14}O_3$	6.41	1.59	1.36	—	—	—	—	—	—
3-phenylpropanoic acid	$C_9H_{10}O_2$	7.12	—	—	4.32	—	—	—	6.05	—
3-hydroxyoctanoic acid	$C_8H_{16}O_3$	7.98	0.74	0.81	—	—	—	—	—	—
3-(4-Methoxyphenyl) propionic acid	$C_{10}H_{12}O_3$	11.49	—	—	2.27	—	—	—	2.39	—
2,3-dihydroxypropyl palmitate	$C_{19}H_{38}O_4$	36.21	—	—	—	0.57	—	—	—	0.54
Derivatives from unsaturated alkanoic acids										
3-phenylprop-2-enoic acid	$C_9H_8O_2$	9.20	—	—	0.54	—	—	—	0.72	—
Saturated alkanoic acids										
Tetradecanoic acid	$C_{14}H_{28}O_2$	16.07	—	—	1.05	0.39	0.20	—	1.51	0.42
Pentadecanoic acid	$C_{15}H_{30}O_2$	18.74	—	—	—	0.41	—	—	—	0.35
Hexadecanoic acid	$C_{16}H_{32}O_2$	21.49	14.64	10.16	11.40	62.84	6.20	3.39	19.03	62.96
Heptadecanoic acid	$C_{17}H_{34}O_2$	24.22	—	—	—	0.86	0.32	0.16	—	0.80
Octadecanoic acid	$C_{18}H_{36}O_2$	26.93	1.96	1.54	2.87	6.09	3.08	0.71	5.07	6.87
Eicosanoic acid	$C_{20}H_{40}O_2$	32.17	—	—	0.55	1.14	—	0.15	0.90	1.00

Table 3. *Cont.*

Compound name	Chemical formula	RT (min)	Abundance (%)							
			Fatty hexane extract				Defatted hexane extract			
			Sapwood	Heartwood	Leaf	CSC	Sapwood	Heartwood	Leaf	CSC
Saturated alkanoic acids										
Docosanoic acid	$C_{22}H_{44}O_2$	37.08	—	—	1.19	0.84	—	—	1.48	0.60
Tetracosanoic acid	$C_{24}H_{48}O_2$	41.71	—	—	5.07	2.80	—	—	3.92	2.12
Saturated diacids										
Hexanedioic acid	$C_6H_{10}O_4$	8.49	0.89	2.16	—	—	—	0.16	—	—
Heptanedioic acid	$C_7H_{12}O_4$	10.33	1.52	—	—	—	—	—	—	—
Octanedioic acid	$C_8H_{14}O_4$	12.44	5.16	2.65	—	0.90	0.18	0.19	—	0.86
Nonanedioic acid	$C_9H_{16}O_4$	14.87	26.89	15.32	1.04	4.20	0.91	1.04	0.95	4.04
Steroids										
Campesterol	$C_{28}H_{48}O$	50.45	—	—	—	—	8.71	9.00	—	—
Stigmasterol	$C_{29}H_{48}O$	51.14	—	1.30	—	0.49	23.43	23.42	1.57	2.64
β-Sitosterol	$C_{29}H_{50}O$	52.32	—	—	—	—	43.74	43.33	—	1.88
Stigmastanol	$C_{29}H_{52}$	52.43	—	—	—	—	1.38	1.74	—	1.44
Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	53.27	17.24	18.79	—	—	—	—	—	—
Sitostenone	$C_{29}H_{48}O$	54.05	—	—	—	—	—	0.60	—	—
Triterpenoids										
β-amyrin	$C_{30}H_{50}O$	52.16	—	—	7.46	—	—	—	16.65	—
Unsaturated alkanoic acids										
9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	25.99	—	—	0.66	—	—	—	2.42	—
9,12,15-octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	26.17	—	—	1.48	—	—	—	6.77	—
9-octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	26.20	—	—	—	—	0.24	0.26	—	—
Others										
6,10,14-trimethylpentadecan-2-one	$C_{18}H_{36}O$	15.91	—	—	0.51	—	—	—	0.64	—
3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	25.11	—	—	—	—	—	—	2.78	—
Octacosanol	$C_{28}H_{58}O$	48.27	—	—	22.67	—	—	—	3.94	—

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4 **Table 4.** Chemical constituents of the dichloromethane and methanolic extracts of cell suspension culture (CSC) and plant of *Eysenhardtia platycarpa* identified by GC–MS

Compound name	Chemical formula	RT (min)	Abundance (%)							
			Dichloromethane extract				Methanolic extract			
			Sapwood	Heartwood	Leaf	CSC	Sapwood	Heartwood	Leaf	CSC
Aromatic compounds										
Vanillin	$C_8H_8O_3$	9.09	0.31	—	—	—	—	—	—	—
Terephthalic acid	$C_8H_6O_4$	14.72	0.30	—	—	—	—	—	—	—
Dibutyl phthalate	$C_{16}H_{22}O_4$	19.14	2.47	2.86	—	0.56	—	—	—	—
1,2-dihydroxyanthraquinone	$C_{14}H_8O_4$	31.87	—	1.56	—	0.41	—	—	—	—
Derivatives from saturated alkanoic acids										
3-phenylpropanoic acid	$C_9H_{10}O_2$	7.12	—	—	1.02	—	—	—	—	—
3-(4-methoxyphenyl) propionic acid	$C_{10}H_{12}O_3$	11.49	—	—	0.65	—	—	—	—	—
2,3-dihydroxypropyl palmitate	$C_{19}H_{38}O_4$	36.21	1.19	0.71	—	0.97	—	—	—	—
Mono and disaccharides										
Ketohexoses	$C_6H_{12}O_6$	*	—	—	—	—	10.05	3.20	3.62	27.75
Aldohexoses	$C_6H_{12}O_6$	*	—	—	—	—	6.52	3.66	2.84	2.79
Furanoses	$C_6H_{12}O_6$	*	—	—	—	—	4.73	—	0.97	—
Disaccharides (glucose with fructose)	$C_{12}H_{22}O_{11}$	*	—	—	—	—	27.93	3.63	1.83	40.98
Polyols										
L-threitol	$C_4H_{10}O_4$	8.77	—	—	—	—	—	—	—	0.42
Xylitol	$C_5H_{12}O_5$	13.52	—	—	—	—	0.40	4.34	—	2.13
D-pinitol	$C_7H_{14}O_6$	16.38	—	—	—	—	36.85	15.89	74.28	—
Ribitol	$C_5H_{12}O_5$	18.11	—	—	—	—	—	—	—	9.23
Sorbitol	$C_6H_{14}O_6$	19.21	—	—	—	—	—	2.13	—	1.84
Inositol	$C_6H_{12}O_6$	*	—	—	—	—	7.04	7.15	6.45	5.21
Saturated diacids										
Hexanedioic acid	$C_6H_{10}O_4$	8.49	—	0.49	—	—	—	—	—	—
Octanedioic acid	$C_8H_{14}O_4$	12.44	—	0.81	—	—	—	—	—	—
Nonanedioic acid	$C_9H_{16}O_4$	14.87	1.87	5.05	0.51	0.63	0.50	1.52	—	—

5 * Several stereoisomers were found at different retention times.

Table 4. *Cont.*

Compound name	Chemical formula	RT (min)	Abundance (%)							
			Dichloromethane extract				Methanolic extract			
			Sapwood	Heartwood	Leaf	CSC	Sapwood	Heartwood	Leaf	CSC
Saturated alkanoic acids										
Tetradecanoic acid	$C_{14}H_{28}O_2$	16.07	—	—	0.36	—	—	—	—	—
Hexadecanoic acid	$C_{16}H_{32}O_2$	21.49	27.24	22.22	5.20	35.33	—	—	2.05	2.06
Heptadecanoic acid	$C_{17}H_{34}O_2$	24.22	0.860	0.85	—	0.36	—	—	—	—
Octadecanoic acid	$C_{18}H_{36}O_2$	26.93	3.22	3.18	1.44	2.59	—	—	1.88	1.18
Eicosanoic acid	$C_{20}H_{40}O_2$	32.17	0.55	—	—	0.43	—	—	—	—
Docosanoic acid	$C_{22}H_{44}O_2$	37.08	0.55	—	—	0.30	—	—	—	—
Tetracosanoic acid	$C_{24}H_{48}O_2$	41.71	1.18	0.90	0.96	1.27	—	—	—	—
Sesquiterpenoids										
β-selinene	$C_{15}H_{24}$	8.31	—	—	0.46	—	—	—	—	—
γ-muurolene	$C_{15}H_{24}$	8.74	—	—	0.32	—	—	—	—	—
β-cadinene	$C_{15}H_{24}$	11.20	—	—	0.77	—	—	—	—	—
11-hydroxy-4βH,5α-eremophil-1(10)-ene	$C_{15}H_{26}O$	13.42	—	—	0.60	—	—	—	—	—
Trans, trans-farnesol	$C_{15}H_{26}O$	15.07	—	—	0.49	—	—	—	—	—
Steroids										
Campesterol	$C_{28}H_{48}O$	50.45	1.69	1.69	—	0.74	—	—	—	—
Stigmasterol	$C_{29}H_{48}O$	51.14	4.35	4.08	—	6.75	—	—	—	—
β-Sitosterol	$C_{29}H_{50}O$	52.32	8.63	7.89	—	6.04	—	—	—	—
Unsaturated alkanoic acids										
9,12-octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	25.99	3.95	5.17	0.72	13.75	—	—	—	—
9,12,15-octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	26.17	—	—	2.29	3.83	—	—	—	—
9-octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	26.20	1.59	2.46	—	0.83	—	—	—	—
Others										
Malic acid	$C_4H_6O_5$	8.30	—	—	—	—	1.94	—	—	—
L-threonic acid	$C_4H_8O_5$	9.79	—	—	—	—	—	—	0.42	—
Galactaric acid	$C_6H_{10}O_8$	20.16	—	—	—	—	—	1.64	—	—
3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	25.11	—	—	1.06	—	—	—	—	—

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All hexane extracts of sapwood, heartwood, leaf, and CSC were composed mostly of saturated alkanoic acids (Saa), saturated diacids (Sd) and steroids (Ste), while only the leaves had triterpenes (Tri) (Figure 4a,b). The fatty and defatted hexane extracts of CCS stood out for producing saturated alkanoic acids (75%), mainly hexadecanoic acid (63%) (Table 3, Figures S4,S8).

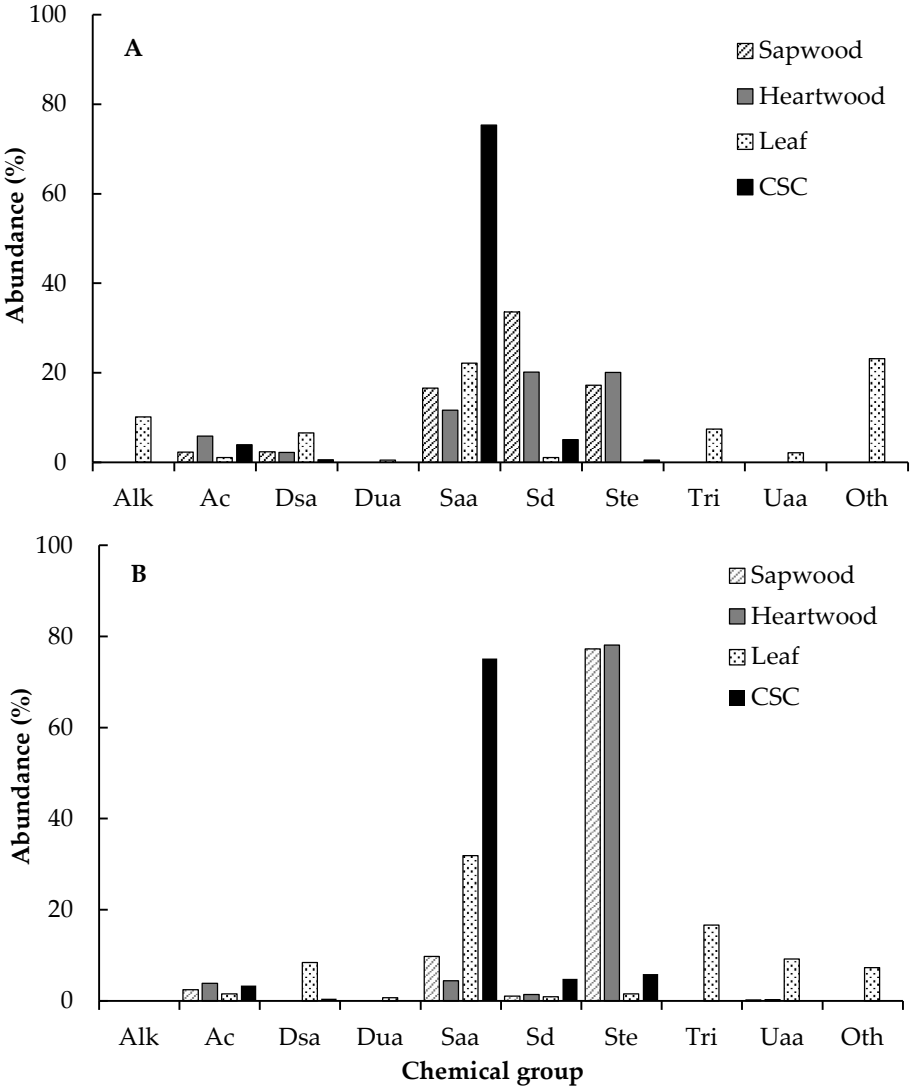

Figure 4. Chemical group summarized from cell suspension culture and intact plant of *E. platycarpa* extracts. (**a**) Fatty hexane extract; (**b**) Defatted hexane extract. Alk: alkanes; Ac: aromatic compounds; Dsa: derivatives from saturated alkanoic acids; Dua: derivatives from unsaturated alkanoic acids; Saa: saturated alkanoic acids; Sd: saturated diacids; Ste: steroids; Tri: triterpenoids; Uaa: unsaturated alkanoic acids; Oth: others.

The largest amount of saturated diacids (nonanedioic acid) were found in the fatty hexane extract of sapwood (26.9%) and heartwood (15.3%) (Figure S1,S2). On the other hand, high amounts of steroids were found in defatted hexane extracts of sapwood (77.2%) and heartwood (78.1%), mainly composed by β-sitosterol (≈ 43%) and stigmasterol (≈ 23%) (Table 3, Figure S5,S6). Stigmasta-3,5-dien-7-one compound was also found in the fatty hexane extracts of sapwood and heartwood in amounts of 17.2 and 18.8%, respectively. Only the fatty and defatted hexane extracts from the leaves contain β-amyrin in amounts of 7.46% and 16.65%, respectively (Table3, Figure S3,S7).

In general, dichloromethane extracts of *E. platycarpa* were dominated mostly by saturated alkanoic acids and steroids, and unsaturated alkanoic acids (Uaa), and lesser amounts of aromatic compounds (Ac), saturated diacids (Sd) and sesquiterpenoids (Ses) (Figure 5a).

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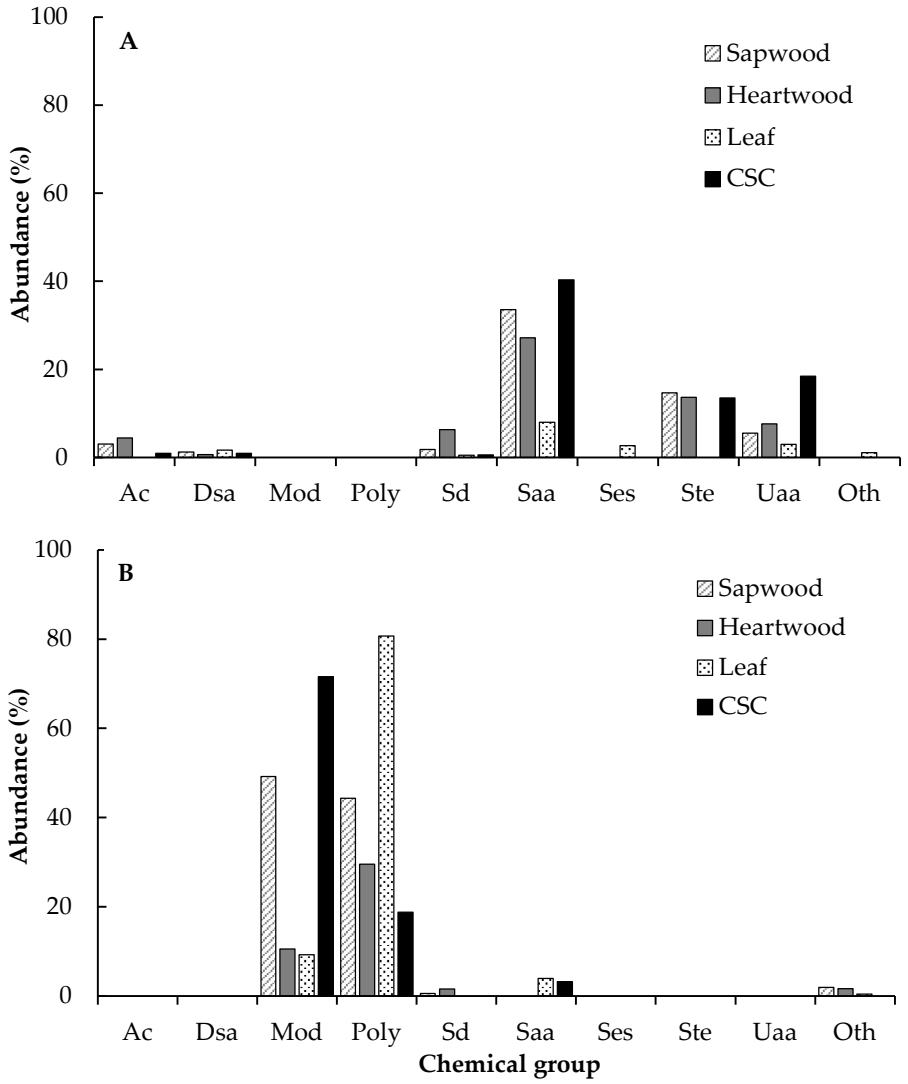

Figure 5. Chemical group summarized from cell suspension culture and intact plant of *E. platycarpa* extracts. (**a**) Dichloromethane extract; (**b**) Methanolic extract. Ac: aromatic compounds; Dsa: derivatives from saturated alkanoic acids; Mod: mono and disaccharides; Poly: polyols; Sd: saturated diacids; Saa: saturated alkanoic acids; Ses: sesquiterpenoids; Ste: steroids; Uaa: unsaturated alkanoic acids; Oth: others.

Dichloromethane extract of CSC showed a greater amount of saturated alkanoic acids (35.3% of hexadecanoic acid) and unsaturated alkanoic acids (13.8% of 9,12-octadecadienoic acid (Z,Z)) (Table 4, Figure S12). The dichloromethane extracts of sapwood, heartwood and CSC had similar amounts of steroids in ranges from 13.5 to 14.7%. Particularly, only the leaves exhibited sesquiterpenoids (*β*-selinene, *γ*-muurolene, *β*-cadinene, 11-hydroxy-4*β*H,5*α*-eremophil-1(10)-ene and trans, trans-farnesol) in amounts <1%. In contrast, all methanolic extracts exhibited polyol-type compounds (Figure 5b), mainly D-pinitol in the leaves extract with 74.3% (Table 4, Figure S13). The rest of the compounds were mostly mono and disaccharides, mainly in the CSC extracts.

The nonanedioic acid, *β*-sitosterol, stigmasterol, stigmasta-3,5-dien-7-one, *β*-amyrin, 9,12-octadecadienoic acid (Z,Z), D-pinitol, hexadecanoic acid and other compounds have been reported for *Eysenhardtia* genus and some Fabaceae species [10,11,31]; however, in this work, the phytochemical screening by GC-MS analysis of plants and cell suspension cultures extracts from *E. platycarpa* is reported for the first time.

 doi:10.20944/preprints202011.0167.v1

2.5. Antifungal Activity

As an assay of biological activity, we evaluate the antifungal potential of the extracts on phytopathogenic fungi available in our laboratory (*Rhizoctonia solani* and *Sclerotium cepivorum*), which have the broad host range and cause significant losses on crop quantity and quality of many crop species. In a previous study, we reported the antifungal activity of extracts of *Eysenhardtia polystachya* [13], a species close to *E. platycarpa*. In the current work, it was found that the antifungal activity of *E. platycarpa* extracts were statistically significant ($p = 0.05$) on the mycelial growth of *R. solani* and *S. cepivorum* (Figure 6,7).

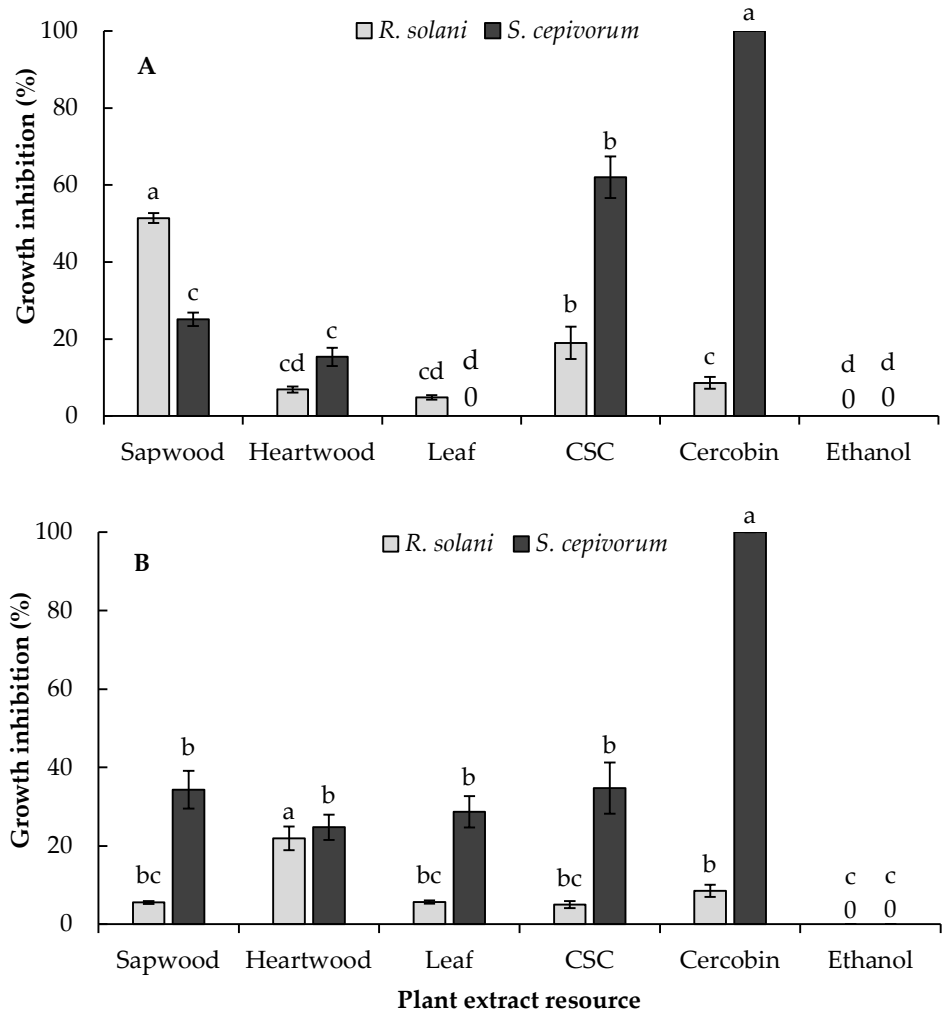

Figure 6. Effect of different source extracts of *Eysenhardtia platycarpa* on inhibition of mycelial growth of *Rhizoctonia solani* and *Sclerotium cepivorum* at 72 h of culture. (**a**) Fatty hexane extract: (**b**) Defatted hexane extract. CSC: cell suspension culture. Error bars represent means ± standard error of three replicates measurements. Bars followed by the same letter between the same fungus are not significantly different ($p = 0.05$) using Tukey's multiple range test.

Of all the 16 extracts, only the fatty hexane and dichloromethane extracts exhibited a better inhibition of both fungi species. The fatty hexanic extract of sapwood had the maximum inhibition of mycelial growth for *R. solani* (51.4%), while the fatty hexane extract of cell suspension culture (CSC) showed 62.0% inhibition for *S. cepivorum* (Figure 6a). The other fatty hexane extracts had values lower than 25.0% inhibition. In the case of defatted hexane extracts, the inhibition percentages for *R. solani* were low (less than 22%), while the values for *S. cepivorum* ranged from 22 to 35% (Figure 6b). The Cercobin fungicide did not affect the growth of *R. solani* but was efficient in inhibiting the growth of

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S. cepivorum. In a previous study, we reported the antifungal activity of *E. polystachya* extracts, in which, the defatted hexane extract of CSC showed 66.0% inhibition for *R. solani* and it was also higher than the Cercobin, while the fatty hexane extracts had low inhibition [13].

Regarding the dichloromethane extracts, only the cell suspension cultures extract showed moderate inhibition on *R. solani* (36.0%) compared with Cercobin (9.0%) (Figure 7a).

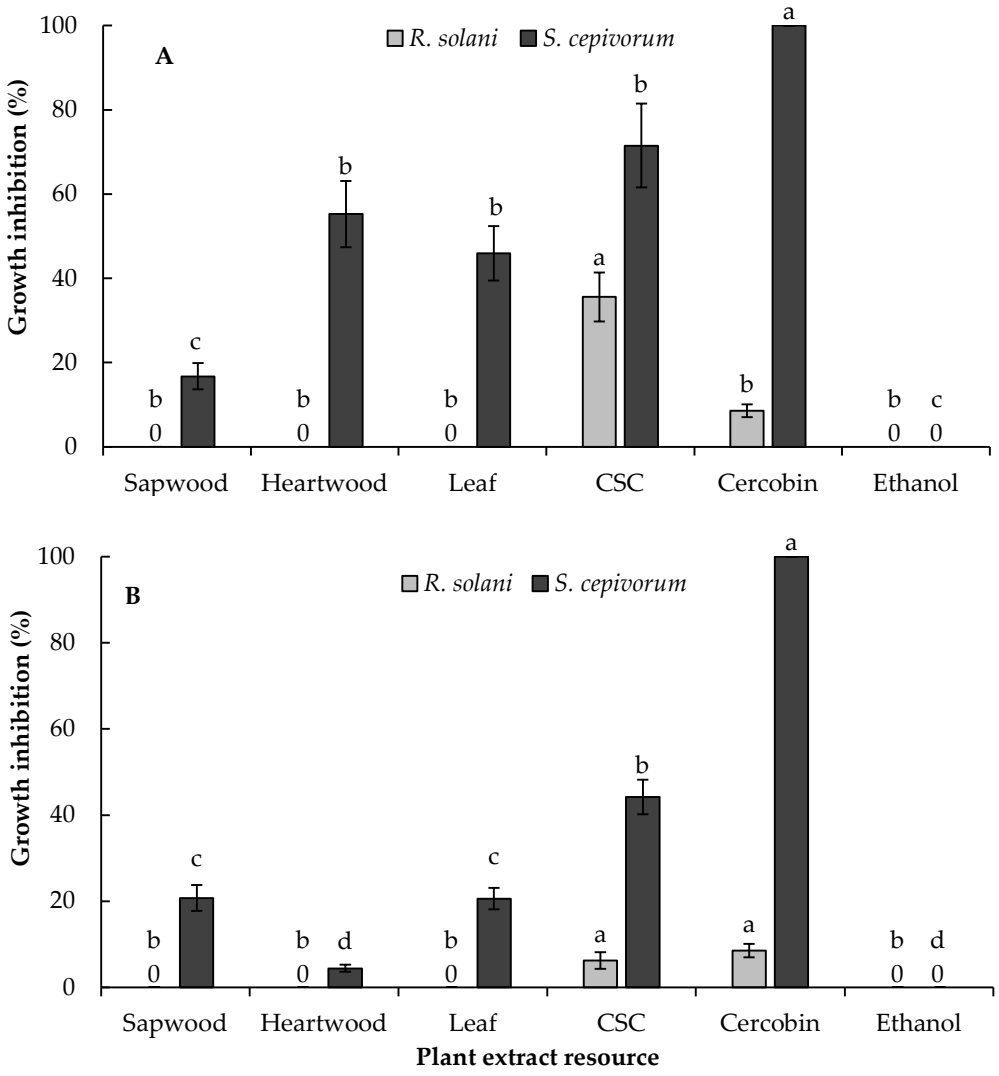

Figure 7. Effect of different source extracts of *Eysenhardtia platycarpa* on inhibition of mycelial growth of *Rhizoctonia solani* and *Sclerotium cepivorum* at 72 h of culture (**a**) Dichloromethane extract; (**b**) Methanolic extract. CSC: cell suspension culture. Error bars represent means ± standard error of three replicates measurements. Bars followed by the same letter between the same fungus are not significantly different ($p = 0.05$) using Tukey's multiple range test.

In contrast, the dichloromethane extract from cell suspension culture showed the most effective inhibition against *S. cepivorum* (71.5%), followed by heartwood (55.2%) and leaves extracts (45.9%) (Figure 7a). In a study carried out for *E. polystachya* we also report that dichloromethane extracts of sapwood and heartwood inhibited the growth of *S. cepivorum* by 73.0 and 80.0%, respectively [13].

On the other hand, the methanolic extracts from *E. platycarpa* had low inhibition compared with Cercobin. Despite, CSC extracts showed the maximum percentage of inhibition for *S. cepivorum* with 44.2% (Figure 7b).

Many plants have been reported to inhibit the *in vitro* growth of phytopathogenic fungi, which promise to be better than commercial fungicides [32,33]. It is possible that the growth inhibition of *R.*

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solani and *S. cepivorum* with the fatty hexane extract of the sapwood and CSC (Figure 6a) is due to a synergism between saturated fatty acids, saturated diacids and steroids, since these are in greater amount in this extract (Figure 4a, Table 3). In fact, the defatted hexanic extracts also contain saturated fatty acids and high amount of steroids, but scarce amount of saturated diacids compared with fatty hexane extract. Therefore, there was also a decrease in the inhibition of fungal growth (Figure 6b).

Several free fatty acids (lauric acid, myristic acid, palmitic acid, oleic acid, and linoleic acid) are known to have an inhibitory effect on fungal germination, mycelial growth, and sporulation [34,35]. The possible mechanisms of antifungal activity have been previously studied and focused on fungal membrane disruption causing an increase in membrane fluidity, causing leakage of the intracellular components and cell death [36] or interfering fungal sphingolipid biosynthesis [37]. In addition, they may influence inhibition of protein and enzyme synthesis related to fatty acid metabolism [38]. On the other hand, the synergism of the aromatic compounds, along with the high amounts of phytosterols (stigmasterol and β-sitosterol), hexadecanoic acid and unsaturated fatty acids ([9,12-octadecadienoic acid (Z, Z)) found in CSC dichloromethane extract of *E. platycarpa* (Table 4) may have increased the inhibition for *S. cepivorum*. On the other hand, the CSC dichloromethane extract was the only one that significantly inhibited the growth for *R. solani*, perhaps due to its high amount of saturated and unsaturated alkanoic acids and steroids compared to the other dichloromethane extracts from intact plant (Figures 5a,7a).

The hexadecanoic acid (palmitic acid) from many plant species has been reported against phytopathogenic fungi, such as *Aspergillus niger*, *Botrytis cinerea*, *Colletotrichum lagenarium*, *Emericella nidulans*, *Fusarium oxysporum* and *Alternaria solani* [34,38,39]. Phytosterols, e.g., stigmasterol and sitosterol have also been reported to be effective against phytopathogenic fungi [40,41]. A mixture of stigmasterol and β-sitosterol isolated from the pericarp of *Areca catechu* markedly inhibited spore germination, mycelial growth, and germ-tube elongation of *Colletotrichum gloeosporioides* [42]. Studies reported in other species of the Fabaceae family (*Tephrosia apollinea*, *Dahlstedt glaziovii*, and *Deguelia duckeana*), have shown that dichloromethane extracts can inhibit fungal growth because they contain high amounts of flavonoids and prenylated phenolic compounds [22,43,44]. Therefore, some plant extracts may be a source of antifungal compounds since they have had to develop compounds to resist infections by fungi present in their environment [45].

3. Materials and Methods

3.1. Collection of Plant Material

The leaves, seeds and the trunk from *Eysenhardtia platycarpa* wild plants were collected in November of 2015, in San Luciano, Jocotepec, State of Jalisco, Mexico, located at 20° 19' 10.23'' north latitude and 103° 24' 12'' west longitude at an elevation of 1950 m.a.s.l. A sample of the plant was used for identification, registered, and deposited in the Luz María Villarreal de Puga Herbarium, Instituto de Botanica, Universidad de Guadalajara (IBUG) with the voucher number 28112017.

3.2. Plantlets Obtainment and Incubation Condition

All *in vitro* culture experiments were conducted at the Laboratorio de Cultivo In vitro de Plantas of the Departamento de Madera, Celulosa y Papel, of the Universidad de Guadalajara, México. First, uncoated seeds of *E. platycarpa* were washed with a soap solution for 10 min, followed by disinfection with 70% (v/v) ethanol for 30 s. Then, the seeds were disinfected with 1.2% (v/v) sodium hypochlorite solution (Cloralex®) for 15 min along with 4 drops of Tween 20® per 100 mL of disinfectant solution. After disinfection, the seeds were rinsed three times with sterile distilled water in a horizontal laminar flow cabinet (CFLH-90E, Novatech).

The disinfected seeds were sown in MS culture medium [46]) supplemented with 3% sucrose (w/v) (Sigma-Aldrich, St. Louis, MO, USA) and only half of the macronutrients were used. Culture medium was adjusted to pH 5.8 and then gelled with 2 g/L of Phytagel® (Sigma-Aldrich). The culture medium was transferred to Gerber flasks of 100 mL capacity and were sterilized in a manual

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autoclave (CV300-A, AESA) at 121 °C, 15 psi, for 18 min. Four disinfected seeds were placed in jars type Gerber containing 25 mL of MS medium.

All cultures were incubated at 25 ± 2 °C under 16-h photoperiod of white fluorescent light with a light intensity of 60 μmol/m²/s. Eight days after germination, the plantlets were transferred to flask of 1 L volume capacity, containing 80 mL of MS culture medium. Plantlets were used for subsequently experiments of callus induction.

3.3. Establishment of Callus Cultures

To induce callus, 30–day–old plantlets grown in *in vitro* conditions were used as a source of explants. Internodal segments of approximately 1 cm in length were sown in Gerber flasks containing 25 mL of semisolid MS culture medium, and plant growth regulators (PGRs). The PGRs consisted of two auxins, 2,4-dichlorophenoxyacetic acid (2,4-D) or naphthaleneacetic acid (NAA), each combined with kinetin (KIN) as a cytokinin; all PGRs were used at 0.0, 0.1, 1.0 and 2.0 mg/L. Each treatment consisted of four flasks with four explants per flask (n = 16) and the experiment was repeated twice. Cultures were maintained under the same incubation conditions as in seed germination and subcultured every 4 weeks with fresh culture medium.

3.4. Cell Suspension Cultures

3.4.1. Growth Kinetics

The callus that visually showed better friable and growth characteristics were used to initiate the establishment of the cell suspension cultures (CSC) using MS liquid medium with the same PGRs that induced callus. Erlenmeyer flasks of 125 mL containing 25 mL of culture medium were inoculated with 3 g of fresh callus (FW). Cultures were incubated in an orbital shaker (PRENDO AGO-6040) at 110 rpm under 16-h photoperiod of white fluorescent light with a light intensity of 60 μmol/m²/s and 25 ± 2 °C. After the established of the culture, cells were subcultured every two weeks for six months in Erlenmeyer flasks of 500 mL to increase the biomass. Kinetic growth was carried out in Erlenmeyer flask of 125 mL containing 25 mL of MS liquid medium and 1.5 g of cells. The biomass contained in three flasks were harvested every two days, filtered, and washed with distilled water to remove excess of the culture medium. The biomass was dried in an oven at 50 °C until constant weight. The experiment was repeated twice, and the biomass dry weight (DW) data were used to plot the growth curve.

3.4.2. Sucrose Consumption Determination

In addition, the culture medium filtered from the CSC of the growth kinetics were used to determine the total sugars. For each sampling, three flasks were harvested, an aliquot of 5 mL was taken for each flask (n = 3) and were stored in freezing until analysis by the phenol-sulfuric method [47]. Aliquot of 250 μL culture medium was diluted in distilled water (1:400); then, 500 μL aliquot was taken and 500 μL of phenol (5%) was added; subsequently, 2.5 mL of concentrated sulfuric acid were added. The sample was vigorously mixed for 3 s and allowed to react at room temperature for 30 min. Samples were read in a spectrophotometer at 490 nm using distilled water as a blank. To carry out the calibration curve, sucrose was used as a standard at concentrations of 1–40 g/L.

3.4.3. Determination of the Total Phenolics and Flavonoids Content

Biomass samples of CSC from growth kinetics and wild plant leaves were used to determine total phenolics and flavonoids content. Samples of 50 mg of CSC or leaves were refluxed using 20 mL methanol (in water bath) at 65 °C for 20 min and 3 extraction cycles were done for each sample. The extracts of the same samples were mixed, filtered, brought to 20 mL, transferred to amber flasks, and stored in freezing until analysis.

Total phenolics content (TPH) were quantified by Folin-Ciocalteu (FC) method [48]. An aliquot of 500 μL methanolic extract was mixed with 125 μL of FC and then 125 μL Na_2CO_3 (20% w/v) was

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added. The mixture was supplemented with distillated water up to 2 mL total volume. The reaction was maintained at room temperature for 60 min under dark conditions. TPH were calculated based on the calibration curve of gallic acid at concentrations of 0 to 50 mg/L. Samples of methanolic extracts from leaves and CSC were analyzed in a Varian Cary® 50 UV-Vis spectrophotometer at 765 nm. The results were expressed in terms of gallic acid equivalents (GAE) in mg/g of dry biomass (DW).

Total flavonoids content (TFL) were quantified with the aluminum chloride colorimetric method [49]. An aliquot of methanolic extracts (240 µL) was mixed with 1.50 mL of distilled water, then 90 µL $NaNO_2$ (5%) was added and allowed to react for 6 min under dark conditions. After the reaction, 180 µL $AlCl_3$ (10%) was added to the mixture and stirred vigorously. After 5 min, 600 µL NaOH (1 M) was added and brought to 3 mL final volume with distilled water. Samples were analyzed in a Varian Cary® 50 UV-Vis spectrophotometer at 510 nm. The calibration curve was performed with quercetin as standard using concentrations of 100 to 1600 µg/mL. The results were expressed in terms of quercetin equivalents (QE) in µg/g of dry biomass (DW).

3.5. Phytochemical Analysis of Cell Suspension Cultures and Intact Plant

3.5.1. Extraction and Samples Preparation

The biomass of the 14-day suspension cell cultures and the intact plant (sapwood, heartwood and leaves,) were ground to a fine powder and dried in an oven at 50 °C. Separately, the dried samples of sapwood (300 g), heartwood (269 g), leaves (238 g) and CSC (9.91 g) were successively extracted (4 each) by maceration at room temperature with hexane, dichloromethane and methanol for 72 h. After extraction of each solvent, the samples were dried before adding the next solvent. The extracts were filtered and concentrated using a rotavapor BÜCHI EL-131 (BÜCHI Labortechnik AG, Switzerland) and dried in an oven at 40 °C to remove traces of the solvent. Each hexane extract was extracted with methanol to obtaining two extracts (a defatted hexane extract and a fatty hexane extract, for each sample). A total of 16 dry extracts were obtained and 2 mg of extracts were dissolved in 100 µL of pyridine and derivatized by adding 100 µL of bis(trimethylsilyl)trifluoroacetamide (BSTFA). The reaction mixture was heated at 50 °C and stirred for 30 min on a heating plate.

2.5.2. Analysis of Extracts by Gas Chromatography-Mass Spectrometry (GC–MS)

The derivatized extracts (1 µL) were immediately injected into an Agilent 6890 instrument coupled to an Agilent-Technologies 5973N Network Mass Selective Detector (GC–MS) using electron impact as the ion source at 70 eV in a mass range of 20-600 DA. The capillary column was a HP-5MS (30 m × 0.25 mm, 0.25 µm film thickness: Agilent-Technologies, Little Falls, CA, USA). The oven temperature was set at 100 °C (1 min), rate of 10 °C/min up to 150 °C, rate of 3 °C/min up to 300 °C (4 min). Helium was used as the carrier gas with a flow rate of 1.0 mL/min and the injector temperature was set at 250 °C. Compounds were identified as TMS derivatives by comparing their mass spectra with the NIST library version 1.7a and by comparing their fragmentation patterns with published data [50,51]). For semiquantitative analysis, peaks were integrated using a GC ChemStation software version C.00.01. The composition was reported as a percentage of the total peak area.

3.6. In Vitro Antifungal Evaluation of Extracts

The phytopathogenic fungi *R. solani* and *S. cepivorum* were provided from Colección del Laboratorio de Patología, Departamento de Producción Agrícola (Universidad de Guadalajara). Seven-day-old strains grown on potato dextrose agar (PDA, BD Bioxon) culture medium was used. The antifungal evaluation was carried out according to the gar disk-diffusion method [52]. The 16 extracts were dissolved in 96% ethanol (1 mg/mL) and the 5 mm diameter filter paper discs (Whatman No. 1) superposed on PDA culture medium were impregnated with 10 µL of each solution. Cercobin® and ethanol were used as positive and negative controls, respectively. After applying solutions extracts or controls on the discs, the solvent was allowed to evaporate for 1 h in a laminar flow cabinet. Mycelium propagules (5 mm^3) were inoculated on discs treated into sterile polystyrene

Petri dishes (Interlux® 90 x 15 mm) containing 10 mL of PDA medium and then incubated at 28 ± 2°C. The results are reported as percentage inhibition (%) of mycelial growth at 72 h of cultures.

3.7. Statistical Analysis

The data corresponding to the percentage of callus induction, dry biomass of cell suspension cultures, total phenolics and flavonoid content, and the inhibition percentage of the mycelial growth were subjected to a normality test and then an analysis of variance (ANOVA) followed by Tukey's multiple range test ($p = 0.05$). SAS 9.0 software (SAS Institute Inc.) was used for the statistical analysis. All the experiments were conducted in triplicate.

4. Conclusions

The obtaining of a biotechnological culture of *E. platycarpa*, the phytochemical profile of cell cultures and intact plant, and their antifungal activity are reported for the first time. The NAA auxin was the most suitable to establish callus and CSC, producing 18.62 g dry biomass/L. Hexane extracts exhibited high amounts of saturated fatty acids, mainly hexadecanoic acid in CSC. Although the methanolic extracts had the best yields, they did not show the best antifungal activity. Dichloromethane extract of CSC showed greater effectiveness to inhibit the *in vitro* growth for *S. cepivorum* (62.0%). The fatty hexane extract of the sapwood had better inhibition of *R. solani* (51%), but in the dichloromethane extracts, CCS was the only one that inhibited *R. solani*. In this paper we demonstrated that CSC extracts could be used as an alternative to synthetic fungicides to control phytopathogenic fungi as *R. solani* and *S. cepivorum*, however, further studies are needed.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: GC-MS chromatogram of the fatty hexane extract of sapwood, Figure S2: GC-MS chromatogram of the fatty hexane extract of heartwood, Figure S3: GC-MS chromatogram of the fatty hexane extract of leaf, Figure S4: GC-MS chromatogram of the fatty hexane extract of cell suspension cultures, Figure S5: GC-MS chromatogram of the defatted hexane extract of sapwood, Figure S6: GC-MS chromatogram of the defatted hexane extract of heartwood, Figure S7: GC-MS chromatogram of the defatted hexane extract of leaf, Figure S8: GC-MS chromatogram of the defatted hexane extract of cell suspension culture, Figure S9: GC-MS chromatogram of the dichloromethane sapwood extract, Figure 10: GC-MS chromatogram of the dichloromethane heartwood extract, Figure S11: GC-MS chromatogram of the dichloromethane leaf extract, Figure S12: GC-MS chromatogram of the dichloromethane cell suspension culture extract, Figure S13: GC-MS chromatogram of the methanolic sapwood extract, Figure S14: GC-MS chromatogram of the methanolic heartwood extract, Figure S15: GC-MS chromatogram of the methanolic leaf extract, Figure S16: GC-MS chromatogram of the methanolic cell suspension cultures extract, Figure S17: Mass spectrum and structure of nonanedioic acid (azelaic acid), Figure S18: Mass spectrum and structure of hexadecanoic acid, Figure S19: Mass spectrum and structure of stigmasta-3,5-dien-7-one, Figure S20: Mass spectrum and structure of octacosanol, Figure S21: Mass spectrum and structure of β-amyrin, Figure S22: Mass spectrum and structure of octadecanoic acid, Figure S23: Mass spectrum and structure of campesterol, Figure S24: Mass spectrum and structure of stigmasterol, Figure S25: Mass spectrum and structure of β-sitosterol, Figure S26: Mass spectrum and structure of 3-phenylpropanoic acid (Benzenepropanoic acid), Figure S27: Mass spectrum and structure of 9,12,15-octadecatrienoic acid, (Z,Z,Z)-(α-Linolenic acid), Figure S28: Mass spectrum and structure of 9,12-octadecadienoic acid (Z,Z), Figure S29: Mass spectrum of D-pinitol.

Author Contributions: A.B.-A. and F.C.-S. conceived, designed and supervised the experiments; A.S.-S., J.M.-C. and A.B.-A. performed the experiments and wrote a draft manuscript; A.R.-E., I.H.-D and L.A. analyzed the data (including GC-MS) and discussed of results; A.R.-E., J.A.S.-G and F.J.F.-T edited and revised manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: This study was partially supported by the Consejo Nacional de Ciencia y Tecnolgía (CONACYT) during the master's degree of Alejandro Sánchez Sánchez, in the Maestría en Ciencia de Productos Forestales (PNPC-CONACYT) of the Universidad de Guadalajara. Also, we thank the Laboratorio Nacional de Estructura de Macromoléculas for the GC-MS analyses.

Conflicts of Interest: The authors declare no conflict of interest.

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