Cell Suspension of Eysenhardtia platycarpa

Subjects: Plant Sciences
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Eysenhardtia platycarpa (Fabaceae) is a medicinal plant used in Mexico. Biotechnological studies of its use are lacking. The objective of this work was to establish a cell suspension culture (CSC) of E. platycarpa, determine the phytochemical constituents by spectrophotometric and gas chromatography–mass spectrometry (GC–MS) methods, evaluate its antifungal activity, and compare them with the intact plant. 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 gallic acid equivalents (GAE)/g dry weight and the total flavonoids content ranged from 56.2 to 104.1 µg quercetin equivalents (QE)/g dry weight. The GC–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 leaf extracts showed mostly sugars and some polyols, mainly D-pinitol (74.3%). Compared with the intact plant, dichloromethane and fatty hexane extracts of CSC exhibited percentages of inhibition higher for Sclerotium cepivorum: 71.5% and 62.0%, respectively. The maximum inhibition for Rhizoctonia solani was with fatty hexane extracts of the sapwood (51.4%). Our study suggests 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; they have been used as flavorings and condiments and for treating health disorders and preventing diseases, including epidemics [1]. Plant products have formed the basis for many useful pharmaceuticals and agrochemicals, and through their rational use, plants can be a potential alternative for obtaining extracts or bioactive compounds to control several diseases in both humans and crops [2][3]. However, many of these extracts or compounds are isolated from wild plants, whose collection generally has a negative impact on the environment [4]. Furthermore, agricultural soil is increasingly limited and, in the future, the production of plants in the field will not be feasible. Although plants can be cultivated in the field, many of them need several years to be harvested, and in many cases, the yields of bioactive compounds and biological activity of cultivated or wild plants are lower compared with cultured plant cells [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, 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 this regard, *Eysenhardtia platycarpa* (*Fabaceae*) is a wild plant extensively exploited as firewood, fodder, or to manufacture utensils and furniture such as “equipales” and fences; in addition, in traditional Mexican medicine, an infusion prepared from the wood is used against kidney and gallbladder diseases [7][8][9]. All these uses, as well as forest fires, are causing a decline in wild populations. Among the few existing investigations on *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 the 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 involving biotechnological studies of *E. platycarpa*. Therefore, it is necessary to look for biotechnological techniques that allow us to obtain bioactive extracts while preserving natural diversity and the environment.

2. Obtaining Plantlets and Callus Induction

In vitro cultures of *Eysenhardtia platycarpa* are shown in Figure 1. The seed germination rate was 98% at 10 days of culture and no microbial contamination was observed. The in vitro plants grew easily without plant growth regulators after being transferred to 1-L jars (Figure 1A).

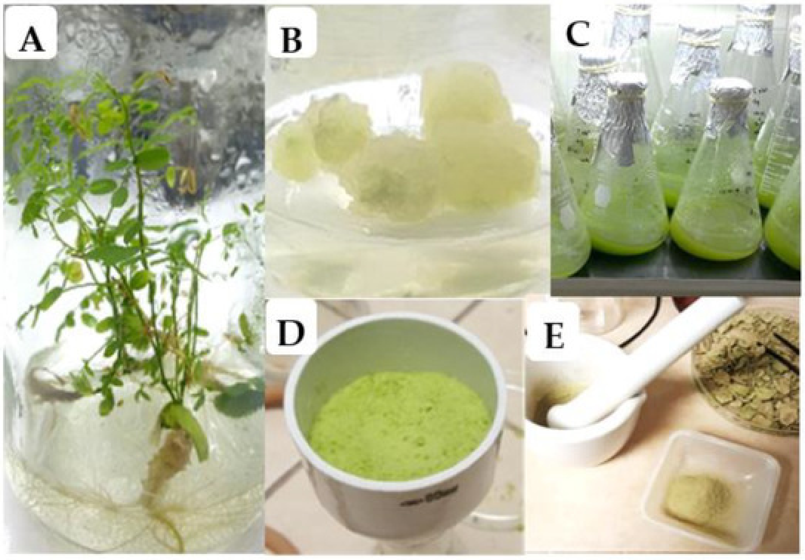

Figure 1. In vitro cultures of *Eysenhardtia platycarpa*. (**A**) Plantlets grown in MS culture medium without plant growth regulators; (**B**) callus production at 15 days of culture; (**C**) cell suspension cultures with 2 mg/L NAA and 0.5 mg/L KIN; (**D**) fresh biomass harvested after 12 days of culture; (**E**) dried biomass used for obtaining extracts.

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 1). This coincides with what has been reported for related species such as *E. polystachya*, in which the highest yields were obtained with methanol and dichloromethane [14]. In *Tectona grandis*, yields of 2.9%, 2.3%, and 3.6% were reported, through a sequential extraction with hexane, ethyl acetate, and methanol, respectively [15]. 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 [16]. For *E. platycarpa*, the highest yield of the methanolic extract was 36.9% of the heartwood and 30.08% for the leaf (Table 1). Other studies carried out on branches of *Severinia buxifolia* also obtained a higher extract yield (33.2%) using methanol [17]. In another study conducted on *Caesalpinia sappan* L., methanolic extracts of heartwood and leaf showed higher yields of 17.60% and 17.05%, respectively [18]. This may be because plants contain large amounts of polar compounds such as proteins and carbohydrates [19]. High amounts of polyols may also have increased the yield of methanolic extracts [20].

Table 1. Yields 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**

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