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Scientific Article

PINEWOOD PRESERVED WITH *Enterolobium cyclocarpum* (JACQ) GRISEB. HEARTWOOD AQUEOUS EXTRACT

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ABSTRACT

Enterolobium cyclocarpum heartwood is prized for furniture making because of its natural durability. In this process, up to 50% of the heartwood is discarded in the sawmill. Which contains extractives that can be used to emulate the natural durability and give protection to soft or rapidly deteriorating wood. Pinewood in service and several items made up mainly of cellulose are subject to deterioration caused by xylophage boring insects and other biotic agents. The purpose of this research was to demonstrate that the *E. cyclocarpum* heartwood aqueous extract protects dry pinewood against the damage caused by the termite *Incisitermes marginipennis* (Latreille) (Isoptera: Kalotermitidae) a borer insect. The removal of pigments from the aqueous extract, which initially stained the wood dark brown, made it possible to preserve the natural color of the wood, an important characteristic for the commercialization of wooden products. In addition to the protective properties, the phenolic compounds in the extract contributed to reducing degradation caused by light and other factors, delaying the aging of the wood. The proposal to use *E. cyclocarpum* residues to prepare a cheap and easy-to-prepare aqueous extract offers a sustainable and economical solution for wood preservation. These compounds can be applied in various biological areas, due to their antitumor, antimicrobial and antioxidant properties. Offering an ecologically correct and economically viable alternative to traditional preservatives, aligning with sustainability and environmental preservation trends. In conclusion, for indoor use, *E. cyclocarpum* heartwood colourless aqueous extract is an alternative to protect pinewood of drywood termite *Incisitermes marginipennis* due to manool presence.

Keywords: Drywood; Manool; Termite

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PINHO CONSERVADO COM EXTRATO AQUOSO DE MADEIRA DE CERNE DE *Enterolobium cyclocarpum* (JACQ) GRISEB.

RESUMO – O cerne de *Enterolobium cyclocarpum* é valorizado na fabricação de móveis devido à sua durabilidade natural. Nesse processo, até 50% do cerne é descartado na serraria. Que contém extrativos que podem ser usados para emular a durabilidade natural e dar proteção à madeira macia ou que se deteriora rapidamente. A madeira de pinus em serviço e diversos itens constituídos principalmente por celulose estão sujeitos à deterioração causada por insetos perfuradores xilófagos e outros agentes bióticos. O objetivo desta pesquisa foi demonstrar que o extrato aquoso do cerne de *E. cyclocarpum* protege o pinus seco contra os danos causados pelo cupim *Incisitermes marginipennis* (Latreille) (Isoptera: Kalotermitidae), um inseto xilofago. A retirada dos pigmentos do extrato aquoso, que inicialmente tingiu a madeira de marrom escuro, possibilitou preservar a cor natural da madeira, característica importante para a comercialização de produtos de madeira. Além das propriedades protetoras, os compostos fenólicos do extrato contribuíram para reduzir a degradação causada pela luz e outros fatores, retardando o envelhecimento da madeira. A proposta de utilizar resíduos de *E. cyclocarpum* para preparar um extrato aquoso barato e de fácil preparo oferece uma solução sustentável e econômica para a preservação da madeira. Esses compostos podem ser aplicados em diversas áreas biológicas, devido às suas propriedades antitumorais, antimicrobianas e antioxidantes. Oferecendo uma alternativa ecologicamente correta e economicamente viável aos conservantes tradicionais, alinhando-se às tendências de sustentabilidade e preservação ambiental. Concluindo, em uso interno, o extrato aquoso incolor do cerne de *E. cyclocarpum* é uma alternativa para proteger a madeira de pinus do cupim de madeira seca *Incisitermes marginipennis* devido à presença de manool.

Palavras-Chave: Madeira seca; Manool; Cupim

1. INTRODUCTION

Wood and wood-based products in service are subject to biodeterioration by insects, such as termites. Biodeterioration reduces the life cycle of these wooden products, including historical monuments, resulting in loss of aesthetic properties, mechanical strength and functionality (Megna and Liotta, 2017). The negative economic impact caused by xylophage insects is unknown in México, but some authors have reported expressive economic losses (Manachini, 2017; Cennamo et al., 2018). This has led to a gradual increase in the domestic consumption of conventionally preserved wood (SEMARNAT, 2018; Carrillo et al., 2017).

It is estimated that in Mexico there are more than 79 species of termites whose biological function in the different ecosystems that they inhabit is extremely important in the maintenance of the delicate natural balance of the soil (Mendez and Equihua, 2001). When boring xylophage insects come into contact with anthropogenic activity, they become a pest that is necessary to control with environmentally friendly methods to avoid damage to ecosystems, wooden and wood-based materials, as well as economic and cultural damage. Such is the case of the drywood termite *Incisitermes marginipennis* (Latreille, 1817 – Kalotermitidae), a lower termite that feeds mainly on pinewood cellulose. It is an insect that forms colonies with a limited number of individuals that in a short lapse of time cause great devastation. This termite is a social insect and lives inside dry pine wood. It is a native insect in NorthAmerica, distributed throughout México and has become an obvious pest seen in historical wooden monuments, such as the national cultural heritage, particularly of those located in central western Mexico (Cibrian et al., 1995).

Wood has been preserved against biodeterioration with a mixture of water-soluble heavy metal salts and formulations, including the main active principle pentachlorophenol (PCP). These chemical formulations cause damage to human health, and the environment (Dufera and Fufa, 2014; Hladik et al., 2018). Alternative formulations can be crude extracts or secondary metabolites from plants with insecticidal properties. Today some botanical insecticides commercially successful are formulated with plant products such as the neem tree derivatives (*Azadirachta indica*) whose active principles are azadirachtin, salanin and meliantrol. They possess properties, such as antifeedant growth regulators, oviposition inhibitors and repellents (Koul et al., 2004; Parrota and Chaturvedi, 1994). Another plant example is *Derris elliptica* from which was extracted rotenone. A compound that acts as contact insecticide post ingestion and as a repellent. Additionally, it was a molecular template for designing rotenoid insecticides (Ambrose and Haag, 1936). Novel proposals like the chemical constituents from *Khaya ivorensis* stem bark and their termiticidal activity were investigated on *Triplochiton scleroxylon* and *Vitex doniana* woods (Adedeji et al., 2018) Also, plant essential oils and terpenes can be alternatives to control drywood termites or other pests (Ramírez et al., 2016; Sen et al., 2017). Terpenes are found in the plant kingdom and play key roles in plant metabolism and development. Gibberillin phytohormones are an example of labdane-type diterpenes. Some labdanes are specialized secondary metabolites with limited taxonomic distribution, whose accumulation may be limited to specific cell types, tissues or organs (Mafu and Zerbe, 2018). Some labdanes show insecticide, antifeedant and other activities against dipterans (Díaz et al., 2015; Geris et al. 2008), lepidopterans (Gonzalez et al. 2011), and also against aphids (Rose et al. 1981).

The furniture and miscellaneous items built with *Enterolobium cyclocarpum* heartwood (common name, parota) are resistant to decay and deterioration. The domestic demand for parota wood furniture generates a significant economic impact in the Mexican Michoacan region and causes a large extraction of the parota and the importation of parota wood from neighbouring states (more than 110 m³/ Roundwood). However, more than 50% of the parota wood is discarded during the production of furniture and tools. The waste has a particle size that is not used to manufacture plywood and that only serves as firewood.

The parota heartwood is resistant to biodeterioration caused by fungi and insects, a characteristic that our group studies from the phytochemical and pharmacological point of view. It has been determined to have a medium deterrent activity against insects that attack processed wood such as the drywood termite *I. marginipennis* (Raya et al., 2013). Putative labdanes from *Enterolobium cyclocarpum* may contribute to heartwood resistance to insect biodeterioration. Pinitol was identified, isolated and purified from parota heartwood, a cyclitol with beneficial effects on glucose metabolism in humans and which inhibits the growth of *Heliothis zea* larvae when it is cultivated in soybean leaves and corncob (Dreyer et al., 1979; Raya et al., 2008). Also, the innocuousness of the heartwood aqueous extract to humans was determined in the case of their potential technological application in the protection of dry pinewood (Martinez et al., 2009). In a continuation of this line of research, the aim of this study was to demonstrate that the application of a colourless aqueous extract of *E. cyclocarpum* heartwood residue is an alternative to protect the pinewood from damage caused by the drywood termite *I. marginipennis* without losing its aesthetic appeal.

2. MATERIAL AND METHODS

2.1 Biological material

Wooden cylinders (15 cm radius X 30 cm length) were obtained from the basal part of the stem of *E. cyclocarpum*, from three sites; Veracruz City, at 10 meters above sea level (masl)], Aguililla, Michoacán, at 350 masl) and Ixtapa, Guerrero West, at 580 masl).

The pinewood lumber from *Pinus cembroides* according to the supplier (12% moisture content) was obtained from the stock of a sawmill located in Morelia, Michoacan at 1941 masl). It was sampled as boards (244 cm X 20 cm X 2.5 cm), then broken down into specimens. Also, it was assured that the pinewood was not preserved.

Drywood termites *I. marginipennis* were collected at three sites; Morelia, at 1840 masl), Maravatio at 1634 masl) and Huandacareo at 1941 masl). Infested pinewood with termites and dry non-infested pinewood were kept for their acclimation in a closed and dark chamber for a month at an average temperature of 25 °C and average relative humidity of 54%. Determination of the termite species was made in accord with the morphology of the soldier and with the taxonomic keys of Krishna and Wessner (1970) and Nickle and Collins (1988). All collection sites are located in Mexico.

2.2 Obtaining *E. cyclocarpum* heartwood aqueous extract

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E. cyclocarpum heartwood was ground in a mill (Thomas Scientific) and the powder was classified into 20 mesh (0.9 mm). The specimen was collected in accord with official Mexican standards (NOM-059-SEMARNAT 2010). The extract was obtained in accord with Martinez et al. (2009). Portions of 100 g of sawdust in 300 mL of deionized water were boiled for 20 min. It was then filtered, the liquid was reserved and the extraction was repeated with the solid: the filtrates were mixed. This process was repeated until 2.5 L were collected. Then, it was reduced 10X with a rotavapor at 40 °C until obtaining at total solids of 56.63 mg/mL that contained 0.2626 mg of total phenolic compound equivalents/mL. The amount of total phenolic equivalents (tpe) was measured as reported by Swain and Hillis method (1959). Briefly, most phenolic compounds are readily attacked by various oxidising agents some form coloured complexes with certain metals, such as, the Folin-Denis reagent (it is a mix of sodium tungstate and phosphomolybdic acid in phosphoric acid). After 1 h, the absorptivity is determined in 1 cm cells at 725 nm, and using as a blank water and reagents.

2.3 Obtaining the diterpene manool by hydrodistillation

Manool was obtained by a homemade hydrodistillation process, 2.5 L of distilled water and 225 g of *E. cyclocarpum* heartwood sawdust (0.9 mm) were used. The duration of the distillation was 256 min. From a volume of 750 mL distilled, the organic phase was extracted with methylene chloride (Baker 9324-03) and 0.1111 g of crude extract was acquired. Compounds were characterized by nuclear magnetic resonance (NMR) spectroscopy analysis. ^{1}H-NMR spectra were obtained with a Varian Mercury Plus spectrophotometer at a frequency of 400 MHz. Deuterated chloroform ($CDCl_3$) was the solvent (Sigma-Aldrich 151858-10G) and tetramethylsilane (TMS) was the internal reference. Manool was purified by column chromatography using 0.5 g silica gel as the stationary phase and 200 mL of hexane and ethyl acetate mixture in different proportions as the mobile phase. Thirty-eight fractions (5 mL) were obtained. Then a thin layer chromatography was performed to verify the presence of manool. The ^{1}H-NMR analysis identified manool in chromatographic fraction 21.

2.4 Impregnation of the pinewood with aqueous extract

Untreated pinewood veneer discs of 4.6 cm in diameter and a thickness of 0.58 mm with 12% relative humidity and specimens of 2 cm x 2 cm x 10 cm were preserved by immersion in 56.63 mg/mL aqueous extract (150 mL), boron salts solution (3.5 g disodium tetraborate and 2.5 g of boric acid, both dissolved in 1 L of deionized water) (positive control) and deionized water (negative control) for 30 min. The excess preservatives were discarded and nine discs and nine specimens were placed in a drying oven at 40 °C to constant weight. The specimens were placed in a desiccator with silica gel for 24 hours and weighed. Then, they were impregnated with the aqueous extract or manool. The impregnated specimens were then placed in a desiccator with silica gel for 24 hours and then weighed. The difference in weight corresponds to the solids retained; it is reported in g/cm^3. The determination of penetration of boron salts into the pinewood specimens was carried out using the turmeric yellow method (See supplementary material. Fig 1) (Erdoiza and Echenique 1980). The red colour from the pinewood specimen surface to the centre of the specimen was measured in millimetres (mm) and it was the penetration value.

2.5 Test of compression parallel to the fibers

Pinewood specimens of 2 cm x 2 cm x 10 cm with 8% relative humidity were placed in a container as follows: a) three specimens preserved with aqueous extract of *E. cyclocarpum*; b) three untreated; c) three specimens without untreated or deteriorated. On the specimens of the treatment a) and b) were placed 15 drywood termites. Immediately, the container was closed. The experiment was repeated three times with three replicates. The damage assessment was done within 77 days of exposure to the termites, measuring the compression parallel to the fibers. It used an universal testing machine (Shimadzu Seisakusho LTD), RH-10 T.V. No. 70133, with capacity was at 0.5 t, 1 t, 5 and 10 t at a loading rate of 0.3 mm/min.

2.6 Leaching test

This test was carried out under the standard ASTM D3987-06 (ASTM 2006). Treated with

Boron salt, aqueous extract and CAE-C101 (each treatment with 9 specimens) and untreated (9 specimens) pinewood veneer discs were placed in deionized water for 5 days collecting aliquots of the leachate every 24 h. After the leaching treatment, the discs were dried to determine the solids retained. Finally, the leachate was concentrated by lyophilization, and the solids content was determined by weight difference. The treated were dried until constant weight.

2.7 The aesthetic appearance of pinewood preserved with *E. cyclocarpum* heartwood aqueous extract

The powder of aqueous extract was dissolved in 1 mL of a mixture of chloroform, methanol and 1-butanol (2:1:1 v/v/v) and was filtrated in a column (6 cm in length and 1.5 cm in diameter) packed with cellulose [cellulose type 20 (C20), 101 (C101), cellulose of fibre median (CFM)], glass fibre (GF) or activated charcoal (AC) and a mixture of chloroform, methanol and 1-butanol (2:1:1 v/v/v) was used as mobile phase. The effluent was collected and named "colourless aqueous extract" (CAE) and in accord with its filter origin these were; CAE-C20, CAE-C101, CAE-CFM, CAE-GF and CAE-AC. Then, they were evaporated in a rotary evaporator (Büchi R-144) at a temperature of 40 °C.

2.8 Staining of pinewood by aqueous extracts

The aqueous extract (1 ml per side) or 50 µg/g manool were applied by brushing to nine pinewood specimens and disc veneers. Then the surface was cleaned with soaked cotton to remove the excess extract. The specimens and discs were dried for 24 h at room temperature. The colour of the wood was compared with the Munsell colour charts (1975).

2.9 Biological tests

Given that the drywood termite *I. marginipennis* colonies have a few individuals, but they cause great damage, treated pinewood veneer disks and specimens were exposed to deterioration with 10 to 15 drywood termites per repetition, with one soldier (Sheffrahn and Rust 1983; ASTM D 3345-74). The feeding conditions were, no-choice (pinewood preserved with *E. cyclocarpum* components: aqueous extract and manool) and choice tests (pinewood with and without 25 µg/g – 50 µg/g manool applied as described in 2.8). Termite survival was registered. Pinewood decay was determined by weight loss every 8 days in the range of 0 to 77 days, as indicated in each experiment.

2.10 Data analysis

All experiments were done three times and each one with three replicates. All the values are expressed as mean±S.D. The statistical significance of differences between the means were determined with the Statistica software using a one-way analysis of variance followed by Tukey post-hoc test. A *p*-value of less than 0.05 was considered to be significant.

3. RESULTS

3.1 Elimination of the pigments *E. cyclocarpum* heartwood aqueous extract and survival of *Insicitermes marginipenis*

In order to remove the pigments without eliminating the bioprotective property of the aqueous extract, it was filtered through filters made with cellulose (type C20, C101 and, CFM) active charcoal (AC) and glass fibre (GF) (Table 1 and Figure 1). The raw aqueous extract stained the pine wood dark brown. In general, the filters retained the pigments that give another colour to the aqueous extract. The better pigment retention rate was observed with CAE-CFM with a retention value of up to 78 times compared to the aqueous extract. All filtered extracts did not change the natural colour of the pinewood (Table 1). However, CAE-C101 caused an antitermite effect of 77% mortality, same as the raw aqueous extract. The raw aqueous extract contains pigments that protect and stain the pinewood dark brown, see Figure 2 (c,e and f).

3.2 Parameters of the pinewood preserved with aqueous extract from *E. cyclocarpum* heartwood

Retention and penetration of aqueous extracts values on specimens and disk veneer pinewood were measured before their exposition to termites. Wood deterioration

Table 1. Effect of depigmented aqueous extracts of *E. cyclocarpum* on the survival of *Incisitermes marginipennis*

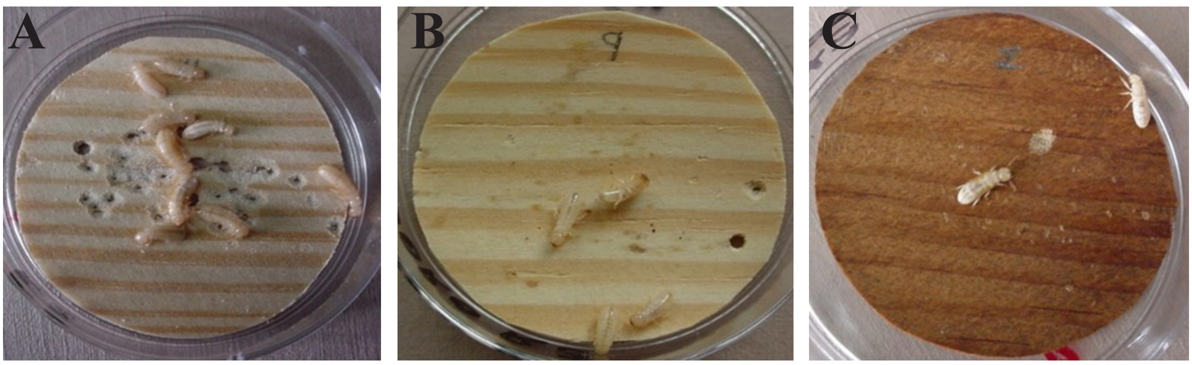
Tabela 1. Efeito de extratos aquosos despigmentados de *E. cyclocarpum* na sobrevivência de *Incisitermes marginipennis*

Filter	Survival termite %±SER	*A600 nm	Discolouration (x)	Colour
Unfiltered	23±1.9a	2.6674±0,1	0	HUE 10YR 6/8 Very pale brown (Color unchanged)
AC	78±6.9c	0.0512±0,004	52	HUE 10YR 8/4 Brownish yellow
CFM	96±2.7b	0.0342±0,002	78	HUE 10YR 8/6 Yellow
C20	92±4.0b	0.0390±0,001	68	HUE 10YR 8/4 Very pale brown (Color unchanged)
C101	23±1.5a	0.0398±0,003	67	HUE 10YR 8/4 Very pale brown (Color unchanged)
GF	87±4.5c	0.0392±0,001	68	HUE 10YR 7/6 Yellow

* A600 = Absorbance at 600 nanometers (nm). Values are means of three replications

Figure 1. Pinewood colour preserved with *E. cyclocarpum* extracts. Treatment sequence from left to right: Control (natural colour), crude extract and extracts without colour (CAE) C101, C 20, CMF, GF and AC

Figura 1. Cor do pinus preservada com extratos de *E. cyclocarpum*. Sequência de tratamento da esquerda para a direita: Controle (cor natural), extrato bruto e extratos sem cor (CAE) C101, C 20, CMF, GF e AC



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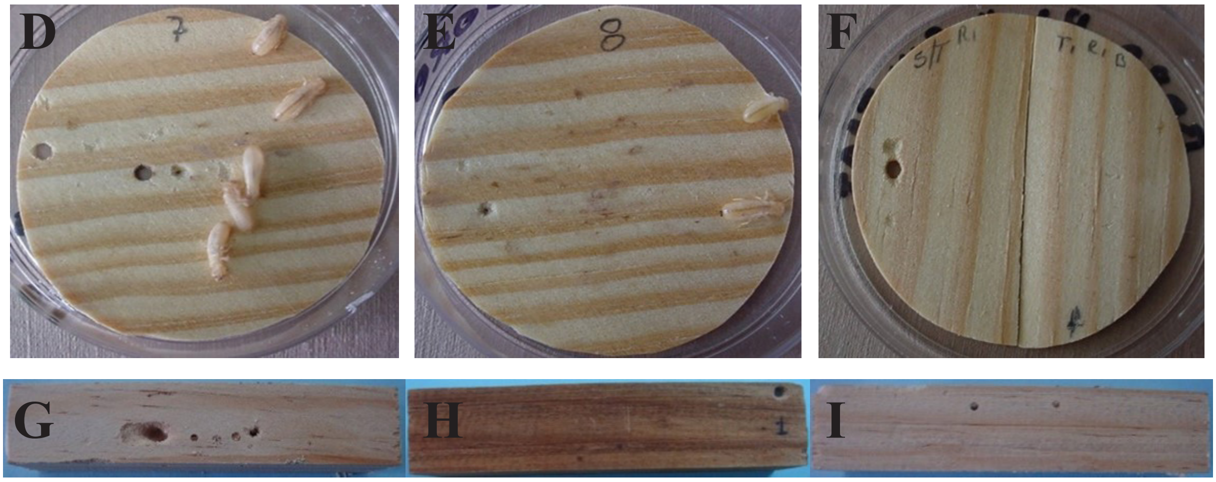

Figure 2. Protective effect of the derivatives from *E. cyclocarpum* heartwood on pine drywood. Discs and specimens from pinewood were treated as follow: untreated (A,G), boron salts (B), plant extract (C,H), colourless extract (cellulose 101) (D). No choice of food (50 µg/g manool w/w) (E). With choice of food (left piece disc without and right piece with 25 µg/g manool) (F,I). Pinewood veneer disks and specimens for 45 days and 77 days were exposed to attack by drywood termite *I. marginipennis*

Figura 2. Efeito protetor dos derivados do cerne de *E. cyclocarpum* na madeira seca de pinus. Discos e corpos de prova de pinus foram tratados da seguinte forma: não tratados (A,G), sais de boro (B), extrato vegetal (C,H), extrato incolor (celulose 101) (D). Sem escolha de alimento (50 µg/g manool p/p) (E). Com opção de alimento (disco da esquerda sem e da direita com 25 µg/g de manool) (F,I). Discos e corpos de prova de madeira de pinus durante 45 dias e 77 dias foram expostos ao ataque do cupim de madeira seca *I. marginipennis*

was determined by loss of relative weight, compressive strength along the fibre and insect survival values were measured after 77 days of exposure to termites. Preserved specimens with aqueous extracts showed solid retention and penetration values of 0.064±0.003 g/cm³ and 7.8 mm, respectively. While for preserved pinewood veneer discs the solid retention values with boron, aqueous extract and filtered extract (cellulose 101) were 0.042±0.001 g/cm³; 0.064±0.003 g/cm³ and 0.014±0.001 g/cm³, respectively (Table 2). The solid retention value of the crude extract in the pinewood probe was 0.021±0.003 g/cm³. In the condition of immersion, the treated pinewood veneer disc had a solid retention of 37.08% and 62.92% was leached.

On the other hand, to see the amount of plant extract that may be kept in the pinewood veneer when subjected to extreme humid conditions. Solid retention into the pinewood probe and leaching in pine wood veneer discs was determined over five days. The result observed was 37% of solid retention in the pinewood under extreme moisture conditions.

Pinewood impregnated with the aqueous extract at a concentration of 0.2626 mg of equivalent total phenolic compounds/mL had a deleterious effect on the drywood termite *I. marginipennis* (Tables 2 and Figure 2).

In the preserved wooden discs with the aqueous extract, the feeding percentage of termites was 23%, while in the negative control, it was 100% and in the positive control it was 37%. Pinewood treated with the aqueous extract shows a decrease in the damage caused by feeding of termites similar to wood treated with boron salts solution. The mortality of termites feeding with pinewood preserved with the crude aqueous extract was 77%, close to the mortality value obtained in the positive control was 83% (Table 2 and Fig. 2c).

It was observed that the probe preserved with the extract of *E. cyclocarpum* was not deteriorated and all the termites placed on them died 58 days post-treatment, only doing 0.15% of damage (Fig. 2e and 2i). In untreated pinewood the termites survived to do galleries in the probe, this caused a decrease in their

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weight of 1.33% (Figs. 2g and 2h). Mechanical test of strength to parallel compression along fibres of the pinewood specimens was as follows (Table 2): untreated wood and not attacked by termites, impregnated wood with aqueous extract or boron salt and attacked by termites had a compressive strength value of 519.18±9.62 kg/cm²; 482.99±20.41 kg/cm² and 430.99±17.29 kg/cm², respectively. But by removing the pigments with C101 filter, the CAE-C101 did not cause a severe change in the natural pigmentation of the wood and preserved its protective quality on pinewood (Figs. 2d and 2i).

3.3 Identification of a major component of CAE-C101 from *E. cyclocarpum*

It is possible that a family of chemical components without colour from the extract is responsible for the protective effect against drywood termites. This possibility was explored by NMR analysis with the assumption that the anti-thermite effect is exerted by the major component (Fig. 3).

Table 2. Parameters of the pinewood preserved with aqueous extract from *E. cyclocarpum* heartwood

Tabela 2. Parâmetros do pinus preservado com extrato aquoso do cerne de *E. cyclocarpum*

Treatment	Initial			
	Retention (g/cm³)		Penetration (mm)	
	Probes	Disks	Probes	Disks
Absolute control	0	0	0	0
Boron Salt	0.013±0.001	0.042±0.001	7.78±1.3	0.58±0.06
Aqueous extract	0.021±0.002	0.064±0.003	7.65±1.0	0.58±0.05
CAE-C 101	0.018±0.002	0.014±0.001	ND	0.58±0.05

Treatment	Final		
	Leaching (Solid retention) %±RSE	Strength compressive along the fibre (kg/cm²)	Termite survival %± SER
Absolute control	96±3.6	519±9.62	-
Boron Salt	25±3.1	500±10.67	17±2.0
Aqueous extract	37±4.0	483±20.41	23±3.1
CAE-C 101	23±3.1	510±10.67	23±2.5

ND = not determined

^{1}H-NMR analysis of the extract shows the characteristic signals of a diterpene (see Figure 2A). While in Figure 2B the spectral signals corresponding to the manool are evident. At 5.91 ppm a double signal of doubles was observed with J_{trans}=17.4 and J_{cis}=10.8 Hz characteristic signal of a vinylic proton with cis and trans couplings corresponding to H-14; at 5.21 ppm a double of doubles signal was observed with a trans coupling of 17.4 Hz and a gem coupling of 1.3 Hz corresponding to H-15'. The H-15 signal was observed at 5.06 ppm as a double-double signal with J_{cis} =10.8 and a J_{gem}=1.3 Hz. In the vinyl proton region, two other signals were observed at 4.37 ppm and 4.80 ppm as corresponding broad single signals to the exo-cyclic methylene hydrogens H-17 and H-17'. The methyl signals 18, 19 and 20 were observed at 0.87 ppm, 0.79 ppm and 0.67 ppm as single signals each. While the methyl 16 signal was found at 1.27 ppm as a single signal; the rest of the signals from the carbon skeleton are between 2.4 ppm and 1.0 ppm.

Manool (50 µg/g) did not cause a change in the colouration of the pinewood and prevented its deterioration caused by the dry termite of the wood. This observation was made when the

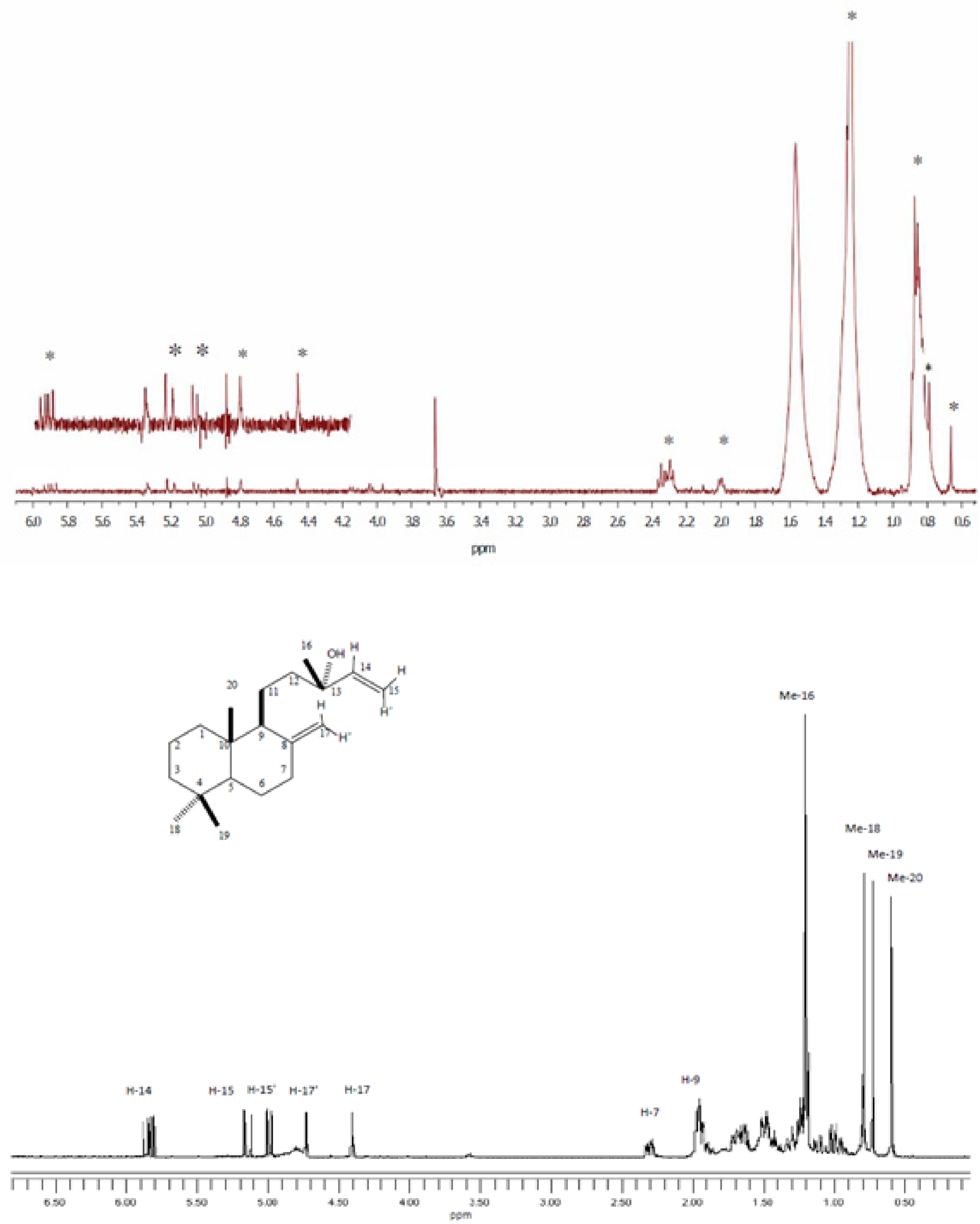

Figure 3. [1]H-NMR analysis of *E. cyclocarpum* components. A. Aqueous extract spectrum. B. Diterpene spectrum. Insert is manool chemical structure (*): Characteristic signals of manool diterpene. H = Hydrogen, Me = Methyl

Figura 3. Análise de [1]H-RMN de componentes de *E. cyclocarpum*. A. Espectro de extrato aquoso. B. Espectro de diterpeno. A inserção é a estrutura química do manool (*): Sinais característicos do diterpeno manool. H = Hidrogênio, Me = Metil

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termite was fed only with wood impregnated with manool and when the insect was fed with two substrates: pinewood with and without 25 µg/g manool (Figures 2e and 2f).

4. DISCUSSION

Great interest has been focused on softwood preservatives with poor durability. An ideal wood preservative is an effective, low-cost chemical with minimal toxic effects on mammals and the environment. The insecticidal property of hardwood and other plant extracts to protect softwood from decay caused by insects and other organisms has been used as a core to develop new so-called eco-friendly wood preservatives. Hardwood extracts from tropical tree species have been successfully applied to protect soft and low-resistance woods such as *Pinus* spp. with some plant components, for example, caffeine (Civelek and Colak, 2008; Tascioglu et al., 2012; Broda et al., 2018). The *E. cyclocarpum* heartwood (common name; parota) is valued for its resistance to biodeterioration. This characteristic led us to study its phytochemical properties and secondary metabolites responsible for protection against biodeterioration. In our group, we determined that extractable compounds from the heartwood of this tropical tree have deterrent components with antifeedant and repellent activities against *I. marginipennis* (Raya et al., 2013). The aqueous extract of *E. cyclocarpum* heartwood did not cause acute toxicity (Martinez et al., 2009). These studies led to the modeling of the application of the aqueous extract from the heartwood of *E. cyclocarpum* in laboratory tests using pinewood.

These solids retention and penetration values in pine wood treated with aqueous extract are higher than those recommended for the preservation of low-durability wood with boron salts for a lower risk of deterioration (NMX-C322-ONNCCE-2003). Some wooden and high cellulose household products are often exposed to moisture. Therefore, it is important to know whether the plant aqueous extract is leached from pine wood preserved in an extreme humidity condition. The pine veneer retains the aqueous extract with solid retention values higher than those recommended by COFAN (1994) for the preservation of wood with boron salts at a low risk of deterioration caused by wood-eating insects. Some coatings, such as varnishes and sealants applied to wooden household items, are transparent, which reveals the natural color of the wood; others contain dyes to stain the wood according to fashion trends. These results are consistent with the observation that wood extracts from European and Malaysian wood species with high content of phenolic compounds have protective properties on pine wood (Diouf et al., 2006; Kadir, 2017).

However, aqueous extract can be applied earlier than other coating additives in the finishing process of domestic wood products to prevent leaching and they can retain 100% of the extract. For this, the aqueous extract as an anti-termite preservative for pine wood will be applied to household goods that will be used indoors because they are not in contact with moisture, and obviously together with sealants, varnishes, lacquers and paints are useful to protect the wood of pine.

When selling preserved wood, an important issue is the color, physical finish and visual appearance of the wood. A feature that allows you to select your application according to your use. Where the natural colors of preserved wood are dictated by fashion trends. Most active compounds and chemical formulations for wood preservation cause a change in the color of the wood, which can cause restrictions in their use, in the manufacture and sale of domestic wood products, as is the case with the mixture of CCA and creosote, used as preservatives. The aqueous extract of *E. cyclocarpum* heartwood contains a high level of phenolic compounds that stain the wood dark brown. The pigments in the aqueous extract were removed with different filters to test the wood preservation effect without altering the natural color of the wood.

E. cyclocarpum heartwood has a high content of phenolic compounds. They were extracted by infusion and measured by the Folin-Denis method. It was demonstrated that the crude extract produces a natural colour variation of pinewood. It is evident that the protection of wood with phenolic compounds has several functions, for example, to reduce the redox degradation caused by light and other physicochemical factors, slowing the ageing and maintaining the natural pigment.

It is clear that monophenols and oligophenols are responsible for the protection of wood since being removed from the polyphenol of the extract, it retains its protective property. Using methods of gas chromatography with a mass detector, a complex mixture of phenolic compounds was found. Also, in the aqueous extract from *E. cyclocarpum*, Raya et al. (2013) observed signals with high relative abundance, they were: butyrate hydroxytoluene (76,22%), 4-methyl phenol (4,91%), 1,2-dimethoxy benzene (2,07%) and 2-methoxy phenol (1,93%).

However, in this work one of the major diterpenes was detected in CAE-C101 and partially purified, also known as manool. It was identified by a comparison of published spectral data. Manool exhibits a wide range of biological properties such as antitumoral, antimicrobial, antioxidant and cytotoxic (Nicolella et al., 2022; Ferreira et al., 2020).

In addition to the repellent effect of the VOCs of *E. cyclocarpum* reported by Raya et al. (2013). The aqueous extract of the plant contains another group of chemicals that cause an anti-feeding effect with accompanying termite mortality due to starvation on the dry wood termite, among them manool stands out. This observation is according to the effect of manool reported by Scheffrahn et al. (1988).

With these data, the use of the residue of *Enterolobium cyclocarpum* produced by sawing its heartwood is proposed for the preparation of a cheap and easy-to-prepare aqueous extract capable of protecting pine wood from biodeterioration by the xylophage *Incisitermes marginipennis* and for the extraction of interesting secondary metabolites such as manool through a hydrodistillation process.

5. CONCLUSION

The removal of pigments from the aqueous extract, which initially stained the wood dark brown, made it possible to preserve the natural color of the wood, an important characteristic for the commercialization of wooden products. In addition to the protective properties, the phenolic compounds in the extract contributed to reducing degradation caused by light and other factors, delaying the aging of the wood. The proposal to use *E. cyclocarpum* residues to produce a cheap and easy-to-prepare aqueous extract offers a sustainable and economical solution for wood preservation. The application of these compounds in various biological areas, include their antitumor, antimicrobial and antioxidant properties. Offering an ecologically correct and economically viable alternative to traditional preservatives, aligning with sustainability and environmental preservation trends.

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AUTHOR CONTRIBUTIONS

Alonso-Hurtado, K. L. and Méndez-Pérez, T.: Conceptualization, Project administration, Data curation and Formal analysis; Martínez-Pacheco, M. M.: Conceptualization; Writing – original draft, Writing – review & editing, Supervision, Investigation, Visualization, Validation, Project administration; Flores-García, A.: Formal analysis; Raya-González, D.: Project administration, Visualization, Validation, Supervision; Velázquez-Becerra, C.: Funding acquisition, Conceptualization.

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