ANTIMICROBIAL ACTIVITY OF THE FIBER PRODUCED BY "POCHOTE" *CEIBA AESCULIFOLIA* SUBSP. *PARVIFOLIA*

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Abstract

Background: The cotton-like fiber from the fruit of Pochote (*Ceiba aesculifolia* subsp. *parvifolia*) can be applied to wounds for healing purposes. As microorganisms can infect wounds and hamper the wound healing process, the aim of this study was to determine the antimicrobial activity and chemical composition of the methanolic extract of Pochote fiber.
Materials and Methods: The methanolic extract was tested against bacteria and fungi. For bacteria, the Kirby-Baüer disc diffusion and broth dilution methods were employed to determine the MIC and MBC. In addition, bactericidal kinetic curves were generated. The antifungal activity was determined by the radial diffusion method. The antioxidant activity, total phenolic content, and the flavonoid content were determined. Bioassay guided fractionation was also performed.
Results: The methanolic extract showed activity against *Enterococcus faecalis*, *Staphylococcus aureus* and *Vibrio cholerae* (cc). The tested *V. cholerae* strains were the most sensitive strains, and exhibited a clear CFU size reduction from the bactericidal kinetic curves. The methanolic extract had activity against *T. mentagrophytes* and *R. lilacina*. The antioxidant activity (SC_{50}= 36.42 μg/mL) was related to the total phenolic (74.4 mg eAG/g) and flavonoid content (21.982 mg (eQ)/g). The bioassay guided fractionation results suggested that the antimicrobial properties of the extract may act through synergism because the total extract had higher activity against bacteria compared to the collected fractions.
Conclusion: This study scientifically validates the application of the fruit fiber from Pochote as a part of a traditional medicine approach to alleviate infections caused by bacteria and fungi.

Key words: antimicrobial, *Ceiba*, Bombacaceae, fruit fiber.

Introduction

Plants have traditionally provided a source of new chemical compounds, and numerous clinical studies have demonstrated therapeutic value of plant origin molecules for human health. Of the more than 350,000 species of higher plants currently recognized, only 5–10% have been investigated; considering that each plant species may contain 500–800 different secondary metabolites, the potential for the discovery of new therapeutic products is considerable (Abreu et al., 2012).

Mexico is well-known for its diversity and endemism and contains twelve centers of diversity as recognized by the international Union for Conservation of Nature (IUCN), such as Tehuacan-Cuicatlan Valley. This valley is located between Puebla and Oaxaca states, has an approximated area of 10,000 km^2 and contains 2,703 plant species, which represents between 10–11.4% of the flora in Mexico. Because of this region's cultural diversity and biological richness, it has been declared a Biosphere Reserve. The lengthy history and interactions between the local population and the native plants have led to the development of important plant knowledge, technological experience in plant populations and the manipulation of plant communities (Avendaño et al., 2006).

San Rafael, a village in the municipality of Coxcatlan, is located southeast in the Valley of Tehuacan-Cuicatlan (Canales et al., 2005). San Rafael has 374 vegetal species belonging to 249 genera and 87 families, and 368 species were registered with one or more uses (18% as medicinal plants) The village is a part of a regional study with the aim of understanding the natural resource conditions in order to improve the tools for its use and preservation (Dávila et al., 2002).

Ceiba aesculifolia subsp. *parvifolia*, known as pochote, is one of the 140 to 250 species that belongs to the *Ceiba* genus in the Bombacaceae family. Local people in the Tehuacan Valley consume the pochote seeds, roots and flowers. In addition, the stem and branches are used as firewood, the cotton-like fruit fiber is used as pillow filler, and the bark spikes are used for making handicrafts. As for the medicinal use of this species, people older than 60 years state that the bark can be used as an infusion for various ailments, such as kidney problems, tumors, gastritis and wound healing (Rosas, 2003; Canales et al., 2005; Avendaño et al., 2006). Moreover, according to orally conveyed information, the cotton-like fiber is applied in wounds and is said to help the healing process. Nevertheless, the literature lacks information about the phytochemistry of this species and its properties. Therefore, the aim of this study is to determine the antimicrobial activity and chemical composition of the methanolic extract of the fruit fiber from *Ceiba aesculifolia* subsp. *parvifolia*.

Materials and Methods
Plant Material

The mature fruits of *C. aesculifolia* subsp. *parvifolia* were collected in San Rafael, Coxcatlan, Puebla, from 30 trees per site (total: 90 trees). A total of 273 fruits were collected from 2003 to 2005 (Avendaño et al., 2008). The plant specimens were determined at Colegio de Postgraduados of Universidad Autonoma de Chapingo, and a voucher was deposited in the IZTA herbarium at Facultad de Estudios Superiores Iztacala with collection number RRL146 and register number 29214.

Extract Preparation

Pochote fiber extracts were obtained by maceration (Domínguez, 1973). The fiber from the mature fruits (384.4 g) was extracted with hexane and methanol. The extracts were filtered and evaporated until dry using a rotary evaporator. The extraction yields were determined for both extracts based on the total weight.

Bioassays
Antibacterial Activity Assay

The following bacteria were used: *Enterococcus faecalis* Hospital Los Angeles (H.A.); *Staphylococcus epidermidis* ATCC 12228; *Staphylococcus aureus* ATCC 25923; *Salmonella typhimurium* FES Cuautitlan; *Enterobacter cloacae* FES Cuautitlan; *Proteus mirabilis* Clinical case (cc); *Vibrio cholerae* (cc); and *Vibrio cholerae* CDC V 12 (El Tor).

The antibacterial activity was measured by the Kirby-Baüer disc-diffusion method (Vanden Berghe and Vlietinck, 1991; Cole, 1994). The microorganisms were grown overnight at 37°C in 10 ml of Mueller Hinton broth (Bioxon 260). The cultures were adjusted to a turbidity comparable to McFarland standard no. 0.5 with a sterile saline solution ($1.5x10^8$ CFU/mL). The microbial suspensions were plated on Müeller Hinton agar plates (Bioxon). Five mm diameter discs (Whatman no. 5) were impregnated with 10 µL of the extract solution (final dose per disc: 2 mg). Discs containing only methanol served as the negative control, whereas discs with 25 µg of chloramphenicol were used as positive controls. The tests were performed in triplicate.

Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC)

Once the sensitive strains were determined, the minimal and bactericidal inhibitory concentrations were determined by the broth dilution method (Koneman et al., 1985). Ninety-six-well ELISA plates were filled with methanolic fiber extract dilutions in DMSO in Mueller-Hinton broth (20- 0.125 mg/mL). The final concentration of DMSO in each well was less than 1%. Next, the plates were inoculated with the bacterial suspensions ($1x10^5$ CFU/mL). After a 24-hour incubation at 35°C, tetrazolium salt (TTC) at a concentration of 0.08% was added to each well. After 30 minutes of incubation, the MIC values were defined as the lowest concentration of the extract that decreased bacterial growth drastically (violet color), and the MBC values were defined as the concentration that completely inhibited bacterial growth (clear color). The appropriate controls with no extract and the solvent alone were used. Each experiment was repeated at least three times.

Bactericidal Kinetic Assay

The bactericidal kinetic assay was performed according to the procedure by Muroi et al., 1993. The most sensitive Gram negative and Gram positive bacterial strains (one from each type with the lowest MIC and MBC values) were used. The growth was determined at nine time points over 24 hours with the extract exposure at different concentrations corresponding to: ½ MIC, MIC, MBC and a control without the extract.

Antifungal Activity Assays
Qualitative Assay
Yeasts

The following strains were used: *Candida albicans* 14065, *C. albicans* 32354 CUSI, *C. albicans* 630, *C. tropicalis* (A.H.), and *Cryptococcus neoformans* (Laboratorio de Micologia y Parasitologia. Facultad de Medicina UNAM).
The qualitative activity of the extract against the different yeasts was determined by the Kirby-Baüer disc-diffusion method (Vanden Berghe and Vlietinck, 1991; Cole, 1994). The yeasts were cultured on PDA (Potato Dextrose Agar) agar at 35°C. The discs were impregnated with the extract (2 mg), nystatin discs (25 µg) were used as positive control, and the discs with methanol (10 µL) served as negative controls. The tests were performed in triplicate.

Filamentous Fungi

The following strains were used: Aspergillus niger CDBB-H-179; Aspergillus sp., donated by Dr. Rodolfo de la Torre (FES-

Iztacala, UNAM); Fusarium moniliforme CDBB-H-265; Fusarium sporotrichioides NRLL3299; Rhizoctonia lilacina CDBB-H-306; and Trichophyton mentagrophytes CDBB-H-1112.

Radial growth inhibition was used to determine the preliminary antifungal activity against the filamentous fungi (Wang and Bun, 2002). Extract discs (2 mg per disc), Ketoconazol (7 µg per disc) discs as a positive control and methanol discs (10 µL per disc) as a negative control were prepared. Petri dishes with PDA Agar were inoculated by point deposition of mycelium (5 mm diameter) and were incubated at 28°C for 72 hours. After mycelium growth, the discs were placed 30 mm from the edge of the mycelium growth. Antifungal activity was reported when there was mycelium reduction or changes in color, sporulation or morphology. No antifungal activity was reported when the fungi grew similar to the control and grew over the discs. The tests were carried out in triplicate.

Quantitative Assay

The quantitative activity assay was performed according a modified version of the Wang and Bun (2002) method. Each well in a 24-well culture plate was filled with 1.5 mL of PDA with the following extract concentrations: 8.0, 6.0, 4.0, 2.0, 1.0, 0.50 mg/mL, and a point (1 mm diameter) deposition of each strain inoculum was placed in the agar at the center of the well. Ketoconazol (40, 50, 60, 70, 80, 90 and 100 µg/mL) was used as the positive control, and agar with DMSO ($\leq$ 1%) was used as the negative control. Standards with 1.5 mL of agar were also used. The plates were incubated at 23°C for 72 hours or until mycelium growth was observed. CFM was the lowest extract concentration resulting in total inhibition of mycelium growth compared to that of the standard. The regressions were measured to determine the CI_{50}, or the extract concentration that delayed 50% of the colony radial extension.

Antioxidant Activity (DPPH Free Radical Scavenging)

The antioxidant activity was determined according to the method by Okusa et al., (2007). Ninety-six-well ELISA plates were filled with extract concentrations ranging from 1-100 µg/mL, HPLC grade methanol served as a blank sample, and a DPPH solution (100 µM) served as a control. The plates were incubated for 30 min at 37°C, and the absorbance values were determined at 540 nm with an ELISA plate reader. The antioxidant activity values were determined according to the following equation: % inhibition = [(absorbance of control-absorbance of sample)/ absorbance of control] *100. The concentration leading to 50% inhibition (SC_{50}) was determined graphically. Quercetin was used as a reference (positive control).

Total Phenolic Content

The Folin-Ciocalteu method (Singleton et al., 1999) was used to determine the total phenolic content of a 0.05 mg/mL concentration of the methanolic extract. Distilled water (6 mL) and 500 µL of Folin-Ciocalteu reagent were added, and after 5 minutes, 1.5 mL of Na_2CO_3 (200 g/L) and distilled water was added. Methanol was used as the blank sample. After incubation at room temperature for 2 hours, absorbance readings were measured using a spectrophotometer at 760 nm. The mean of three readings was used to interpolate with the gallic acid curve (6.25, 12.5, 25, 50, 100, 200 µg/mL), and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/g of extract.

Total Flavonoid Content

The Dowd method, as adapted by Ramamoorthy and Bono (2007), was used to determine the flavonoid content. A solution of 2% aluminum trichloride ($AlCl_3$) in HPLC grade methanol and an extract concentration of 0.2 mg/mL was used. Readings at 415 nm using spectrophotometer were taken after 10 minutes against a blank sample (5 mL extract solution and 5 mL methanol without $AlCl_3$). A quercetin (1-100 µg/mL) calibration curve was used as the standard. The mean of three readings was expressed as mg of quercetin equivalent QE/g of extract.

Bioassay Guided Fractionation
Column Chromatography (CC)

Three grams of the extract were used for the column chromatography fractionation. Silica gel was used as the stationary phase (mesh: 70-230 µm, Sigma 5-2509), and increasing concentrations of hexane, ethyl acetate, methanol was used as the eluents. A sample of each 20 mL collected fraction was applied to thin layer chromatographic plates (Kieselgel 60 F254 Merck), and the spots were visualized with UV light (λ= 254 nm and λ= 360 nm), and the stains vanillin-sulfuric acid and ceric sulfate. Fractions with similar spots were grouped, and the yields were determined for use in the susceptibility tests. For determination of the crystal melting points a Fisher-Johns apparatus was used.

Susceptibility Test

The Kirby-Baüer disc-diffusion method was used to determine the activity of each fraction. The fractions with the highest yields were used for this test as 2 mg was required for each disc. As *V. cholerae* (cc) was the Gram-negative bacteria that was most sensitive to the methanolic extract, this strain was used to test the activity of the fractions.

High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-ME)

The methanolic extract and the fractions with biological activity were loaded onto the HPLC HP Series 1100 separations module from Hewlett-Packard (Wilmington, DE, USA), equipped with an All-sphere ODS-1 column, at 269 bar pressure and a

temperature range of 22°C-23°C. The mobile phase consisted of methanol: acetonitrile: H_2O (25:25:50) for 20 minutes. A DAD detector was used at a wavelength of 260 nm with a full scan of 200-400 nm. The mobile phase for the active fractions (32, 41, 49, 74, 77) was MeOH: acetonitrile: $H_2O:H_3PO_4$ (25:25:50:0.1).

The methanolic extract and the low polarity fractions (biologically active) were injected into a Gas chromatograph 6850 (China) equipped with a RTX column (30 m x 0.25 mm i.d., film thickness, 0.25 µm). The temperature of the column was programmed starting at 70 °C for 2 min, and then the temperature was increased 8 °C/min to 270 °C. At 270 °C, a programmed linear gradient increased the temperature 10 °C/min to 290 °C. The injector and detector temperatures were 250 °C and 290 °C, respectively. The gas carrier was helium at a flow rate of 0.9 mL/min. The peak areas were measured by electronic integration. The relative amounts of the individual components were based on the peak areas. GC-MS analysis was performed on a AGILENT 5975C (China) mass spectrometer. The mass spectra were recorded at 70 eV. The hexane extract components were identified by comparison of the retention indices and the mass spectra with the NIST/EPA/NIH Mass Spectral Library.

Statistical Analysis

A descriptive analysis was performed for the qualitative antibacterial test data. The mean, median, quartile 1 (Q_1), quartile 3 (Q_3), minimum and maximum measurements were determined for each species, extract and bacterial type to obtain the box plots. Factorial ANOVA was used to determine significance. The factors considered were species, extract and bacterial strain. All of the statistical analyses were conducted using Minitab 16 software. Linear and logarithmic regression analysis were used for analysis of the antifungal extract activities.

Results

The extraction yields of pochote fiber hexane and methanol extracts were 0.56% and 3.85%, respectively. The bioassays used the methanolic extract due to the higher yield. Table 1 shows that all of the strains were sensitive to chloramphenicol. The methanolic extract had antibacterial activity against two Gram-positive bacteria (*E. faecalis* H. A. = 10.4 mm; *S. aureus* 25923 = 9 mm) and one Gram-negative bacterium (*V. cholerae* cc = 10 mm). ANOVA analysis showed that there were significant differences between the strains (F= 327.66, P=0.0001).

Table 1: Methanolic extract and chloramphenicol (positive control) inhibition zones.

Bacteria	Inhibition zones (mm)	
	Extract	Chloramphenicol
E. faecalis H.A.	10.4 ± 0.2	30.3 ± 1.52
S. aureus 25923	9.0 ± 1.2	13.6 ± 1.15
S. epidermidis 12228	na	13.0 ± 1.0
E. cloacae	na	22.3 ± 0.57
P. mirabilis cc	na	11.3 ± 0.57
S. typhimurium	na	12.6 ± 1.15
V. cholerae (cc)	10.0 ± 1.0	29.0 ± 0.57
V. cholerae CDC V12 (El Tor)	na	21.6 ± 0.57

na: no activity

The MIC and MBC of the extract were determined for *E. faecalis* (MIC= 2 mg/mL and MBC= 14 mg/mL) and *V. cholerae* (MIC= 8 mg/mL and MBC= 12 mg/mL). In addition, there was a clear morphological change (reduced colony forming unit size) in both of the evaluated strains after application of the extract (Figure 1).
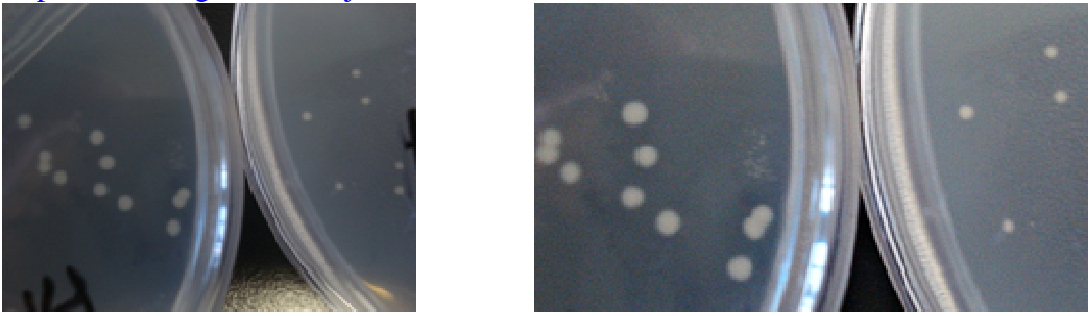
Figure 1: Reduction of colony forming unit size. The standard CFU size (without extract) (a), and the CFU size after extract application (b) is shown. Left: *E. faecalis*; Right: *V. cholera*.

Figures 2 and 3 show the effect of the methanolic extract on the survival curve for a Gram-positive (*E. faecalis*) and a Gram-negative bacterium (*V. cholerae* clinical strain). In *E. faecalis*, the MBC reduced bacterial growth more than what was observed with the MIC, ½ MIC or the standard (Figure 2). Within four hours of exposure to the extract, the bacteria displayed a growth decline, but the growth rebounded by 24 hours. For *V. cholerae,* the growth decreased after extract exposure for four hours at all three of the assayed concentrations but displayed similar growth to the standard (Figure 3).
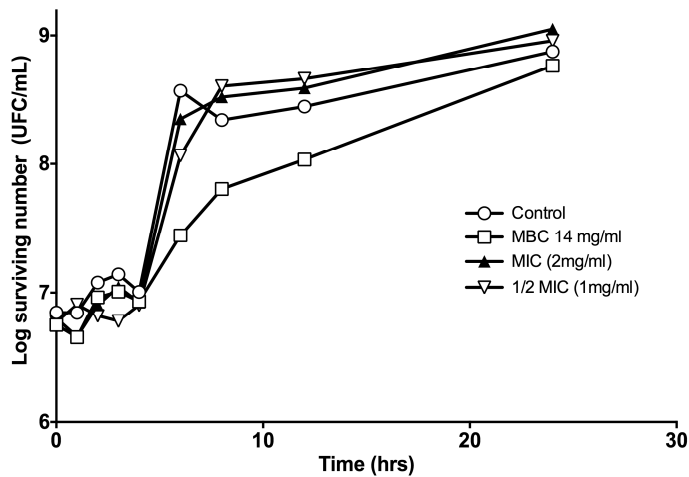

Figure 2: Survival curve of *E. faecalis* exposed to the methanolic extract of *C. aesculifolia* subsp. *parvifolia*. The methanolic extract was added to each experimental culture at the zero time point. The concentrations used were as follows: 1 mg/mL (½ MIC), 2 mg/mL (MIC) and 14 mg/mL (MBC). Control: without the methanolic extract.
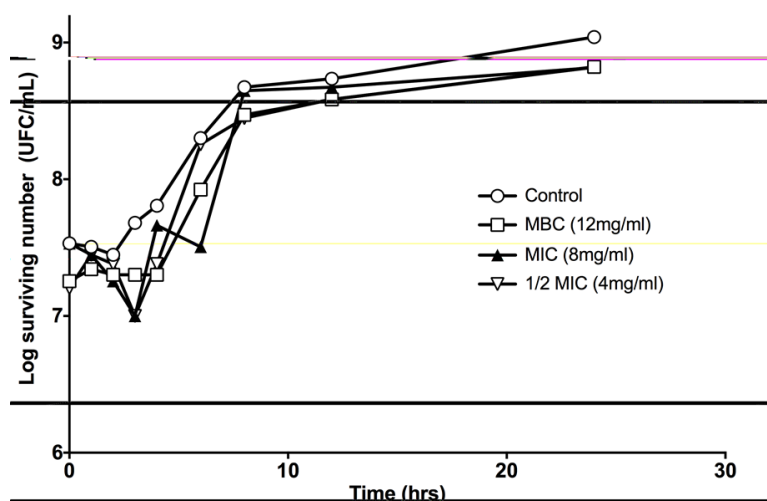
Figure 3: Survival curve of *V. cholerae* exposed to the methanolic extract of *C. aesculifolia* subsp. *parvifolia*. The methanolic extract was added to each experimental culture at the zero time point. The concentrations used were as follows: 4 mg/mL (½ MIC), 8 mg/mL (MIC), 12 mg/mL (MBC). Control: without the methanolic extract

     The qualitative tests demonstrated that the methanolic extract had no activity against the yeasts but had an effect on all of the filamentous fungi strains. The highest values of inhibition were for *T. mentagrophytes* (IC_{50}= 1.52 mg/mL) and *R. lilacina* (IC_{50}= 2.02 mg/mL), with MFC= 6.0 mg/mL in both strains, which was followed by *F. moniliforme* (IC_{50}= 1.08 mg/mL and MFC >8 mg/mL) (Table 2). The *Aspergillus* strains showed the highest IC_{50} values (6.48 and 7.70 mg/mL) without reaching an MFC. However, exposure of the extract to *Aspergillus* led to a clear reduction in spore production and vertical, but not horizontal (over agar), mycelium growth as in the standard (control).

     In terms of antioxidant activity, the extract had a SC_{50}=36.42 µg/mL compared with the SC_{50}=4.6 µg/mL of quercetin (positive control). The total phenolic content of the methanolic fiber extract was determined from the gallic acid curve. The result was 74.4 mg GAE/g, which represents 0.5% of total extract. The total flavonoid content of the extract was 21982.76 µg (QE)/g, which represents 0.022% per gram of the methanolic extract.

     The chromatographic analysis by GC-MS of the methanolic extract demonstrated the presence of palmitic acid, methyl ester of oleic acid, methyl isohexadecanoate and methyl ester of linoleic acid. Chalcones, flavonoids and phenols were also present according to the HPLC analysis.

From the bioassay guided fractionation, a total of 353 aliquots (20 mL) were grouped in 88 fractions. Based on sufficient extraction yields, 75 fractions were tested against *V. cholerae*. The hexane-ethyl acetate fractions (7:3 ratio) contained the greatest antibacterial activity. Table 3 shows the inhibition zones of the 13 active fractions. A 60°C fusion point was presented by fractions 12, 14, 16 and 19. However, fraction 17 was an exception (fusion point=110-115°C).

Table 2: Quantitative antifungal activity of the methanolic extract.

Filamentous Fungi	Extract		Ketoconazol
	IC_{50} (mg/mL)	FMC (mg/mL)	IC_{50} (µg/mL)
Aspergillus niger	6.48	>8.0	15.29
Aspergillus sp.	7.70	>8.0	9.75
Fusarium moniliforme	1.08	>8.0	7.55
Fusarium sporotrichioides	3.78	>80	3.90
Rhizoctonia lilacina	2.02	6.0	21.56
Trichophyton mentagrophytes	1.52	6.0	1.16

Table 3: Antibacterial activity of methanolic extract fractions against *V. cholerae* cc.

Extraction solvent	Proportion	Fraction number	Inhibition zone (mm)	Compounds
	8:2	7	7.0	
		10	7.0	Gamma-sitosterol, campesterol, n-hexadecanoic acid, ergost-5-en-3-ol (3beta)
		11	8.0	
		12	7.5	
		14	7.0	
Hex-AcOEt	7:3	**15**	**8.1**	
		16	8.0	
		17	**8.5**	Aromatic compounds
		19	7.9	Hexatriacontane, hentriacontane, tetratetracontane
	6:4	22	8.0	
	3:7	32	8.0	Phenols, phenylpropanoids, xanthone
	2:8	35	7.0	
	1:9	37	7.0	

Bold= higher values of inhibition zones.

Discussion

The potential of secondary plant metabolites (phytochemicals) as antibacterials when used alone and as synergists or potentiators of other antibacterial agents has been previously demonstrated (Abreu et al., 2012). Based on traditional knowledge of pochote fiber in wound healing, the antimicrobial activity the fiber extracts were measured.

The extraction yields of the hexane and methanol extracts of pochote fiber are dependent on secondary metabolite production, which depends on the growth conditions, physiological and development state of the plant and environmental conditions and are frequently low (less than 1% dry weight) (Rao and Ravishankar, 2002; Ramakrishna and Ravishankar, 2011; Anaya, 2004). It is also known that the seeds and fruits of *Ceiba* and *Chorisia* genera contain fatty acids and steroids (Refaat et al., 2012), so high amounts of non-polar substances were expected to be extracted. However, the methanolic extract showed the highest extraction yield (3.85%), principally because of the presence of polar compounds.

As wounds are exposed to the external environment where microbial attack is possible (Reddy et al., 2008), and because Gram-negative and Gram-positive bacteria are implicated in wound infection, the antibacterial activity of the fiber extract was measured. All of the tested strains were sensitive to chloramphenicol, and the strains most sensitive to the methanolic extract were *E. faecalis* H. A., *S. aureus* 25923 and *V. cholerae* cc. For Gram-positive bacteria, *E. faecalis* showed the highest inhibition zone. This bacterium is normally found in the gastrointestinal tract but is known to cause wound infections in immunocompromised patients (Brooks et al., 2008). The extract only showed activity against *V. cholerae* cc in the tested Gram-negative bacteria. Nearly a third of *Vibrio* species are pathogens to humans, and disease is generally transmitted through contaminated water or food or by wound exposure to aquatic environments where *Vibrio* species are present (Fernández and Alonso, 2009).

According to a descriptive analysis, the median of Gram-positive bacteria is higher due to the less-complicated cell wall structure compared to Gram-negative bacteria (Oyedeji et al., 2011). The peptide-glucans in Gram-positive bacteria are inside a matrix of teichoic, teichuronic and lipoteichoic acids, which provides a negative charge to assimilate divalent cations. This characteristic allows polar compounds from the extract to target the cell, which is different than the hydrophobic characteristics of the Gram-negative bacteria membrane providing resistance to organic dissolvents (Fook and Kheng, 2009).

Studies examining the antimicrobial activity of fruits of the Bombacaceae family are rare; however, Chekuboyina et al., (2012) analyzed seed oil activity against *S. aureus* and reported on the antibacterial activity of unsaturated fatty acids, carotenoids, flavonoids, tannins and phenolic compounds.

V. cholerae (MIC= 8 mg/mL and MBC= 12 mg/mL) and *E. faecalis* (MIC= 2 mg/mL and MBC= 14 mg/mL) had the lowest MIC and MBC values, and a clear CFU size reduction was observed in the bactericidal kinetic curves. The morphological change may be due to compounds in the extract interfering with a metabolic pathway due to the minimum antibacterial concentration. Application of an additional dose at four hours may improve antibacterial activity. This may also allow the immune system to attack the pathogenic microorganisms once the antibiotic is administered (Madigan et al., 2009).

Fungi can hamper the normal wound healing process (Reddy et al., 2008), and phytopathogenic fungi are also relevant because they cause economic losses (Madigan et al., 2009). The extract showed no activity against yeasts, which is likely due to the increased cell wall protein composition of yeasts (30-50% dry weight) compared to that of filamentous fungi (20-30% dry weight) and presence of

glucan and chitin in their structure that may interfere with the extract activity (Pontón, 2008). However, the extract activity in all filamentous fungal strains may be due to constitutive antifungal substances, such as fatty acids, long-chain alkanes, phenols and flavonoids (Montes, 2009). These substances have reported antifungal activities, which could interfere in cell wall synthesis, alter cell permeability, electron transport, nutrient absorbance, adenosine triphosphatase or other metabolic pathways, cellular enzymatic deactivation and/or denaturation of cell proteins (Cowan, 1999; Feng and Zheng, 2007).

Furthermore, the antifungal compounds in pochote fiber could be produced as a defense mechanism to enhance seed viability (Freeman and Beattie, 2008) against a fungus reported by Avendaño et al., (2008), which is related to *Moniliophthora* and *Crinipellis* genera. This fungus is a causal agent of fruit number reduction and fruit deformation in San Rafael and against *Arsenura polyodonta* Jordan larvae specific for pochote (Avendaño et al., 2006).

Fungistatic activity of the extract was demonstrated for *F. moniliforme* (CF_{50}= 1.08 mg/mL) without complete inhibition of mycelium. This fungus is widely distributed, has a wide hosts and is causal agent of pathogenesis of economic importance, as cob rot and premature germination of maiz in Tlaxcala, Puebla and Mexico (Márquez 1985; García and Martínez, 2010).

Total inhibition was reached with 6.0 mg/mL for *T. mentagrophytes* and *R. lilacina* with the lowest CF_{50} values (1.52 mg/mL and 2.02 mg/mL, respectively). *T. mentagrophytes* is known for its sensitivity to fatty acids, which affects membrane permeability by displacing phospholipids (Vila et al., 2013). As for the *Aspergillus* strains, there was clear evidence of spore reduction, which could be related to an inhibition of cellular respiration in filamentous fungi (Inouye et al., 1998).

The extract had a SC_{50}=36.42 µg/mL, which was less than quercetin with a SC_{50}= 4.6 µg/mL. This difference could be due to the absence or a reduced number of OH groups serving as hydrogen donors to the DPPH radical compared to quercetin, which can reduce more DPPH molecules per one molecule quercetin (Molyneux, 2004).

There is little information available on the antioxidant activity, phenol content and flavonoid content of Bombacaceae family fruits. Nevertheless, there is a report on the antioxidant capacity of *C. pentandra* fruits in which a methanolic extract had an activity of 82.95%, which was near the 92.91% value of ascorbic acid (positive control) (Nagamani et al., 2012). This greater antioxidant activity of the fruit extract compared with the pochote fiber extract may be due to the presence of seeds, mesocarp and epicarp in the young fruits resulting in increased phenolic content and antioxidant activity (Nagamani et al., 2012) as they act as reducing agents, hydrogen donors and singlet oxygen capturers (Fook and Kheng, 2009).

In addition, the antioxidant activity of the extract is related to the total phenolic content (7.44% per gram of extract) and the flavonoid content. Flavonoids have a variable number of hydroxyl phenolic groups and excellent ferric and other transition metal chelation properties, which confer a significant antioxidant capacity (Martínez et al., 2002) and play an important role in plant defense and signaling for reproduction, pathogenesis and symbiosis (Rijke et al., 2006),

The sterols identified through chromatographic techniques, such as campesterol and stigmasterol, have demonstrated antioxidant properties *in vitro* and *in vivo* (Madawala et al., 2012). These sterols are also associated with pathogen defense, which could be related to the activity against bacteria and fungi observed in this study (Ramakrisha and Ravishankar, 2011).

Secondary metabolites are generally present as mixtures, and parasites or pathogens could be affected differentially by individual compounds or by mixtures at specific concentrations or proportions in the plant (Montes, 2009). For that reason, it is important to determine the chemical composition of the extract to determine if synergism is occurring. The previously demonstrated biological activity may be related to the presence of palmitic acid (highest area percentage), esters of linoleic and oleic acids and isohexadecanoate. HPLC analysis demonstrated the presence of chalcones, flavonoids, and phenols, which are well-known for their antimicrobial properties (Araujo and Salas, 2008; Ramírez et al., 2012).

Based on the limited available data in the literature, there is no apparent relationship from a phytochemical point of view among species of the same genus, mainly because so few have been studies (Paula et al., 1997). Nevertheless, palmitic acid has been reported in the seeds of *Adansonia*, *Chorisia* and *Ceiba*; oleic and linoleic acid has been reported in the seeds of the *Adansonia* genus; and campesterol, cholesterol, stigmasterol and C_{12}-C_{35} alkanes have been reported in the seeds of *Adansonia*, *Chorisia* and *Ceiba* (Refaat et al., 2012).

The inhibition diameter zones were larger with the extract than with the fractions, which is likely because the compounds have a synergistic activity resulting in reduced inhibition when tested separately.

Finally, traditional use has gradually been lost, and the population of this plant species is decreasing in the Valley's communities (Avendaño et al., 2006; Montes, 2009), highlighting the importance of further studies in this species from all points of view.

Conclusions

In this study we found that *C. aesculifolia* subsp. *parvifolia* has sterols, flavonoids, phenolic compounds, fatty acids and esters that may be acting as synergists for antimicrobial activity, especially against fungal strains. This study provides scientific evidence supporting the traditional knowledge associated with medicinal *C. aesculifolia* subsp. *parvifolia* fiber use and provides a basis for future studies.

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