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ALLELOCHEMICAL POTENTIAL OF *Metopium brownei*

ANA LUISA ANAYA,[1,*] RACHEL MATA,[2]
FAUSTO RIVERO-CRUZ,[2]
BLANCA ESTELA HERNÁNDEZ-BAUTISTA,[1]
DANIEL CHÁVEZ-VELASCO,[2] and ARTURO GÓMEZ-POMPA[3]

[1]*Instituto de Fisiología Celular*
UNAM, Ciudad Universitaria
Apdo. Postal 70-243. México 04510, D.F.

[2]*Facultad de Química, Departamento de Farmacia*
UNAM. Ciudad Universitaria
México 04510, D.F.

[3]*Department of Botany and Plant Sciences*
University of California, Riverside
Riverside, California 92521

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Abstract—*Metopium brownei* is a tree that grows in coastal tropical forests along the Gulf of Mexico and in the Yucatan Peninsula. This medicinal species produces a strongly irritant exudate, and sometimes forms pure populations favored by fire. The bioactivity of the aqueous leachates, organic extracts (leaves, bark, and wood), and mixtures of urushiols and flavonoids from *M. brownei* were evaluated on the growth of two plants: *Amaranthus hypochondriacus* and *Echinochloa crusgalli*, and four phytopathogenic fungi: *Fusarium oxysporum*, *Helminthosporium* sp., *Alternaria* sp., and *Pythium* sp. Alkylcatechols (urushiols) were isolated from an acetone extract of the bark. Dihydroquercetin and eriodictyol were isolated from the chloroform–methanol extract of the wood. In addition, masticadienoic acid was isolated from the leaves. The aqueous leachates, organic extracts, and the mixtures of flavonoids and urushiols were inhibitory to the growth of test plants and phytopathogenic fungi. The allelochemical role of the bioactive compounds from *M. brownei* is discussed in relation with other results reported in some studies on Anacardiaceae family and *M. brownei*.

Key Words—*Metopium brownei*, Anacardiaceae, dihydroquercetin, eriodyctiol, masticadienoic acid, urushiols, alkylcatechols, allelochemicals, allelopathics.

*To whom correspondence should be addressed.

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INTRODUCTION

Metopium brownei (Jacq.) Urban. (Anacardiaceae), commonly known as "chechém negro," "boxchechém," "kabal chechém," and "madera negra venenosa," is a tree (8–25 m high) that grows in tropical forests on the Gulf of Mexico coast from Veracruz to the Yucatan Peninsula. The genus *Metopium* is one of the most important in some plant communities (Smith and Vankat, 1992). *M. brownei* is considered a representative arboreal species of the Anacardiaceae that can grow in flooding areas. *M. brownei* is very resistant to fire, and sometimes forms pure populations favored by fire. It has a thin, gray–brown bark, composite smooth leaves (3–7 oval folioles, 3–8 cm), green yellowish small inconspicuous flowers in axillary panicules, and characteristic brilliant red drupe fruits (1–2 cm) (Cabrera-Cano et al., 1982).

Most of the leaves of *M. brownei* fall between April and May, simultaneous with flowering (Rzedowsky, 1978; Cabrera-Cano, et al., 1982; Ramamoorthy et al., 1993). These leaves are almost free of pathogens and herbivorous damage. Soils covered with fallen leaves of this tree show very scarce growth of understory plants. *M. brownei* is used medicinally as an antiviral, cathartic, diaphoretic, antiinflammatory, and sedative; this tree also has economic importance in the region. Its hard, beautiful wood is utilized in the fabrication of wood floors and staves (Martinez, 1979; Amo, 1979; Mendieta and Amo, 1981; Gómez-Pompa et al., 1991).

In a previous study (Rivero-Cruz et al., 1997), the composition of *Metopium brownei* urushiol was established. Three 3-*n*-pentadec(en)ylchatechols (urushiols) with antifungal properties and toxicity to brine shrimp (LC_{50} = 89.13 μg/ml) were identified. Rivero-Cruz et al. (1997) suggested that urushiols could be of importance in the defense mechanisms of the plant by preventing the attack of some fungi and bacteria.

The present study was undertaken to continue the study on the secondary compounds produced by *M. brownei* and to examine the effect of some of them on the growth of plants and fungi, and their possible ecological role.

METHODS AND MATERIALS

Plant Material. The aerial bark and wood of *M. brownei* were collected in the Municipio of Tulum, Quintana Roo, in November 1995 (voucher specimen AL95-16. National Herbarium, MEXU, Instituto de Biología, UNAM). The plant was dried at 30°C. Fallen leaves were collected at the El Eden Ecological Reserve, Quintana Roo, Mexico in June of 1996.

Preparation of Aqueous Leachates. Aqueous leachates of leaves, fallen leaves, bark, and wood were prepared by soaking the dry plant material in dis-

tilled water (2% w/v) for 4 hr. Leachates were filtered and their osmotic pressure measured in a freezing-point osmometer (Osmette A. Precision Systems, Inc.) in order to prevent the negative osmotic effects of a highly concentrated solution on germination and growth of the test plants (Anaya and Rovalo, 1976).

Bioassays with Seeds. Aqueous leachates were mixed (1 : 1) with 1.5% pure agar to obtain a 1% w/v test solution. Pure agar (0.75%) was used as the control. Germination and seedling radicle growth bioassays for the aqueous leachates were carried out according to previously published procedures (Anaya et al., 1990) with *Amaranthus hypochondriacus* L. (Amaranthaceae) and *Echinochloa crusgalli* (L.) Beauv. (Poaceae). The seeds of *A. hypochondriacus* were purchased from Mercado de Tulyehualco, Mexico, D.F., and those of *E. crusgalli* from Valley Seed Service, Fresno, California. The bioassays were set up in 5-cm Petri dishes, 10 seeds per dish, following a complete random block design with four repetitions per treatment. Petri dishes were kept in darkness at 27°C (4). Germination and radicle growth were measured after 24 hr for *A. hypochondriacus* and 48 hr for *E. crusgalli.*

Preparation of Organic Extracts. Organic extracts were prepared by macerating the dry leaves, bark, and wood of the plant in hexane, chloroform, methanol, or chloroform–methanol (1 : 1) at room temperature, for 72 hr. After evaporation of the solvent, each extract was resuspended in order to obtain 50, 100, and 200 μg/ml solutions.

Bioassays with Seeds. Bioassays with organic extracts were set up in the same conditions as with the aqueous leachates, but filter paper was used instead of agar. Filter paper in Petri dishes was impregnated with 1.5 ml of the solutions at 50, 100, and 200 μg/ml, and after total evaporation of the solvent, the paper was moistened with 1.5 ml of distilled water. Test seeds for these bioassays were *A. hypochondriacus* and *E. crusgalli.* Following this same procedure, the crude extract of urushiols from the bark and the crude extract of flavonoids from the wood of *M. brownei* were tested at 50, 100, 200, 700, and 1000 μg/ml on the same species of seeds. In this last bioassay, a positive control of 2,4-D was used.

Bioassays with Phytopathogenic Fungi. Organic extracts and a mixture of urushiols and flavonoids were tested on the radial growth of *Pythium* sp., *Alternaria* sp., *Fusarium oxysporum,* and *Helminthosporium longirostrastum.* The bioassays were carried out in 9-cm Petri dishes by procedures previously described (Castañeda et al., 1992; Hamburger and Cordell, 1987). Organic extracts and flavonoids were tested at 50 and 200 μg/ml. Urushiols were tested at 50, 100, 150, 250, 500, and 700 μg/ml, and Captan, a commercial fungicide, was used as a positive control. The measurements were made after 72 hr of incubation. Bioassays were set in a complete random block design with three repetitions per treatment.

All data were analyzed with ANOVA and Tukey's test.

Phytochemical Experimental Procedures. Brine shrimp lethality test (BST)

bioassays were used as a tool for the biodirected fractionation of *M. brownei* as described by Anderson et al. (1991). Melting points were determined by means of a Fischer Johns apparatus and were uncorrected. IR spectra were measured on a Perkin Elmer 599B spectrometer with KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-3005 instrument operating at 300 MHz and 75 MHz, respectively, in $CDCl_3$, C_5D_5N, or C_6D_6 with TMS as an internal standard. UV spectra were taken on a Shimadzu UV 160U spectrophotometer.

Isolation of Masticadienonic Acid from Leaves of M. brownei. Dried and ground leaves (5.045 kg) were extracted at room temperature with $CHCl_3$–MeOH (1 : 1). After filtration the extracts were combined and concentrated in a vacuum. The resulting residue (396.2 g, BST LC_{50} > 1000 μg/ml) was submitted to open column chromatography on silica gel with step gradient elution: hexane, hexane–$CHCl_3$ (9 : 1, 8 : 2, 7 : 3, 6 : 4, 1 : 1, 3 : 7), $CHCl_3$, and $CHCl_3$–MeOH (99 : 01, 95 : 5, 9 : 1, 9 : 2, 7 : 3, 6 : 4, 1 : 1). A total of 274 fractions (1 liter each) were collected and pooled based on TLC profiles to yield 13 major fractions (FL_1–FL_{13}). FL_3 was subjected to repeated silica gel column chromatography to yield 300 mg of masticadienoic acid, identical to a standard sample previously isolated from *Ampypterygium adstringens* (Navarrete, 1986).

Isolation of Dihydroquercetin and Eriodyctiol from Wood of M. brownei. The air-dried pulverized plant material (1.3 kg) was extracted with a mixture of $CHCl_3$–MeOH (1 : 1). The resulting extract was concentrated on a rotavaporator to give a residue (30 g, BST LC_{50} = 214 μg/ml). This residue was subjected to open column chromatography on silica gel (300 g) and eluted with a gradient of hexane and $CHCl_3$–MeOH. A total of 189 fractions was combined on the basis of their TLC patterns to yield 10 primary fractions (FW_1–FW_{10}). Biological testing of the primary fractions showed that FW_4 was toxic (BST LC_{50} = 181.98 μg/ml). Similarly, using the direct bioautographic TLC assay for detecting antifungal substances, only fraction FW_4 contained the fungitoxic activity. FW_4 (1.92 g) was rechromatographed on a flash silica gel (45 g) column by using benzene with increasing amounts of ethyl acetate to yield dihydroquercetin (80 mg), mp 235–240°C [lit. mp, 240–242°C] and eriodyctiol (8 mg), mp = 265–267°C [lit. mp, 267°C].

RESULTS

In Figures 1 to 12, only the most significant results of the present study are shown.

Bioassays with Seeds. The aqueous leachates (1%) of fresh and fallen leaves and bark inhibited radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli*, but the aqueous leachate of wood did not produce any effect on radicle growth of either target species (Figure 1). All aqueous leachates

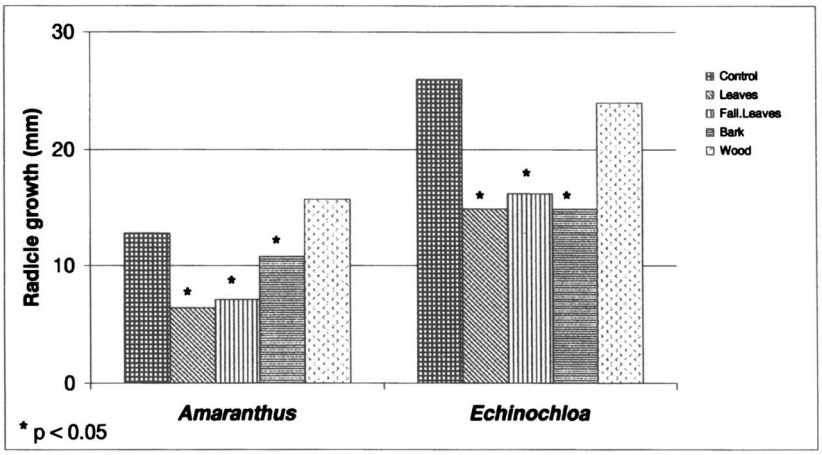

FIG. 1. Effects of aqueous leachates leaves, fallen leaves, bark, and wood of *Metopium brownei* on the radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli* ($N = 4$).

(1%) showed very low osmotic pressure values (14–35 mosm/liter). These osmotic pressure values do not affect the growth of the seeds as previous studies have shown (Anaya and Rovalo, 1976). The results of bioassays carried out with hexane extracts of leaves are shown in Figure 2. The hexane extract of leaves inhibited radicle growth of *A. hypochondriacus* at 100 and 200 μg/ml, but had a nonsignificant effect on *E. crusgalli*. Compared with the effect of the aqueous leachate of leaves, the hexane extract of leaves had a less inhibitory effect than the aqueous leachate of leaves (Figure 1). Methanol and chloroform extracts of leaves had no effect on either test plant species (results not shown). Frequently, the phytotoxic effect of plant aqueous leachates is lost when the bioactive compounds from the plant are extracted with organic solvents. Figure 3 shows the effects of the chloroform–methanol (1 : 1) extracts from the bark and the wood at three concentrations on the radicle growth of both test plants. The extract had a significant inhibitory effect on the radicle growth of *E. crusgalli* at 100 and 200 μg/ml but no effect on *A. hypochondriacus*. The chloroform–methanol extract from the wood inhibited radicle growth of *E. crusgalli* only at 200 μg/ml.

Phytochemical fractionation of a $CHCl_3$–MeOH extract of the leaves of *M. brownei* allowed the isolation and identification of masticadienoic acid (Figure 4c). This compound has no effect on the radicle growth of *Amaranthus* and *Echinochloa* (results not shown).

The $CHCl_3$–MeOH extract from the wood was fractionated by column chromatography to render a flavonoid mixture, and eriodyctiol and dihydroquercetin were isolated (Figure 4a and b). These compounds were identified

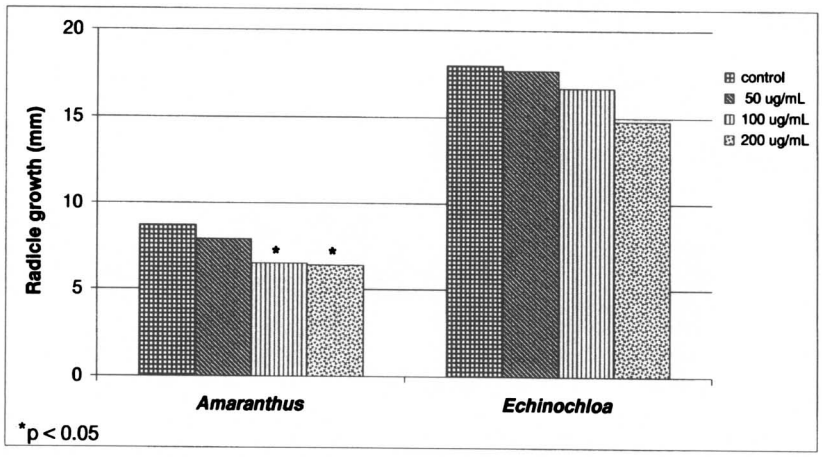

FIG. 2. Effects of the hexane extract of leaves of *Metopium brownei* (50, 100, and 200 μg/ml) on the radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli* ($N = 4$).

by spectral means (Wagner et al., 1976; Shen and Chang, 1993). The acetone extract from the stem bark was subjected to column chromatography on silica gel to yield an urushiol fraction that contained 3-pentadecylcatechol (Figure 5a), 3-(10′*Z*-pentadecenyl)catechol (Figure 5b), and 3-(10′*Z*, 13′*E*-pentadecadi-

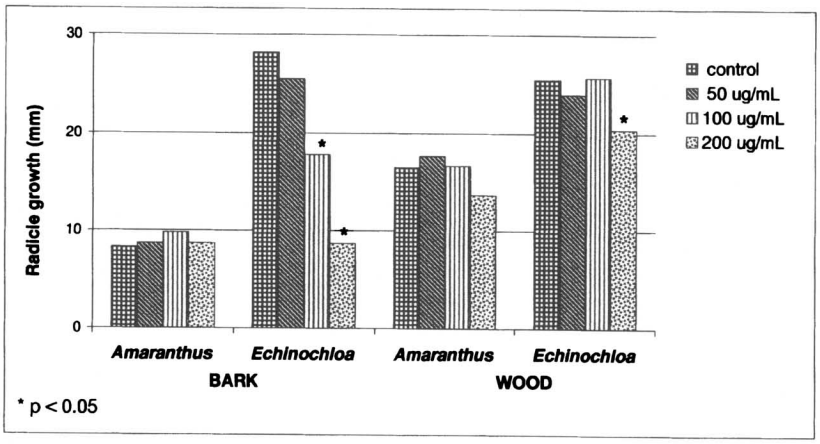

FIG. 3. Effects of organic extracts of bark and wood of *Metopium brownei* (50, 100, and 200 μg/ml) on the radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crusgalli* ($N = 4$).

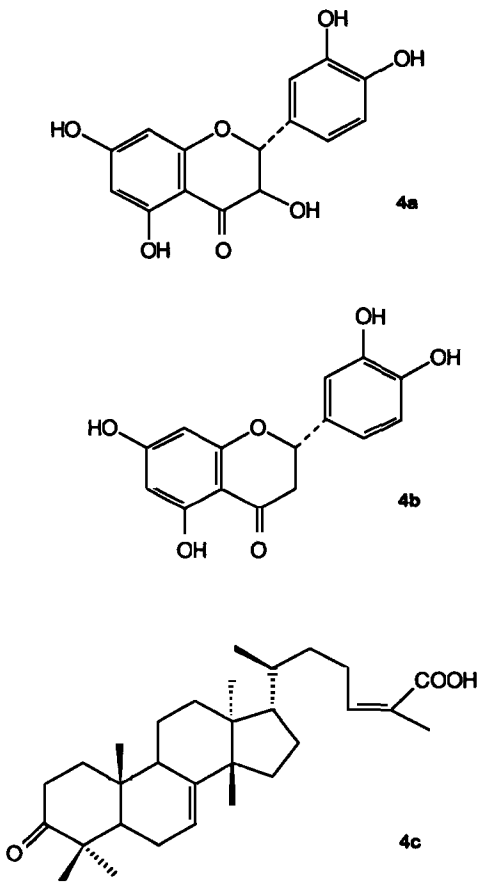

FIG. 4. Structures of dihydroquercetin (a) eriodictyol (b), and masticadienoic acid (c) from *Metopium brownei*.

enyl)catechol (Figure 5c). The separation and identification of these alkyl catechols were previously described (Rivero-Cruz et al., 1997).

Figure 6 shows the effects of five concentrations of flavonoids and urushiols, and 2,4-D on the radicle growth of *A. hypochondriacus*. Flavonoids inhibited radicle growth at 200–1000 μg/ml. Linear regression analysis indicated an $IC_{50} > 1000$ μg/ml. Urushiols inhibited *Amaranthus* radicle growth only at 700 and 1000 μg/ml ($IC_{50} > 1000$ μg/ml) and had a less inhibitory effect compared with flavonoids. 2,4-D used as a positive control had a strong inhibitory effect on *Amaranthus* at 50 μg/ml and totally inhibited radicle growth at 100 μg/ml ($IC_{50} = 16.1$ μg/ml).

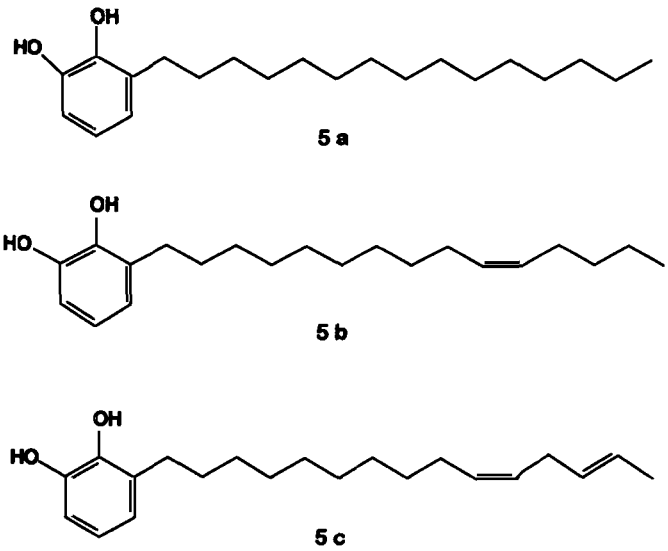

FIG. 5. Structures of urushiols, 3-pentadecylcatechol (a), 3-(10′*Z*-pentadecenyl)catechol (b), and 3-(10′*Z*, 13′*E*-pentadecadienyl)catechol (c) from *Metopium brownei* bark.

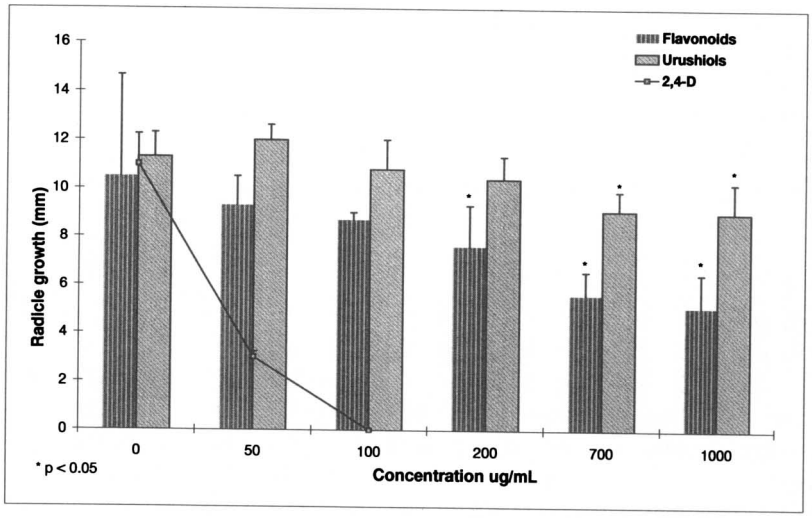

FIG. 6. Effects of flavonoids and urushiols from *Metopium brownei* at six concentrations, and 2,4-D on the radicle growth of *Amaranthus hypochondriacus* ($N = 4$).

Figure 7 shows the results of the bioassays testing flavonoids, urushiols, and 2,4-D on the radicle growth of *E. crusgalli*. At low concentrations (50–200 μg/ml) flavonoids did not inhibit radicle growth. Flavonoids inhibited only at 700 and 1000 μg/ml (IC_{50} = 628.3 μg/ml). Urushiols inhibited *Echinochloa* from 50 to 1000 μg/ml (IC_{50} > 1000 μg/ml). However, the effect of flavonoids at high concentrations was stronger than the effect of urushiols. *Echinochloa* was more resistant to 2,4-D (IC_{50} = 35.2 μg/ml) than *Amaranthus*.

Bioassays with Phytopathogenic Fungi. A methanol–chloroform (1 : 1) extract from the leaves of *M. brownei* had a significant but moderate inhibitory effect only on *Fusarium* radial growth (results not shown). The methanol extract of the bark inhibited the radial growth of *Helminthosporium* and *Pythium* (50 and 200 μg/ml), and also inhibited *Fusarium growth* at 200 μg/ml (Figure 8). The methanol extract of the wood of *M. brownei* inhibited *Pythium* (200 μg/ml) and *Helminthosporium* (50 and 200 μg/ml) (Figure 9). A significant inhibitory effect of two concentrations of flavonoids was observed on *Pythium* and *Alternaria* at 200 μg/ml (Figure 10).

All concentrations of urushiols produced a significant inhibitory effect on the radial growth of *Fusarium* (30–64% inhibition) (IC_{50} = 150 μg/ml) (Figure 11). The fungicide Captan, used as a positive control in this bioassay, had higher inhibitory effect compared with the urushiols at low concentrations (50 and 100

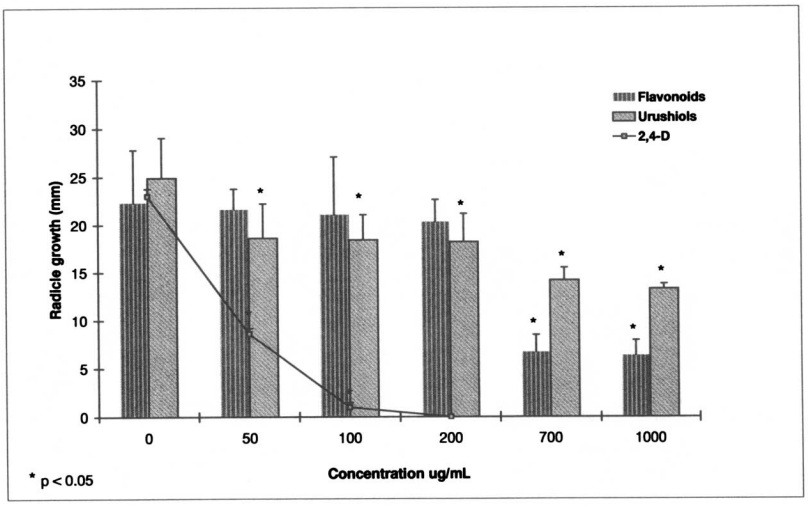

FIG. 7. Effects of flavonoids and urushiols from *Metopium brownei* at six concentrations, and 2,4-D on the radicle growth of *Echinochloa crusgalli* (*N* = 4).

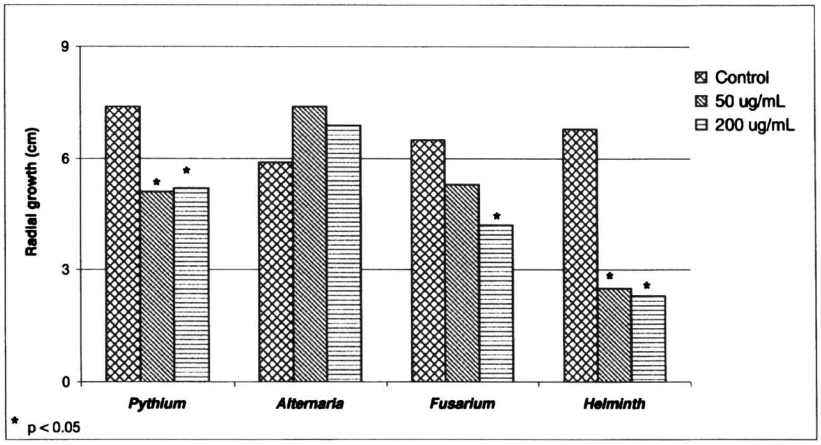

FIG. 8. Effects of methanol extract (1 : 1) of bark from *M. brownei* at 50 and 200 μg/ml on the radial growth of four phytopathogenic fungi (N = 3).

μg/ml) and high concentrations (500 and 700 μg/ml), but both had a very similar effect at 150 and 200 μg/ml (IC_{50} = 85.86 μg/ml).

Helminthosporium was also significantly inhibited by the urushiols at all concentrations tested (IC_{50} = 224.9 μg/ml) (Figure 12). The effects of Captan and urushiols were very similar on the growth of *Helminthosporium* from 150 to

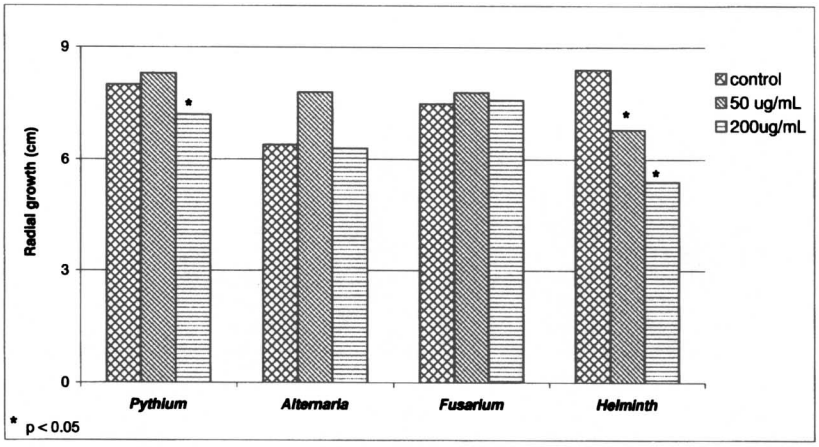

FIG. 9. Effects of the methanol extract (1 : 1) of wood from *M. brownei* at 50 and 200 μg/ml on the radial growth of four phytopathogenic fungi (N = 3).

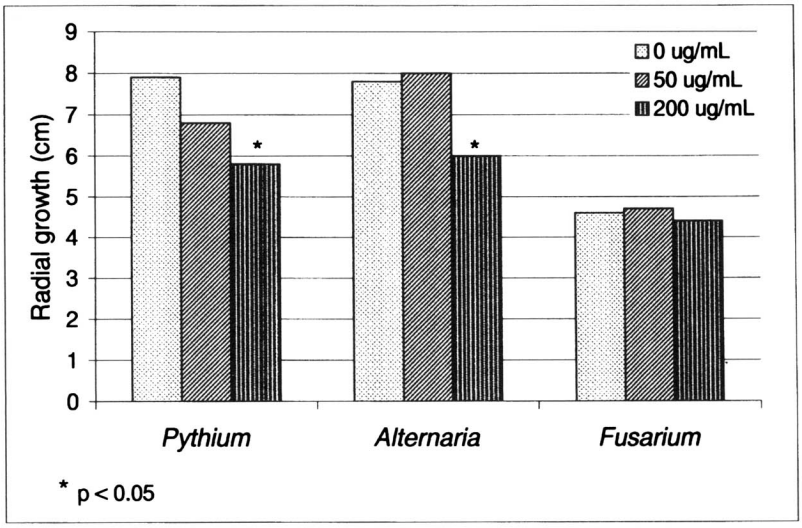

FIG. 10. Effects of flavonoids from *M. brownei* wood at 50 and 200 μg/ml on the radial growth of three phytopathogenic fungi (N = 3).

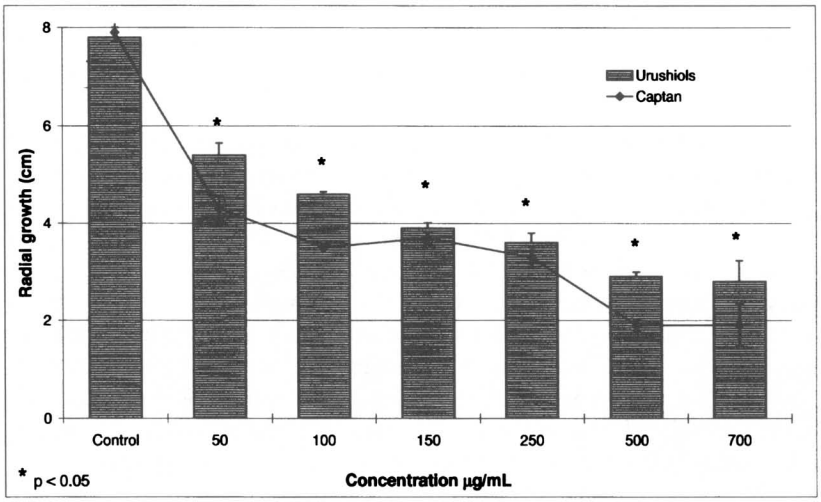

FIG. 11. Effects of urushiols from *M. brownei* bark at six concentrations on the radial growth of *Fusarium oxysporum* (N = 3).

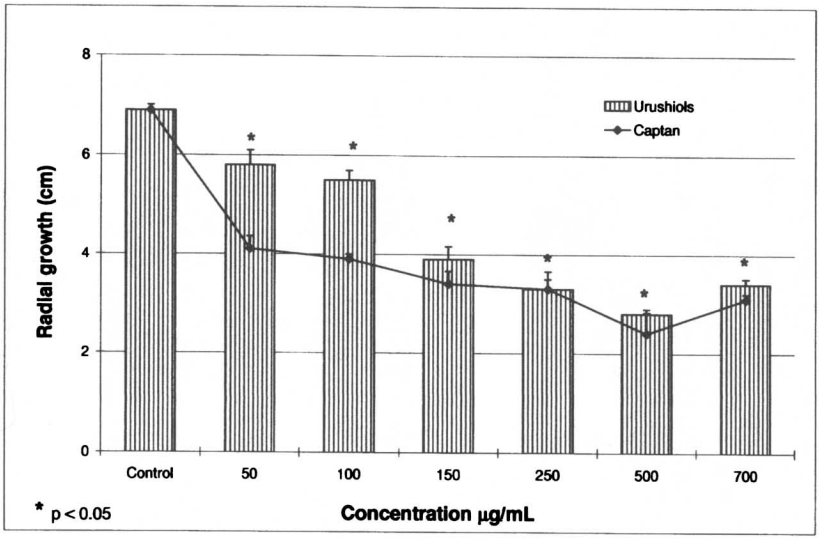

FIG. 12. Effects of urushiols from *M. brownei* bark at six concentrations on the radial growth of *Helminthosporium longirostratum* ($N = 3$).

700 µg/ml (IC_{50} = 155.3 µg/ml). Compared with *Fusarium*, *Helminthosporium* was more resistant to urushiols, particularly at lower concentrations.

DISCUSSION

The great majority of flavonoids, the best-known group of polyphenols, comprises some 4000 structures of plant origin. Most phenolics are toxic to living organisms. Eriodictyol and dihydroquercetin in *M. brownei* belong to the group of minor flavonoids (Harborne and Baxter, 1993) that have more limited natural distribution than anthocyanins, flavones, and flavonols. Eriodictyol possesses antibacterial activity. It shows growth inhibitory activity against larvae of *Heliothis zea*, and induces nodulation gene expression in *Rhizobium leguminosarum* in the symbiosis between this bacterium and its legume host, *Pisum sativum*. Dihydroquercetin (Taxifolin) has many biological activities. It is antimicrobial, antiinflammatory, antihepatotoxic, and antioxidant. It stimulates RNA synthesis, inhibits different enzyme activities, and inhibits the growth of *Heliothis zea* (Harborne, 1991). The influence of plant flavonoids on herbivore feeding (Coleoptera: Chrysomelidae), mainly *Rhus* spp., has been noted (Furth and Young, 1988). In the present study we observed a phytotoxic activity of flavonoids from the wood of *M. brownei* on radicle growth of *A. hypochondri-*

acus and particularly on that of *E. crusgalli*. Flavonoids also had a significant inhibitory effect on the radial growth of *Pythium* sp. and *Alternaria* sp.

The literature on Anacardiaceae shows that regardless of the geographical locality, different species of this family cause problems for mammals, including humans. The most common effect is allergic dermatitis. Poison ivy, mangoes, cashews, an indigenous Anacardiaceae at the Southern Transvaal, *Smodingium argutum*, and many other species, including *M. brownei*, are involved in health problems (Geller, 1989; Gorst-Allman et al., 1987; Wahlberg and Lovell, 1996; Rivero-Cruz et al., 1997). Sometimes pistachio nuts can provoke anaphylaxis, but the allergens involved are still poorly characterized (Fernandez et al., 1995). Rivero-Cruz et al., (1997) confirmed that *Metopium brownei* produces alkenyl phenols (with a mono-, di-, or triunsaturated side chain), as do most of the allergenic Anacardiaceae species that have been studied. In the Navassa Islands, West Indies, *M. toxiferum* causes contact dermatitis in goats (Brown et al., 1973). It has been demonstrated that urushiols inhibit arachidonic acid metabolism and could be used as anti-allergic agents in hyposensitization therapy (Gross et al., 1975; Watson et al., 1981, 1983; Duke, 1985; Harborne and Baxter, 1993).

Saps of Anacardiaceae contain mixtures of catechols and are used to produce lacquer ware. Japanese lacquer originates from the sap of *Toxicodendron vernicifluum* (syn. *Rhus verniciflua*), the lacquer tree (Vogl et al., 1995; Vogl and Mitchell, 1996; Qin et al., 1996). The antimicrobial effects of the saps probably are related with their content of catechols. The gum exudate of the cashew tree (*Anacardium occidentale*) inhibited growth of some fungi and bacteria, and the gum also prevented oviposition and reduced the number of larvae of the Bruchid beetle *Callosobruchus maculatus*. The feeding of the larvae of the chrysomelid beetle *Crimissa cruralis* was also strongly affected by the gum (Marques et al., 1992). Reyes-Chilpa et al. (1987) found that extractives from different tree species, including Anacardiaceae *Astronium graveolens*, are primarily involved in heartwood resistance to brown rot fungus *Lenzites trabea*. Several studies also showed that the presence of antifungal compounds in *Rhus glabra* branches and mango fruits, and the effect of phytopathogenic fungi on mangiferin production in the twigs of mango (Droby et al., 1985, 1986; Chakrabarty and Ghosal, 1985; McCutcheon et al., 1994). Furthermore, under field conditions, water extracts of *Metopium brownei* diminished the damage produced to a corn crop by the fall armyworm, *Spodoptera frugiperda* (Gonzalez-Gaona and Lagunes-Tejeda, 1986). These results, including the fungitoxic activity of *M. brownei* urushiols that we observed in the present study, suggest an active role for bioactive metabolites of the gum, latex, and sap of various Anacardiaceae in defense mechanisms of the plants.

Anacardiaceae is one of the plant families from which the native bees *Melipona rufiventris paraensis* and *Frieseomelitta varia* collected pollen in Amazonia (Marques-Souza et al., 1995). A study of nectar sources of European and

Africanized honey bees (*Apis mellifera* L.) in the Yucatan Peninsula during the wet and dry seasons showed that the most common species in honey samples was *M. brownei* (Villanueva, 1994). In addition, *M. toxiferum* (poison-wood) is one of the five dominant species of the diet of nestling white-crowned pigeons (*Columba leucocephala*) from July through September (Bancroft and Bowman, 1994). These examples call attention to different ecological adaptations of several organisms in their interactions with Anacardiaceae species. Different bioactive secondary compounds (including flavonoids and urushiols) are involved in these biotic interactions. The bioactivities differ depending on the type of biotic relation that the plant establishes.

Coley and Barone (1996) suggested that leaves of tropical forests have both higher overall levels of defense and a greater diversity of defense compared to their temperate counterparts. This greater commitment to defense is an evolutionary response to elevated pressure from herbivores. In the tropics, mature leaves are long-lived and must therefore be resistant to both abiotic and biotic damage. These defense mechanisms are present in other organs of the plant, such as stem bark, that has great herbivory pressure, mainly from microorganisms. This is also true for *M. brownei*, whose bark possesses defensive urushiols that can be effective on different herbivores (microorganisms to mammals) and they have phytotoxic effects.

Urushiols and flavonoids, among other secondary metabolites of *M. brownei*, afford different kinds of protection to the plant, as antibiotics, antifeedants, allergens, and probably allelopathics. Further studies on biotic interactions between *M. brownei* and their neighboring plants, endophytic and pathogen fungi, insects, and also vertebrates, will shed more light on the ecological role of the different bioactive secondary metabolites that this important Anacardiaceae species of the Peninsula of Yucatan, Mexico produces.

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