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Antimicrobial activity of *Schinus lentiscifolius* (Anacardiaceae)

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ABSTRACT

Ethnopharmacological relevance: *Schinus lentiscifolius* Marchand (syn. *Schinus weinmannifolius* Engl) is a plant native to Rio Grande do Sul (Southern Brazil) and has been used in Brazilian traditional medicine as antiseptic and antimicrobial for the treatment of many different health problems as well as to treat leucorrhea and to assist in ulcer and wound healing. Although it is a plant widely used by the population, there are no studies proving this popular use.

Material and methods: The crude aqueous extract, the crude neutral methanol extract, fractions prepared from this extract (*n*-hexane, ethyl acetate, and *n*-butanol), pure compounds isolated from these fractions, and derivatives were investigated *in vitro* for antimicrobial activities against five Gram positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis, Staphylococcus saprophyticus*, *Streptococcus pyogenes*, three Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa*, and *Shigella sonnei*, and four yeasts: *Candida albicans, Candida tropicalis, Cryptococcus neoformans, and Saccharomyces cerevisiae*. The isolated compound moronic acid, which is the most active, was tested against a range of other bacteria such as two Gram positive bacteria, namely, *Bacillus cereus*, *Enterococcus* spp, and six Gram negative bacteria, namely, *Burkholderia cepacia*, *Providencia stuartii*, *Morganella morganii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Proteus mirabilis*.

Results: The leaf aqueous extract (decoction) of *Schinus lentiscifolius* showed a broad spectrum of antibacterial activity, ranging from 125 to 250 μg/ml (MIC) against the tested bacteria and fungi. The *n*-hexane extract, despite being very little active against bacteria, showed an excellent antifungal activity, especially against *Candida albicans* (MIC=25 μg/ml), *Candida tropicalis* (MIC=15.5 μg/ml), and *Cryptococcus neoformans,* (MIC=15.5 μg/ml). From the acetate fraction (the most active against bacteria), compounds **1–6** were isolated: nonadecanol (**1**), moronic acid (**2**), gallic acid methyl ester (**3**), gallic acid (**4**), quercetin (**5**) and quercitrin (**6**). The minimal inhibitory concentration (MIC) of moronic acid between 1.5 and 3 μg/ml against most of the tested bacteria shows that it is one of the metabolites responsible for the antibacterial activity of *Schinus lentiscifolius*.

Conclusion: The antimicrobial activity and some constituents of *Schinus lentiscifolius* are reported for the first time. The results of the present study provide scientific basis for the popular use of *Schinus lentiscifolius* for a number of different health problems.

1. Introduction

The genus *Schinus* (Anacardiaceae) encompasses about 600 plant species typical of tropical and subtropical regions. The species *Schinus terebinthifolius* Raddi, *Schinus molle* Hort. ex Engl., *Schinus polygama* (Cav.) Cabr., and *Schinus lentiscifolius* March are native and common species in the forest of Rio Grande do Sul (Biome Campos Sulinos, RS, Brazil), Uruguay, Paraguay, Argentina and Peru. The first three species above mentioned have been used in South American folk medicine as antiseptic, antimicrobial and repellent (Siddiqui et al., 1996; Wimalaratne et al., 1996; Guerra et al., 2000; Ferrero et al., 2006, 2007; Lima et al., 2006; Deveci et al., 2010; Santos et al., 2010) for the treatment of many different health problems. Moreover, they have also been used to treat leucorrhea, to heal ulcers and wounds (Bacchi, 1986; Fenner et al., 2006), and to fight uterine inflammation (Amorin and Santos, 2003), as well as analgesic and central depressant (Barrachina et al., 1997). Previous phytochemical studies of this species have resulted in the isolation of various sesquiterpenes, triterpenes, flavonoids, tannins, steroidal saponins, sterols, gums, resins and essential oils (Yueqin et al., 2003; Erazo et al., 2006; Diaz et al., 2008).

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Although *Schinus lentiscifolius* has a different morphology from *Schinus molle*, it is often confused and used in folk medicine as *Schinus molle*. Although little has been documented about the popular use of *Schinus lentiscifolius*, the book Medicinal Plants in Brazil (Lorenzi and Matos, 2002) mentions that the medicinal application of *Schinus lentiscifolius* is the same as *Schinus molle*. Phytochemical studies of *Schinus molle* have resulted in the isolation of mono-sesqui and triterpenes, flavonoids, gallotannins and fatty acids (Hänsel et al., 1994), triterpeoidal ketoacids and biflavone (Pozzo-Balbi et al., 1978; Yueqin et al., 2003), antioxidant flavonol glycosides (Marzouk et al., 2006), alkaloids, tannins, and essential oils (Diaz et al., 2008). There are only few reports on the chemical composition of the essential oil of the species *Schinus lentiscifolius* (Rossini et al., 1996). Thus, due to lack of information about the chemical components and activity of *Schinus lentiscifolius*, the aim of this study was to describe the composition and the antimicrobial activity of different extracts, fractions, and isolated compounds from the leaves of this specie.

2. Material and methods

2.1. Material and reagents

Melting points of the isolated compounds and derivatives were determined with a "MQAPF-301" apparatus and are uncorrected. Optical rotations were taken on a Perkin Elmer 341 digital polarimeter. Low resolution ESI-MS was recorded on an Agilent LC/MS/MS model 6460. ^{1}H- and ^{13}C-NMR spectra were recorded at 400.1/100.6 MHz on a Bruker DPX-400 spectrometer using $CDCl_3$, CD_3OD and DMSO-d_6 as solvent and TMS as internal standard. Thin layer chromatography was performed on pre-coated TLC plates (Merck, silica 60 F-254) by spraying with Liebermann–Burchard's reagent and 10% H_2SO_4/EtOH, followed by heating.

2.2. Plant material, preparation of the extracts, and isolation

The aerial parts of *Schinus lentiscifolius* were collected in May 2010 in Ijui (latitude 28°23'16" south and longitude 53°54'53" west), RS, Brazil, and authenticated by Prof Mara Lisiane Tissot Squalli from the Department of Biology and Chemistry of the University of Ijuí (DBQ-INIJUI) where a specimen sample (6.376) is retained.

The dried aerial parts of *Schinus lentiscifolius* (400 g) was powdered and extracted (300 g) four times with MeOH at room temperature. The MeOH extract (ME) was filtered and concentrated in vacuum to obtain a crude extract (50.1 g). Part of this extract (40 g) was dissolved in H_2O and extracted successively with *n*-hexane (HF, 14 g), ethyl acetate (AF, 10 g) and *n*-butanol (BF, 8 g). A portion of the ethyl acetate fraction (5 g) was applied to silica gel column (400 g) which was eluted with *n*-hexane containing increasing amounts of acetone (up to 100%) to give 20 fractions. Fractions 2–3 (*n*-hexane:acetone 99:1) were combined to yield **1** (120 mg). Fractions 6 and 8 (*n*-hexane:acetone 90:10) were combined (250 mg) and submitted to preparative TLC (*n*-hexane:acetone 90:10, two elutions) to yield **2** (220 mg). Fraction 12 (*n*-hexane:acetone 70:30) was submitted to preparative TLC (150 mg) (*n*-hexane:acetone 60:40, two elutions) to yield **4** (115 mg). Fraction 18 (*n*-hexane:acetone 30:70) was submitted to preparative TLC (40 mg) (ethyl acetate: MeOH, 93:7, two elutions) to yield **5** (16 mg). Fraction 21–22 (*n*-hexane:acetone 10:90) were combined and evaporated to yield 3 (180 mg). Compounds **1,3–6** were identified by direct comparison (TLC, GC, and HPLC) with authentic samples of nonadecanol (**1**), gallic acid methyl ester (**3**), gallic acid (**4**), quercetin (**5**) and quercitrin (**6**). Identification of moronic acid (2) {mp 210–211 °C, $[\alpha]_D^{25}$+50.2 (c 0.1, $CHCl_3$)} was made by comparison of its spectral data (EIMS, ^{1}H NMR and ^{13}C NMR, ^{1}H-^{1}H-COSY, DEPT 135, HMQC, and HMBC) with reported values in the literature (Ito et al., 2001).

To prepare the aqueous extract (decoction), a portion of the dry ground powder leaves (100 g) was extracted in distilled water (boiled in water for 5 min) in the ratio 1:5(w/v). The resulting solution was lyophilized into dry fine powder yielding an aqueous crude extract (16.4 g).

2.3. Preparation of the alcohol derivatives of moronic acid

The amount of 100 mg (0.209 mmol) of **2** was transferred to a round-bottomed flask and dissolved in 9 ml of absolute ethanol (10 ml) and THF (2 ml). The mixture was then cooled in ice water bath and added sodium borohydride (4 mg, 0.109 mmol). The reaction was left to react for 12 h, and the solvent was evaporated. Ice water was added, and the reaction was extracted three times with ethyl acetate. Next, the organic layer was collected, dried with Na_2SO_4, and evaporated. The resulting residue was chromatographed on a silica gel column yielding two products with yields of 60% and 20%, which were analyzed by NMR spectroscopy and characterized as diastereoisomeric alcohol morolic acid (7) {mp 200–201 °C, $[\alpha]_D^{25}$+29 (c 0.059, $CHCl_3$)}, and acridocarpusic acid D (**8**) {mp 242–244 °C, $[\alpha]_D^{25}$+15.6 (c 0.1, MeOH)}. The identification of the compounds **7** and **8** was made by comparison of their spectral data (EIMS, ^{1}H NMR and ^{13}C NMR, ^{1}H- DEPT 135, HMQC, and HMBC) with reported values in the literature (Ito et al., 2001; Cao et al., 2004).

2.4. Preparation of moronic acid methyl ester

For methylations with diazomethane, 10 mg (0.02 mmol) of **2** was treated with an excess of ethereal diazomethane. After the mixture had been kept at room temperature until the evolution of nitrogen had ceased (about 30 min) the excess of diazomethane and the ether were removed in vacuum yielding 9.8 mg of **9** {mp 168 °C, $[\alpha]_D^{25}$+60 (c 0.1, $CHCl_3$)}.

2.5. Antimicrobial activity

Compounds were also evaluated for their antimicrobial activity against seven Gram positive bacteria: *Bacillus cereus* (ATCC 33019), *Bacillus subtilis* (ATCC 6633), *Enterococcus* spp (ATCC 6589), *Staphylococcus aureus* (ATCC 6538p), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus saprophyticus* (ATCC 15305); nine Gram-negative bacteria: *Burkholderia cepacia* (ATCC 17759), *Enterobacter aerogenes* (ATCC 13048), *Enterobacter cloacae* (ATCC 1304), *Escherichia coli* (ATCC 25922), *Morganella morganii* (ATCC 8019), *Proteus mirabilis* (ATCC 25933), *Providencia stuartii* (ITB 1971), *Pseudomonas aeruginosa* (ATCC 17759), and *Shigella sonnei* (ATCC 15305), and three yeasts: *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformans* (ATCC 208821), and *Saccharomyes cerevisiae* (ATCC 2601).

The minimal inhibitory concentration (MIC) was determined on 96-well culture plates by a micro dilution method using a microorganism suspension at a density of 10^5 CFU ml^{-1} with casein soy broth incubated for 24 h at 37 °C for bacteria, and Sabouraud Broth incubated for 48 h at 25 °C for yeasts. Cultures that did not present growth were used to inoculate plates of solid medium (Muller Hinton Agar and Sabouraud Agar) in order to determine the minimal lethal concentration (MLC). Proper blanks were assayed simultaneously and samples were tested in triplicate. The reference antibiotics used were levofloxacin, chloramphenicol and ampicillin for bacteria, as well as nistatin for yeast (Sigma). Technical data have been described previously

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