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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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(National Committee for Clinical Laboratory Standards (NCCL), 2002a for yeast and National Committee for Clinical Laboratory Standards, (NCCL), 2002b for bacteria).

2.6. Bacteriolytic activity

2.6.1. Bacteria and culture conditions

Bacterial cells were cultivated in casein soy broth (17 g of enzymatic digest of casein, 3 g of enzymatic digest of soybean meal, 2.5 g of dextrose, 5 g of sodium chloride, 2.5 g of dipotassium phosphate per liter, pH 7.3) at 27 °C. The overnight bacterial culture cells were harvested by centrifugation (10 min, 3000 rpm), resuspended in fresh casein soy broth, and the number adjusted to approximately 5×10^7 cells per ml ($OD_{600\ nm}$~0.2 with a SP-22 spectrophotometer, Biospectro, Brazil). Bacterial culture in broth, solutions of antibiotics, and test samples were mixed in wells of a 96-well plate for optical density measurements.

2.6.2. Optical density (OD) measurements

Bacterial culture (180 μl) was added in a 96-well microplate. The 96-well microplate was placed in a Spectra Max M2 microplate spectrophotometer (Molecular Devices Corp.) programmed to obtain measurements at 23 °C. Optical densities of cell cultures were read using a 620 nm emission filter each 15 min with shaking (30 s) between each reading measurement. Cells were further cultivated to mid-logarithmic phase. Solutions of each test samples and antibiotic (20 μl) at different concentrations in fresh casein soy broth were added in a 96-well microplate. The 96-well microplate was placed in a Spectra Max M2 and OD of cell cultures containing test samples and antibiotic were read after 10 h of incubation. Technical data have been described previously (Lehtinen et al., 2006).

2.7. Statistical analysis

The effects of different concentrations of moronic acid and ampicillin used as positive control on bacteria proliferation were determined using one-way analysis of variance (ANOVA) repeated measures followed by Bonferroni post hoc test. OD is presented as percent of control no treated group. A p value of less than 0.05 was considered significant. Analyses were carried out using the SPSS statistical software, version 18.0 (SPSS Inc., Chicago, IL).

3. Results and discussion

Results of the antimicrobial activity of the decoction, methanol crude extract, fractions of this extract, isolated metabolites of *Schinus lentiscifolius*, and moronic acid derivatives are summarized in Tables 1–3. The leaf methanol crude extract of *Schinus lentiscifolius* exhibited a significant inhibitory activity against some of the microorganisms tested. The methanol extract was partitioned between water and *n*-hexane, ethyl acetate and *n*-butanol, respectively. The *n*-hexane fraction showed an excellent antifungal activity against the strains of the tested fungi. This activity is possibly due to the content of volatile constituents of this fraction (mono-and sesquiterpenes). The fraction ethyl acetate was the most active against a panel of tested bacteria. A bioassay-guided study of the ethyl acetate fraction led to the isolation and identification of nonadecanol (**1**), moronic acid (**2**), gallic acid methyl ester (**3**), gallic acid (**4**), quercetin (**5**) and quercitrin (**6**). Moronic acid (**2**) was previously isolated from *Ozoa mucronata* (Hostettmann-Kaldas and Nakanishi, 1979), *Rhus javanica* (Kurokawa et al., 1999) and from Brazilian propolis (Ito et al., 2001); however, this is the first report of the isolation of **2** from *Schinus sp*. Although **2** is a known triterpene, its presence in a medicinal plant is interesting because of the various biological activities assigned to it, such as Anti-Herpes Simplex Virus (Kurokawa et al., 1999), anti-tumor (Rios et al., 2001), anti-AIDS agent, (Ito et al., 2001; Yu et al., 2006), anti-diabetic (Ramirez-Espinosa et al., 2011), antimicrobial (Hostettmann-Kaldas and Nakanishi, 1979), inhibition of the Epstein–Barr virus (Chang et al., 2010), among others. In addition, **2** was subjected to a stereoselective reduction of the carbonyl group to the corresponding alcohol morolic acid (**7**) and acridocarpusic acid D (3-*epi*-morolic acid, **8**) in a 3:1 ratio (Fig. 1), both pentacyclic triterpenes found naturally. Morolic acid was originally isolated from the heart-wood of *Mora exelsa* (Barton and Brooks, 1950) and

Table 1
Antibacterial activity of *Schinus lentiscifolius*: Extracts, fractions, isolated compounds and derivatives (μg/ml).

Tested sample	Microorganisms															
	Gram positive										Gram negative					
	Bacillus subtilis		*Staphylococcus aureus*		*Staphylococcus epidermidis*		*Streptococcus pyogenes*		*Staphylococcus saprophyticus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Shigella sonnei*	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
H_2O crude ext.	250	> 500	125	> 500	125	> 500	125	> 500	125	> 500	125	> 500	250	> 500	125	> 500
MeOH crude ext.	250	> 500	250	> 500	500	> 500	250	> 500	250	> 500	500	> 500	250	> 500	> 500	> 500
n-Hexane fraction	> 500	> 500	> 500	> 500	500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	500	> 500	> 500	> 500
EtOAc fraction	125	> 500	125	> 500	250	> 500	125	> 500	125	> 500	250	> 500	250	500	125	> 500
n-Butanol fraction	250	> 500	250	> 500	250	> 500	250	> 500	250	> 500	250	> 500	250	> 500	250	500
Nonadecanol	50	> 500	200	> 200	200	> 200	50	> 500	50	> 500	200	> 200	> 200	> 200	100	> 200
Moronic acid	1.52	25	1.52	25	100	> 200	1.52	25	1.52	25	3.12	25	100	> 200	1.52	25
Morolic acid	100	> 200	200	> 200	100	> 200	50	> 200	50	> 200	100	> 200	100	> 200	100	> 200
Moronic acid OMe	200	> 200	200	> 200	NT	NT	NT	NT	> 200	> 200	200	> 200	100	> 200	NT	NT
Acridocarpusic acid D	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200
Quercetin	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200	100	200	100	> 200	100	> 200
Quercitrin	200	> 200	200	> 200	200	> 200	200	> 200	200	> 200	200	> 200	200	> 200	100	> 200
Gallic acid	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200	100	> 200
Gallic acid OMe	> 200	> 500	> 200	> 500	> 200	> 500	> 200	> 500	> 200	> 500	> 200	> 500	> 200	> 500	> 200	> 500
Levofloxacin	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52	6.25	1.52	1.52
Chloramphenicol	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12
Ampicillin	10	NT	15	NT	NT	NT	NT	NT	NT	NT	31	NT	NT	NT	31	NT

MIC (minimum inhibitory concentration in μg/ml) and MLC (minimum lethal concentration in μg/ml), NT (not tested).

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Table 2
Antifungal activity of *Schinus lentiscifolius*: extracts, fractions, isolated compounds and derivatives (μg/ml).

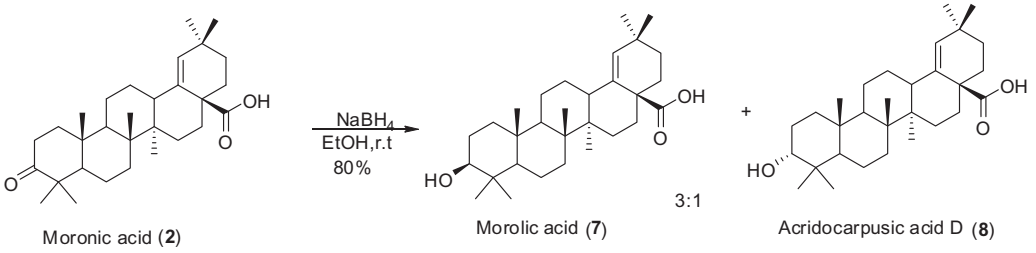
Tested sample	*Candida albicans*		*Cryptococcus neoformans*		*Candida tropicalis*		*Saccharomyces cerevisiae*	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
H_2O crude ext	250	250	250	250	125	250	250	500
MeOH crude ext	500	500	500	> 500	500	> 500	250	500
n-Hexane fraction	25	250	15.5	250	15.5	125	125	> 500
EtOAc fraction	250	500	500	500	500	> 500	500	500
n-Butanol fraction	500	> 500	250	> 500	125	> 500	500	> 500
Nonadecanol	200	> 200	100	200	100	200	200	> 200
Moronic acid	> 200	> 200	200	> 200	> 200	> 200	200	200
Morolic acid	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Moronic acid OMe	200	> 200	50	200	100	200	200	> 200
Acridocarpusic acid D	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Quercetin	100	200	100	200	100	200	100	> 100
Quercitrin	100	200	100	200	100	> 200	200	> 200
Gallic acid	200	200	100	200	100	200	100	> 200
Gallic acid OMe	100	> 200	100	> 200	50	> 200	125	> 200
Nistatin	1.52	6.25	1.52	6.25	3.12	6.26	1.52	6.25

MIC (minimum inhibitory concentration in μg/ml) and MLC (minimum lethal concentration in μg/ml).

Table 3
Antibacterial activity of moronic acid (μg/ml).

Compound	Microorganisms															
	Gram positive						*Gram negative*									
	Bacillus cereus		*Enterobacte aerogenes*		*Enterococcus* spp		*Burkholderia cepacia*		*Enterobacter cloacae*		*Morganella morganii*		*Proteus mirabilis*		*Providencia stuartii*	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
Moronic acid	100	> 200	100	> 200	1.52	100	1.52	100	100	> 200	200	> 200	3.12	50	100	> 200
Levofloxacin	1.52	1.52	1.52	1.52	1.52	1.52	1.52	6.25	1.52	1.52	1.52	1.52	0.62	1.52	1.52	1.52
Chloramphenicol	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12
Ampicilin	NT	NT	NT	NT	300	NT	300	NT	NT	NT	NT	NT	NT	NT	NT	NT

MIC (minimum inhibitory concentration in μg/ml) and MLC (minimum lethal concentration in μg/ml).



Moronic acid (**2**) Morolic acid (**7**) Acridocarpusic acid D (**8**)

Fig. 1. The reduction of moronic acid (**2**) in the presence of $NaBH_4$ and ethanol to form morolic acid (**7**) and acridocarpusic acid D (**8**).

acridocarpusic acid D was previously isolated from *Acridocarpus vivy* (Cao et al., 2004). Morolic acid and acridocarpusic acid D exhibited important biological activities, such as anti-inflammatory (Giner-Larza et al., 2002), anti-HIV and inhibitory activity against glycogen phosphorylase (Zang et al., 2009). With the aim of observing the importance of the carboxyl group in its antimicrobial activity, moronic acid was converted into its methyl ester (**9**) by treatment with diazomethane (–COOH→–COOMe). Results of the antimicrobial activity in Table 1 show that moronic acid (**2**), with a MIC of 1.52–3.12 μg/ml against most of the tested bacteria, is the most active component of all isolates of *Schinus lentiscifolius*. Compared to it, the three derivatives are less active (MIC≥100 μg/ml), showing that the carbonyl and carboxyl functions are important for its antimicrobial activity. The reduced activity of **6** and **7** is in agreement with the results of Mendoza et al. (1997) who found that increasing the hydrophilicity of kaurene diterpenoids by the introduction of a 3-OH group their antimicrobial activity reduced drastically. As shown in Table 2, the isolated compounds and derivatives showed weak antifungal activity (MIC≥100 μg/ml) against the tested fungal strains. Exceptions were the methylated derivatives of gallic and moronic acids, which had their activities increased by the ester function. Moronic acid OMe showed for instance an increased activity against *Cryptococcus neoformans* (MIC=50 μg/ml) compared to moronic acid (MIC > 200 μg/ml), while the methyl gallate was twice as active against *Candida tropicalis* (MIC=50 μg/ml) as gallic acid (MIC=100 μg/ml). Comparing the activities of gallic and moronic acids and their ester derivatives (Tables 1 and 2), it was observed that the ester function decreased the antibacterial activity (particularly moronic acid) but increased the antifungal activity.

Because this plant is often used as tea, we also decided to analyze the antimicrobial activity of this leaf aqueous extract (decoction). The aqueous extract was prepared (boiled in water for 5 min), lyophilized, and subjected to an antimicrobial screening

with the same strains of bacteria and fungi used previously. The crude aqueous extract exhibited a broad spectrum of antimicrobial activity, ranging from 125 to 250 μg/ml against the tested bacteria and fungi (Tables 1 and 2). The analysis of this extract shows the presence of moronic acid (**1**) (GC, derivatization with diazomethane) and gallic acid methyl ester, gallic acid, quercetin and quercitrin (analysis by HPLC), which could explain its activity. According to the literature (Guerra et al., 2000; Amorin and Santos, 2003; Lima et al., 2006; Deveci et al., 2010; Santos et al., 2010), the essential oil and extracts of leaves of various species of *Schinus* mainly *Schinus* molle and *Schinus terebinthifolius* have clinically demonstrated antibacterial activity *in vitro* against several strains of bacteria (which probably explains why it is used as herbal remedy for different infectious diseases in their countries of origin). These results encouraged us to test moronic acid, which is the most active isolated compound against a range of other bacteria, such as two Gram positive and six Gram negative bacteria (see Table 3). Moronic acid shows excellent activity against *Enterococcus* spp (MIC=1.5 μg/ml), and against *Burkholderia. cepacia* (MIC=1.5 μg/ml), and *Proteus mirabilis* (MIC=3.25 μg/ml). None of the extracts, fractions and pure compounds were bactericidal–fungicidal against the tested microorganisms (considering MBC–MFC⩾4 × MIC). Kinetic measurement of the bacteriolytic activities of moronic acid against the bacteria *Enterococcus* spp, *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive) and *Escherichia coli*, *Burkholderia cepacia* and *Shigella sonnei* (Gram negative) were determined by OD based on real time essay (Lehtinen et al., 2006). Comparing the mode of action of moronic acid against the selected bacteria, we suggest that its mechanism of action is bacteriolytic with different intensities. Moronic acid significantly decreased the *Staphylococcus aureus* density from 2 h of exposition at ≥2 μg/ml concetration ($p=0.0001$). However, from ≥4 μg/ml the density was >50% comparing to the treated control group. The ampicillin showed effect on bacterial density just at higher concentration (1000 μg/ml) from 4 h of exposition ($p=0.01$) (Fig. 2). As can be seen in Table 4, *Burkholderia cepacia* and *Bacillus subtilis* presented susceptibility to moronic acid after 6 h of exposition. At ≥2 μg/ml concentration we observed a decrease in its density ($p=0.001$). However, the density response was not dependent on the moronic acid concentration and was around 20% in relation to control. The ampicillin did not present effect on *Burkholderia cepacia* density, whereas in *Bacillus subtilis* ≥300 μg/

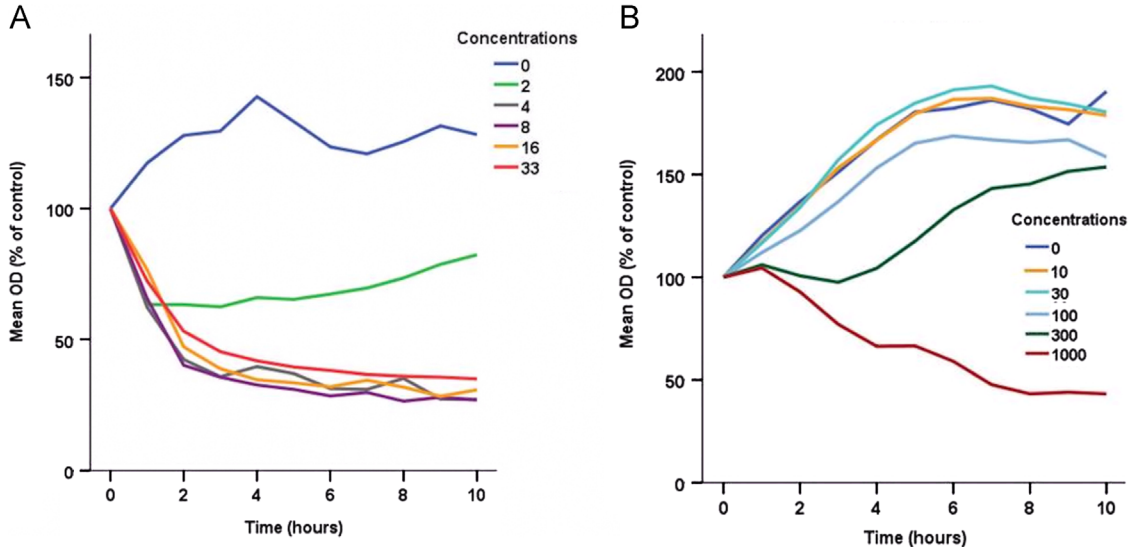


Fig. 2. *Staphylococcus aureus* mean optical density (OD % of control) over 10 h for exposition to different concentrations of moronic acid (A) and ampicillin (B).

Table 4
Bacteriolytic activity (%) of moronic acid and ampicillin against Gram positive (*Bacillus subtilis and Staphylococcus aureus)* and Gram negative bacteria (*Burkholderia cepacia, Escherichia coli, Enterococcus spp, and Shigella sonnei).*

Compound (μg/ml)	Microorganisms											
	Gram positive				*Gram negative*							
	Bacillus subtilis		*Staphylococcus aureus*		*Burkholderia cepacia*		*Escherichia coli*		*Enterococcus* spp		*Shigella sonnei*	
	6 h	10 h	6 h	10 h	6 h	10 h	6 h	10 h	6 h	10 h	6 h	10 h
Moronic acid												
2	20.0 ± 3.3	30.4 ± 1.0	32.5 ± 13.3	17.8 ± 12.0	27.2 ± 0.6	33.1 ± 0.9	–	–	–	–	–	–
4	24.3 ± 1.8	27.5 ± 7.2	68.7 ± 0.9	72.9 ± 2.5	27.4 ± 1.0	36.2 ± 0.5	–	–	–	–	–	–
8	29.1 ± 4.8	33.2 ± 1.8	71.5 ± 0.7	73.0 ± 2.1	23.2 ± 2.0	30.9 ± 1.6	–	–	–	–	–	–
16	26.3 ± 7.3	30.3 ± 4.7	68.0 ± 1.1	69.1 ± 8.8	23.1 ± 0.6	30.3 ± 0.3	–	–	–	–	–	–
33	27.3 ± 5.7	30.6 ± 5.2	62.0 ± 3.5	65.0 ± 3.4	24.6 ± 1.8	37.7 ± 4.9	5.5 ± 1.1	6.4 ± 1.0	–	–	3.9 ± 0.6	5.9 ± 1.5
Ampicillin												
10	–	–	–	–	–	–	–	–	–	–	6.7 ± 1.6	1.7 ± 0.2
30	–	–	–	–	–	–	–	–	–	–	–	–
100	–	–	–	–	–	–	–	–	–	–	1.3 ± 3.6	8.6 ± 3.5
300	35.1 ± 5.2	1.8 ± 10.5	–	–	–	–	47.5 ± 1.8	48.0 ± 1.8	–	–	0.9 ± 0.8	1.3 ± 1.2
1000	58.6 ± 10.8	54.9 ± 19.2	40.8 ± 13.4	56.9 ± 7.1	–	–	48.2 ± 1.7	51.8 ± 1.8	–	–	2.5 ± 1.8	14.4 ± 4.4

–=No activity was observed.

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ml decreased approximately 40% after 4 h of exposition. Moronic acid presented no significant bacteriolytic activity on *Escherichia coli* and *Shigella sonnei* at the highest concentration tested (5.5–6.6%). The ampicillin presented very little effect on *Shigella sonnei* density, whereas in *Escherichia coli* ≥300 μg/ml decreased approximately 48% after 6 h of exposition. Neither moronic acid nor ampicillin showed effect on *Enterococcus* spp density.

4. Conclusion

This work provides new information about the antimicrobial potential of *Schinus lentiscifolius*. Therefore, the results of the present study provide scientific basis for the popular use of *Schinus lentiscifolius* for a number of different health problems. It has been used as antiseptic and antimicrobial as well as to treat leucorrhea, to heal ulcers and wounds, to treat uterine inflammation and urinary tract infections, and to relieve symptoms of rheumatoid arthritis. Further experiments especially regarding toxicity of *Schinus lentiscifolius*.are needed to prove the safety of the traditional preparations.

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