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Artigo

Salacia crassifolia (Celastraceae): CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY

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The phytochemical study of hexane extract from leaves of *Salacia crassifolia* resulted in the isolation of 3β-palmitoxy-urs-12-ene, 3-oxofriedelane, 3β-hydroxyfriedelane, 3-oxo-28-hydroxyfriedelane, 3-oxo-29-hydroxyfriedelane, 28,29-dihydroxyfriedelan-3-one, 3,4-*seco*-friedelan-3-oic acid, 3β-hydroxy-olean-9(11):12-diene and the mixture of α-amirin and β-amirin. β-sitosterol, the polymer *gutta-percha*, squalene and eicosanoic acid were also isolated. The chemical structures of these constituents were established by IR, ^{1}H and ^{13}C NMR spectral data. Crude extracts and the triterpenes were tested against *Entamoeba histolytica, Giardia lamblia* and *Trichomonas vaginalis* and no activity was observed under the *in vitro* assay conditions. The hexane, chloroform, ethyl acetate and ethanol crude extracts, and the constituent 3,4-*seco*-friedelan-3-oic acid and 28,29-dihydroxyfriedelan-3-one showed *in vitro* antimicrobial activity against *Salmonella typhimurium*, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Streptococcus sanguinis* and *Candida albicans*.

Keywords: *Salacia crassifolia*; Celastraceae; pentacyclic triterpenes; antimicrobial property.

INTRODUCTION

Species of the *Salacia* genus are widespread throughout different countries of South America, including Brazil, where, to date, twenty-one species have been identified.[1] Species of this genus are known to produce pentacyclic triterpenes (PCTT), mainly those of the friedelane,[2] lupane,[3] oleanane, ursane,[4] and quinonemethide series.[5] Other secondary metabolites, such as steroids,[6] have also been isolated from *Salacia spp*. Biological activities, such as bactericidal, fungicidal, larvicidal,[7] trypanocidal,[8] antimalarial,[9,10] hypoglycemic, antioxidant,[10,11] cardioprotection, antitumor,[11] anti-inflammatory,[10,12] anti-rheumatoid,[12] antiarthritic, and antimutagenic properties[10] have been reported for PCTT class compounds.

Different species of the *Salacia* genus are used in traditional Brazilian medicine to treat diabetes and as an anti-inflammatory drug.[1] *Salacia crassifolia* Mart, G. is popularly known in Brazil as bacupari, bacupari de caapuêra, and saputá,[13] and its fruits are used as a source of human nourishment.[14] This species is a native tree of the Brazilian Cerrado, which is one of the richest of all tropical savanna regions. In traditional medicine, the leaves, stems, seeds, and fruits of *S. crassifolia* are used to treat pediculosis,[15] common kidney diseases, gastric ulcers, skin cancer, malaria, chronic cough, and headaches.[16,17] A crude ethanol extract obtained from the bark of *S. crassifolia* presents antifungal activity against *Trichophyton rubrum* and *T. mentagrophytes*.[18] Genotoxic and cytotoxic properties have also been observed in this crude extract through mouse bone marrow micronucleus assays, and these results represent important data for the development of new cancer therapies.[17]

There is only one study related to the isolation of the chemical constituents of *S. crassifolia* in which the isolation of a pristimerin mixture, considered to be the main constituent, was reported to have been identified by infrared (IR) spectrum comparison of the mixture with spectral data reported for this PCTT.[19] This mixture underwent *in vitro* assay and showed activity against different bacteria.[19]

Several studies have been published aimed at demonstrating the different pharmacological properties attributed to species of the Celastraceae family. Anti-inflammatory,[20] anti-HIV,[21] antitumor,[22] antibacterial,[23] antifungal,[24] larvicidal,[25] and antioxidant[26] activities were described for species in this family.

Intestinal parasitism is one of the most serious public health problems in Brazil, primarily among those associated with malnutrition in children, which may affect their physical, psychosomatic, and social development.[27] In this context, the *Entamoeba histolytica* and *Giardia lamblia* parasites have been considered the main etiologic agents in amebiasis and giardiasis, respectively. According to recent estimates, *Amoeba spp* infects approximately 500 million people worldwide, resulting in 40,000 to 100,000 deaths annually, mainly in tropical and subtropical countries.[28] Different *Giardia* species inhabit the digestive tract of a large variety of domestic and wild animal species, as well as humans, and is considered a common cause of gastroenteritis in humans, affecting approximately 280 million people worldwide each year. PCTT activity against *G. lamblia* was reported in the literature.[29] In addition, extracts from branches of different species of the Celastraceae family, with high triterpene content, also showed activity against this parasite.[30]*Trichomonas vaginalis* represents an important flagellated protozoan that causes trichomoniasis, one of the most common sexually transmitted diseases in women worldwide. Trichomoniasis has been associated with the

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transmission of human immunodeficiency virus (HIV), pelvic inflammatory disease, cervical cancer, and infertility.[31] Metronidazole is an antimicrobial agent that has been used in clinical medicine for more than 45 years. It was originally recommended to manage infections caused by *T. vaginalis*. Later, this compound also proved to be effective against other agents of protozoal infections, such as *Ameba sp.* and *Giardia sp*.[32] Conversely, short-term exposure or exposure to sublethal levels of metronidazole, when prescribed for prophylaxis or to supply noncompliance treatment, represents processes that establish conditions that induce resistance to this compound.[33]

This paper reports a first phytochemical study of hexane extracts from the leaves of *S. crassifolia*, through which it was possible to isolate pentacyclic triterpenes 3β-palmitoxy-urs-12-ene (**3**), 3-oxofriedelane (**4**), 3β-hydroxyfriedelane (**5**), a mixture of α-amirin (**6-I**) and β-amirin (**6-II**), 3-oxo-28-hydroxyfriedelane (**8**), 3-oxo-29-hydroxyfriedelane (**9**), 3,4-*seco*-friedelan-3-oic acid (**10**), 3β-hydroxy-olean-9(11):12-diene (**11**), 28,29-dihydroxyfriedelan-3-one (**13**), β-sitosterol steroid (**7**), the *gutta-percha* polymer (**2**), a long chain hydrocarbons squalene (**1**), and eicosanoic acid (**12**) (Figure 1).

The extracts and some of their constituents underwent *in vitro* antimicrobial assay in an attempt to evaluate their properties against *Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Streptococcus sanguinis*, and *Candida albicans*, as well as against the parasites *Entamoeba histolytica, Giardia lamblia*, and *Trichomonas vaginalis.*

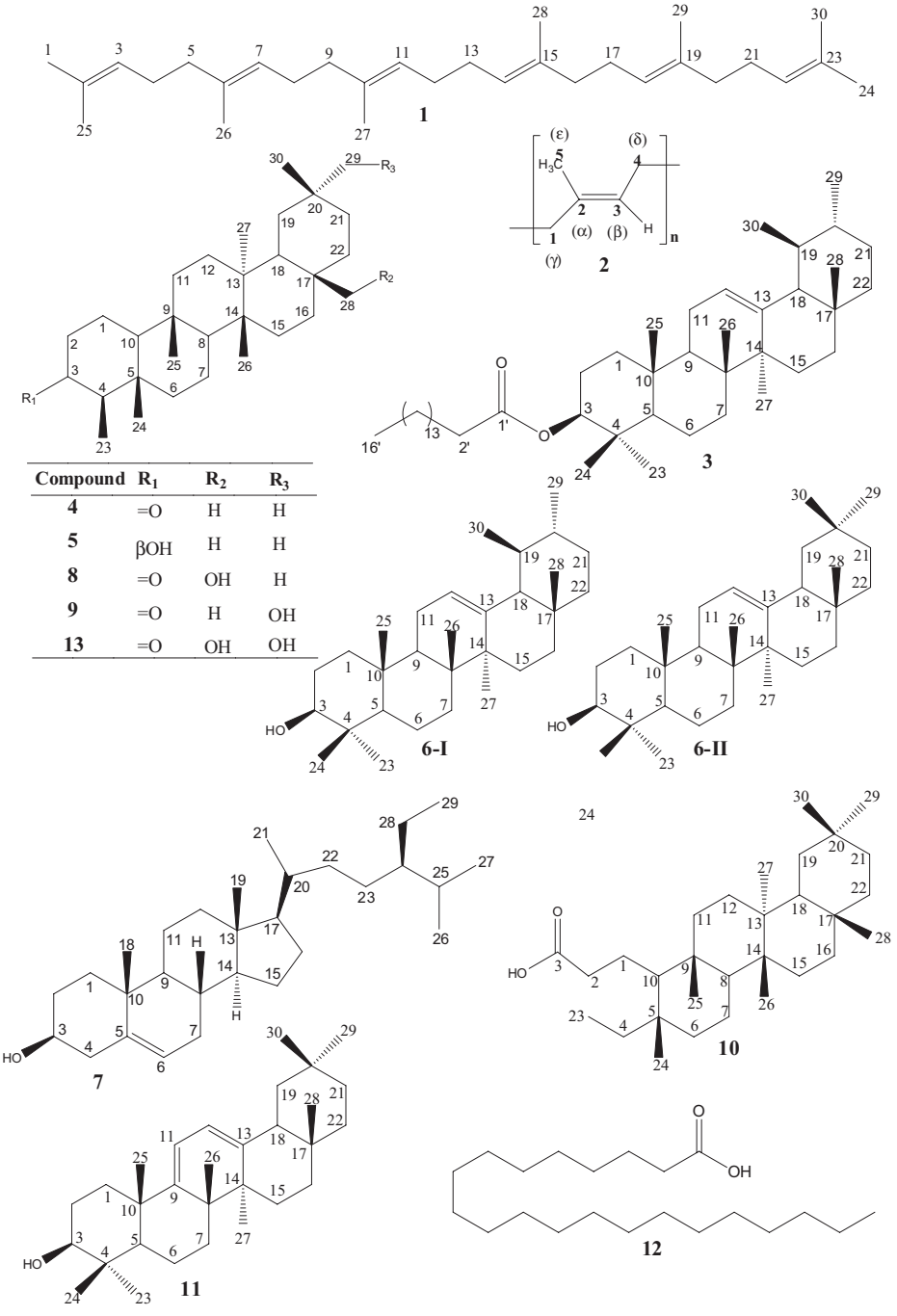

Figure 1. *Chemical structures of compounds isolated from leaves of S. crassifolia*

EXPERIMENTAL

Plant material

Samples of leaves of *S. crassifolia* (Celastraceae) were carefully collected in the region of Montes Claros municipality. The botanic material was identified by Dr. Maria Olívia Mercadante-Simões, botanist of Departamento de Botânica da Universidade de Montes Claros (UNIMONTES), Minas Gerais, Brazil. A voucher specimen (Nº 144624) was deposited in the collection of the *Herbarium* of Departamento de Botânica (BHCB), Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG).

General procedures

Column chromatography (CC) processes were carried out using silica gel 60 (0.063-0.200 mm) as stationary phase and organic solvent pure or in mixtures of crescent polarity were used as mobile phase. Silica gel 60 (Merck) was used to prepare plates (0.25 mm) for analytic thin layer chromatography (TLC).

The ^{1}H and ^{13}C NMR spectra were obtained on Bruker *Avance* DPX-200 or DRX-400, operating at 300 K. The chemical shifts (δ) were expressed in parts-per-million (ppm) and coupling constants (*J*) were registered in Hertz (Hz). Tetramethylsilane (TMS) was used as internal standard ($\delta_H = \delta_C = 0$). The infrared spectra (IR) (1% KBr soln, 400-4000 cm^{-1}) were obtained on Shimadzu IR408 spectrometer. Melting points were determined on MQAPF-302 apparatus (Microquímica Equipamentos Ltda).

Extraction and isolation of compounds

The dried leaves were fragmented in a knife mill and the resulting powder (1.35 kg) underwent exhaustive extraction (maceration) with hexane, chloroform, ethyl acetate, and finally ethanol. After recovering the solvent, using a rotary evaporator, extracts with hexane (EHS), chloroform (ECS), ethyl acetate (EAS), and ethanol (EES) were obtained.

EHS was obtained as a semi-solid material that was then subjected to TLC, eluted with hexane, together with compounds commonly isolated from species of the Celastraceae family. The presence of *gutta-percha* was identified through this process as being the main EHS constituent. EHS (14.6 g) was subjected to CC in an attempt to eliminate the majority of the *gutta-percha*. Thus, the column was first eluted with MeOH to obtain EHS1 (7.2 g) without *gutta-percha*. Next, it was eluted with $CHCl_3$ to isolate *gutta-percha* (7.3 g).

Constituents **1** to **13** were isolated from EHS1 (7.2 g) (Figure 1). Initially, EHS1 was subjected to silica gel (420.0 g) CC eluted with hexane-$CHCl_3$-EtOAc-MeOH, either in pure form or in mixtures of enhancement polarity. Fractions (1 to 302) of 200 mL each were obtained and grouped according to the similar profiles observed through TLC. In fraction (fr) 1 (eluted with hex/$CHCl_3$, 7:13), a colorless oil (18.0 mg) was extracted and identified as squalene (**1**). The fr 2-3 (also eluted with hex/$CHCl_3$, 7:13) provided a white solid, with a characteristic consistency of plastic tape (51.8 mg), which was identified as g*utta-percha* (**2**). A colorless viscous liquid (777.0 mg) was isolated from fr 45-64 (eluted with hex/$CHCl_3$, 3:22), which was identified as being 3β-palmitoxy-urs-12-ene (**3**).

Fr 65-71 (eluted with $CHCl_3$/EtOAc, 49:1) was isolated as a brown amorphous solid (424.2 mg), which was subjected to another silica gel CC eluted with hexane, $CHCl_3$, EtOAc, and MeOH, either in pure form or in mixtures of increasing polarity, yielding 140 fractions (fr^a) of 25 mL. After eluent evaporation, fr^a 66-70 (eluted with hex/$CHCl_3$, 1:1) produced a white solid material identified as 3-oxofriedelane (**4**) (49.8 mg, mp 250.0-255.0 °C). Fr^a 71-75 (also eluted with hex/$CHCl_3$, 1:1) yielded another white solid material that was identified as 3β-hydroxyfriedelane (**5**) (14.5 mg, mp 270.0-274.0 °C).

Fr 72-84 (eluted with hex/$CHCl_3$, 31:19) was obtained additional amount (204.0 mg) of 3β-hydroxyfriedelane (**5**).

Fr 85-90 (eluted with hex/$CHCl_3$, 14:11) produced a yellow solid (370.0 mg), which was subjected to silica gel CC eluted with hexane, EtOAc and MeOH, either in pure form or in mixtures of increasing polarity, yielding 103 fractions (fr^b) of 20 mL each. After solvent evaporation, from fr^b 53-74 (eluted with hex/EtOAc, 49:1→19:1) was obtained as a white flakes. This solid was identified as a mixture of the α-amirin (**6-I**) and β-amirin (**6-II**) (149.0 mg, mp 160.0-164.0 °C).

Fr 91 (eluted with hex/$CHCl_3$, 14:11) produced a yellow solid (113.0 mg), which was subjected to silica gel CC eluted with hexane, EtOAc and MeOH, either in pure form or in mixtures of increasing polarity, yielding 83 fractions (fr^c) of 25 mL. After solvent evaporation, an additional amount (7.3 mg) of α-amirin (**6-I**) and β-amirin (**6-II**) was isolated from fr^c 39-53 (eluted with hex/EtOAc, 49:1→19:1). Fr^c 56-64 (eluted with hex/EtOAc, 47:3→93:7) yielded a white solid, which was identified as β-sitosterol (**7**) (10.1 mg, mp 130.0-133.0°C).

After solvent evaporation, an additional amount (23.2 mg) of the mixture of α-amirin (**6-I**) and β-amirin (**6-II**) was obtained from fr^d 6-14 (eluted with hex/$CHCl_3$, 1:1→1:4). Fr^d 30-65 (eluted with hex/$CHCl_3$ 1:9→$CHCl_3$/AcOEt 9:1) yielded an additional amount (71.2 mg) of β-sitosterol (**7**). Fr^d 69-75 (eluted with $CHCl_3$/EtOAc, 9:1) was obtained as a white solid material, which was identified as being 3-oxo-28-hydroxyfriedelane (**8**) (20.0 mg, mp 255.0-260.0 °C). Fr^d 77-99 (eluted with $CHCl_3$/EtOAc, 4:1→3:2) produced a white solid that was identified as 3-oxo-29-hydroxyfriedelane (**9**) (27.2 mg, mp 233.0-236.0 °C).

Fr 104-149 (eluted with hex/$CHCl_3$, 14:11→ 5:15) was characterized as a yellow solid (370.0 mg), after subjected to extraction with hexane producing additional amount of β-sitosterol (**7**) (38.8 mg), 3-oxo-29-hydroxyfriedelane (**9**) (45.4 mg) and a mixture of 3-oxo-28-hydroxyfriedelane (**8**) with 3-oxo-29-hydroxyfriedelane (**9**) (25.4 mg).

Fr 150-242 (eluted with hex/$CHCl_3$ 5:15→$CHCl_3$/EtOAc 19:1) produced a yellow solid (405.3 mg), which was subjected to silica gel CC eluted with hexane, $CHCl_3$, EtOAc and MeOH, either in pure form or in mixtures, yielding 163 fractions (Fr^e) of 25 mL. After solvent evaporation a white solid was obtained from Fr^e 34-52 (eluted with hex/$CHCl_3$, 1:9→1:19), which was identified as 3,4-*seco*-friedelan-3-oic acid (**10**) (25.7 mg, mp 142.0-145.0°C). An additional amount (8.3 mg) of 3-oxo-29-hydroxyfriedelane (**9**) was obtained from Fr^e 67-77 (eluted with $CHCl_3$→$CHCl_3$/EtOAc 99:1).

Fr 243-270 (eluted with $CHCl_3$→$CHCl_3$/EtOAc 9:1) produced a green solid (461.0 mg), which was subjected to silica gel CC eluted with hexane, $CHCl_3$, EtOAc and MeOH, either in pure form or in mixtures, yielding 119 fractions (Fr^f) of 25 mL. After solvent evaporation, a white solid was produced from Fr^f 50-57 (eluted with hex/$CHCl_3$, 3:7→1:9), which was identified as 3β-hydroxy-olean-9(11):12-diene (**11**) (9.8 mg, mp 210.0-216.0°C).

Fr 272-283 (eluted with $CHCl_3$/EtOAc, 9:1→3:2) produced a white solid (7.8 mg), mp 72.0-75.0 °C, which was identified as icosanoic acid (**12**).

Fr 284-291 (eluted with $CHCl_3$/EtOAc 3:2→ EtOAc) was characterized as a brown solid (278.7 mg) which was subjected to silica gel CC eluted with hexane, $CHCl_3$, EtOAc and MeOH, either in pure form or in mixtures of increasing polarity, yielding119 fractions (Fr^g) of 25 mL. After solvent evaporation, a white solid material was produced from Fr^g 38-42 (eluted with $CHCl_3$/EtOAc, 7:3), which was identified as 28,29-dihydroxyfriedelan-3-one (**13**) (26.3 mg, mp 281.0-284.0°C).

Biological assays

Antibacterial and antifungal activity

The microdilution method was used to evaluate the antibacterial and antifungal properties and determine the minimum inhibitory concentration required to inhibit the growth of 50% of organisms (MIC_{50}), following procedures set forth by Zacchino and Gupta (2007).[34] Extracts EHS, ECS, EAS, and EES, as well as triterpenes **3, 4, 5, 6, 7, 9, 10, 11**, and **13** were submitted to *in vitro* assay against *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 25723), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 15313), *Streptococcus sanguinis* (ATCC 49456), and *Candida albicans* (ATCC 18804). The bacteria and fungus were maintained in a brain heart infusion (BHI) culture medium at 7 °C. The samples were tested at ten concentrations (25.00, 12.50, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195, 0.098, and 0.049 μg mL^{-1}) to determine the MIC_{50}. All microdilution assays were carried out in quintuplicate, using a 96-well microplate. Ampicillin was used as a positive control for bacteria and miconazole for *C. albicans*. The inocula of bacteria and fungus used in the experiments contained 4.16 x 10^3 cells mL^{-1}. At the end of the incubation time (24 h), the results were obtained using a Microplate TP-Reader (Thermoplate, Brazil).

The samples were dissolved in DMSO to reach a concentration of 50.0 mg mL^{-1} for extracts and 12.5 mg mL^{-1} for triterpenes. From each solution, 40.0 μL were transferred to assay tubes containing 960 μL of BHI culture medium. Next, the tubes were incubated at 37 °C for 18 h. To obtain the microbial inoculum to be used in the experiments; 500.0 μL of pre-microbial inoculum was added to the assay tube containing 4.5 mL of sterile distilled water. The solution was homogenized and the turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity, corresponding to 0.5 McFarland standards (108 CFU mL^{-1}). The assays were performed in a 96-microwell plate in quintuplicate. To each well were added 100 μL of BHI culture medium. In well 1, 100.0 μL of the sample solution was added. The solution was homogenized, and 100.0 μL was transferred to the next well, and so on until ten concentrations levels had been prepared. Next, 100.0 μL of inoculum of the microorganism was added to the wells. The growth of microorganisms was induced by incubation at 37 °C for 24 h.

Antiparasitic activity

Extracts EHS, ECS, EAS, and EES, as well as triterpenes **3, 4, 5, 6, 7, 8, 9, 10, 11,** and **13** were submitted to screening assays to determine activity against trophozoites of *Entamoeba histolytica*, *Giardia lamblia,* and *Trichomonas vaginalis*. Assays were carried out on triterpenes that produced activity against some of the above parasites to establish the inhibitory concentration that reduces the growth of trophozoites by 50% (IC_{50}). Extracts and triterpenes were dissolved in 1 mL of dimethylsulfoxide (DMSO). Aliquots from the resulting solutions were diluted in 5 mL of adequate culture medium YI-S-32, for *Amoebae* and *Trichomonas sp*., and TYI-S-33 for *Giardia sp*. Each solution was sterilized through filtration in nitrocellulose membrane (0.2 μm) and transferred to glass tubes (Pyrex® 13 × 100 mm) containing trophozoites in axenic cultures (2.4 x 10^5 trophozoites of *E. histolytica*, 1.2 x 10^5 *G. lamblia,* and 6 × 10^4 *T. vaginalis*), producing a final assay concentration of 34.0 μg mL^{-1} for extracts and 100.0 μM for triterpenes.

Samples were maintained in a bacteriological incubator at 37 °C for 48 h. Negative controls (culture medium), positive controls (Metronidazole, Sigma®), and controls with DMSO were developed for each type of assay. All assays were done in triplicate and repeated twice. Viability was determined qualitatively by observing trophozoite mobility and adhesion, using an inverted microscope (Olympus IX51); vitality was measured using an adapted leukocyte count technique in the Neubauer chamber.[35]

RESULTS AND DISCUSSION

Phytochemical study

Herein is reported the first phytochemical study of EHS from *S. crassifolia* that resulted in the isolation of secondary metabolites [Compounds **1** to **13** (Figure 1)]. The chemical structures of these constituents were identified based on the respective IR, ^1H, ^{13}C (with DEPT-135) NMR spectral data and those obtained by mass spectrometry (Supplementary material). The spectral results were in accordance with previously reported data. In the IR spectra of the constituents were observed absorption band correspondent to functional groups characteristic of each compound and the data were in according to the literature.[36,37]

In the ^1H NMR spectrum of **1** were observed signals correspondent to olefinic hydrogen atom [δ 5.12 (6H)], methylene hydrogen [δ 2.01 (20H)] and hydrogen of methyl group [δ 1.60, δ 1.68 (24H)]. In the ^{13}C NMR spectrum of **1** were observed signals associated to 6 CH (δ 124.32 to δ 124.44) and 6 non-hydrogenated carbon atom (δ 131.30 to δ 135.15).[38]

In the ^1H NMR spectrum of **2** were observed signals characteristic of olefinic hydrogen atom [δ 5.12 (t, J = 6.3 Hz, 1H)], methylene hydrogen [δ 2.06 (4H)] and methyl hydrogen [1.60 (3H)]. Based on the ^{13}C NMR spectrum and the data obtained from DEPT-135 experiment were identified two signals which were assigned to CH (C-3; δ 124.26) and to non-hydrogenated carbon atom (C-2; δ 134.93).[39]

In the ^1H NMR spectrum of **3** were observed the signal at δ5.13 (t, J = 3.6 Hz, 1H) associated to olefinic hydrogen characteristic of compounds of the ursane series; and at δ 4.50 (m) which was attributed to hydrogen bonded to carbon of ester group. The carbon signals at δ 124.33 (CH) and at δ 139.63 (C) were associated to carbon of double bond, the signal at δ 173.68 to (C=O) of ester and δ 80.60 was attributed to C-3.[40] In the mass spectrum (LCMS-IT-TOF) of **3,** the peak at m/z 409.3852 g mol^{-1} (calculated 409.3834 g mol^{-1}) characterize PCTT of the ursane series due to the protonation of the oxygen of ester and fragmentation of C-O bond, with subsequent loss of the side carbon chain. The peak at m/z 255.2314 g mol^{-1} (calculated 255.2324 g mol^{-1}) was associated to the mass of side carbon chain, loss of a proton at C-2, followed by a double bond formation together with fragmentation of C-O bond.

In the ^1H NMR spectrum of compound **4** six signals [δ 0.73 (3H); δ 0.87 (6H); δ 0.95 (3H); δ 1.01 (6H); δ 1.05 (3H) and δ 1.18 (3H)] were correlated with eight methyl groups and the carbon signal at δ 213.21 was attributed to carbonyl group (C-3).[41]

In the ^1H NMR spectrum of **5** the presence of a multiplet at δ 3.81 correspondent to hydrogen bonded to hydroxylated carbon together with the carbon signal at δ 71.59 (C-3) were associated to carbon attached to hydroxyl group.[42]

In the ^1H NMR spectrum of **6** the triplet at δ 5.18 and 5.13 (J = 3.6 Hz) correspondent to H-12 of C=C bond and the signals at δ 3.24 and 3.21 are typical of H-3 of hydroxylated carbon of β-amirin and α-amirin, respectively.[41] By the spectral data obtained from ^{13}C NMR together with DEPT-135 of **6** were listed 60 signals of carbons (16 CH_3, 19 CH_2, 12 CH and 13 C). In accordance with previously reported data, the signals at δ 121.74 (C-12) and δ 145.20 (C-13) were attributed to β-amirin and δ 124.43 (C-12) and 139.60 (C-13) to α-amirin.[41]

The multiplet at δ 3.52 (m, 1H), in the spectra of compound **7,** was associated to carbynolic hydrogen (H-3) and the signal at δ 5.36

(s, 1H) to olefinic hydrogen (H-6). The carbon signal at δ 71.84 was attributed to hydroxylated carbon (C-3), and at δ 121.73 (C-6) and 140.79 (C-5) were correlated to olefinic carbons.[43]

In the spectra of PCTT **8**, the signal at δ 3.63 was attributed to hydrogen bonded to hydroxylated carbon, the carbon signal at δ 213.13 to carbonyl group (C-3) and at δ 68.07 associated to hydroxylated carbon (C-28).[41]

In the ^{1}H NMR spectrum of **9** were observed a duplet signal at δ0.88 (J = 5.6 Hz), attributed to methyl C-23 and a signal at δ 3.27 correlated to hydrogen bonded of hydroxylated. In the ^{13}C NMR spectrum were observed a carbon signal at δ 213.19 that was associated to carbonyl (C-3) and the carbon signal at δ 74.77 (C-29) correspondent to hydroxylated carbon.[7]

In the ^{1}H NMR spectrum of **10**, the signal at δ 2.38 was attributed to hydrogen atoms bonded to a carbon atom in a (alpha) position relative to a carbonyl group. The carbon signal at δ 178.72 was correlated to carboxyl group (C-3). [44]

In the ^{1}H NMR spectrum of **11** were observed signals at δ 5.57 and δ 5.50 which were associated to hydrongen H-11 and H-12, respectively and at δ 3.24 that is typical of hydroxylated carbon (H-3). Based on the ^{13}C NMR and DEPT-135 spectral data of **11**, the signals at δ 154.30 (C-9), δ 115.70 (C-11), δ 120.67 (C12) and δ 147.11 (C-13) were correlated to compound of the oleanane series with conjugated double bond.[41]

In the ^{1}H NMR spectrum of **12** were observed a triplet at δ 2.34 (t, J = 7.2 Hz, 2H) correspondent to α-carboxylic hydrogen, signal at δ 1.63 attributed to β-carboxylic hydrogen and at δ 0.88 (t, J = 6.4 Hz, 3H) correspondent to terminal methyl hydrogen.[45] The intense signal at δ 1.25 (m, 34H) was associated to internal methylene groups of a long chain hydrocarbon. And, in the ^{13}C NMR spectrum of **12** were the signal at δ 179.35 was correlated to carboxylic carbon (C-3).[46]

In the ^{1}H NMR spectrum of **13** were observed a duplet signal at δ 0.87 (J = 6.8 Hz) attributed to methyl C-23 and signals at δ 3.35 (H-29) and δ 3.71 (H-28), which were associated to hydrogen bonded to hydroxylated carbon. In the ^{13}C NMR spectrum of **13,** the carbon signal at δ 213.07 (C-3) was attributed to carbonyl group and the carbon signals at δ 73.87 (C-29) and at 66.97 (C-28) were associated to hydroxylated carbon.[41]

Evaluation of antibacterial and antifungal activity

The level of resistance or susceptibility of the bacteria and the fungus to the samples from *S. crassifolia* was determined by the presence or absence of growth. The results of MIC_{50}, corresponding to extracts and triterpenes subjected to antimicrobial assay, are shown in Table 1. Based on the results, it was possible to establish that extracts EHS, ECS, EAS, and EES, as well as PCTT **10** and **11** present antibacterial and antifungal properties. All extracts showed high activity against *P. aeruginosa*, *S. aureus*, *B. cereus,* and *S. sanguinis* (MIC_{50} 0.195 μg mL^{-1}).

Evaluation of antiparasitic activity

The extracts and triterpenes isolated from *S. crassifolia* were *in vitro* evaluated for their amoebicidal, giardicidal and trichomonicidal property. All extracts and triterpenes showed no activity *in vitro* until maximum concentration tested (34.0 μg mL^{-1} and 100.0 μ mol L^{-1}, respectively).

CONCLUSION

A variety of substances were isolated and identified from the leaves of *S. crassifolia*: 3β-palmitoxy-urs-12-ene, 3-oxofriedelane, 3β-hydroxyfriedelane, 3-oxo-28-hydroxyfriedelane, 3-oxo-29-hydroxyfriedelane, 3,4-*seco*-friedelan-3-oic acid, 3β-hydroxy-olean-9(11):12-diene and 28,29-dihydroxyfriedelan-3-one, and a mixture of α-amirin and β-amirin. The steroid β-sitosterol, *gutta-percha*, squalene and eicosanoic acid were also identified.

Table 1. Minimal inhibitory concentrations that inhibit 50% of the growth (MIC_{50}) determined for extracts and constituents isolated from *S. crassifolia* against pathogenic microorganisms

Sample	Minimal inhibitory concentration (MIC_{50}) (μg mL^{-1})							
	S. typhimurium	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. cereus*	*L. monocytogenes*	*S. sanguinis*	*C. albicans*
Extract								
EHS	25.00	25.00	0.195	0.195	0.195	50.00	0.195	25.00
ECS	25.00	25.00	0.195	0.195	0.195	25.00	0.195	25.00
EAS	25.00	25.00	0.195	0.195	0.195	25.00	0.195	25.00
EES	25.00	25.00	0.195	0.195	0.195	25.00	0.195	25.00
Compound								
3	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–
10	62.50	125.00	62.50	62.50	62.50	125.00	125.00	250.00
11	250.00	500.00	500.00	500.00	500.00	500.00	500.00	500.00
13	–	–	–	–	–	–	–	–
ampicillin	0.049	0.098	0.049	0.098	0.098	0.049	0.098	–
miconazole	–	–	–	–	–	–	–	3.32

– = Not Detected in the assay conditions.

Extracts obtained with hexane, chloroform, ethyl acetate and ethanol, and the triterpenes 3,4-*seco*-friedelan-3-oic acid and 3β-hydroxy-olean-9(11):12-diene showed antimicrobial property through *in vitro* assays against *S. typhimurium, E. coli, P. aeruginosa, S. aureus, B. cereus, L. monocytogenes, S. sanguinis* and *C. albicans*. All extracts and triterpenes showed no activity against *E. histolytica, G. lamblia* and *T. vaginalis*. The results of this study open the door to new perspectives on the use of *S. crassifolia* as a source of substances with antimicrobial property.

SUPPLEMENTARY MATERIAL

^{1}H and ^{13}C NMR spectra including DEPT-135 of all compounds and mass spectra only of compound **3** isolated from hexane extract of *S. crassifolia* leaves are shown in Figure 1S to 41S. This supplementary material is available free of charge at http://quimicanova.sbq.org.br, as PDF file.

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