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Original Article

ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF STEROIDS AND TRITERPENES ISOLATED FROM AERIAL PARTS OF JUSTICIA ACUMINATISSIMA (ACANTHACEAE)

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ABSTRACT

Objective: Phytochemical investigation of aerial parts of *Justicia acuminatissima* (Acanthaceae) was directed to isolation of triterpenes and steroids

Methods: Chromatographic fractionation, spectroscopic characterization, anti-inflammatory and antimicrobial activities of the constituents were carried out by standard methods.

Results: *β*-Sitosterol, stigmasterol, lupeol, friedelin, *β*-friedelinol, *α*-amyrin, *β*-amyrin, betulin, erythrodiol, betulinic acid, glycosylated sitosterol, glycosylated stigmasterol, 4-hydroxybenzoic acid, *α*-glucose, *β*-glucose, and sucrose were described for the first time in *J. acuminatissima*. Some of these phytoconstituents have not been yet described in the genus *Justicia.* Dichloromethane fraction exhibited high antimicrobial action against all tested microorganisms. Ethyl acetate fraction exhibited high antimicrobial action against *E. coli* and *C. albicans*. Dichloromethane and ethyl acetate fractions, mixture of *β*-sitosterol and stigmasterol, lupeol, and mixture of glycosylated *β*-sitosterol and glycosylated stigmasterol exhibited significant reduction in rat paw edemas.

Conclusion: Aerial parts of *J. acuminatissima* mainly provided steroids and triterpenes. Some fractions and phytoconstituents exhibited high antimicrobial and anti-inflammatory activities. Glycosylated sterols exhibited low antimicrobial activity, but high anti-inflammatory activity, indicating a significant effect of the glycosylated residue on their biological activities.

Keywords: Acanthaceae, Anti-inflammatory activity, Antimicrobial activity, *Justicia acuminatissima*, Pentacyclic triterpenes, Sterols.

INTRODUCTION

Acanthaceae family, order Scrophulariales and superorder Lamiiflorae (sensu Dahlgren), comprises about 2,500 species in 250 genera, which are widespread in tropical regions around the world and poorly represented in temperate regions. *Justicia* is the largest genus of Acanthaceae, containing about 600 species. Species of *Justicia* are widely used in folk medicine to treatment of respiratory, gastrointestinal, and heart diseases. Some of them are used to treatment of tuberculosis, diabetes, cancer, inflammation, rheumatism, and arthritis, also exhibiting antimalarial, antibacterial, antiviral, giardicidal, anti-HIV, and anthelminthic properties. Compounds of different chemical classes have been isolated from species of *Justicia*, such as coumarins, flavonoids, alkaloids, iridoids, diterpenes, and triterpenes. However, *Justicia* is characterized by isolation of a large variety of lignans, mainly glycosylated lignans and those ones containing arylnaphthalide skeleton [1]. *Justicia acuminatissima* (syn. *Justicia calycina*) is popularly known as "sara-tudo" in the Amazon Region. Its leaves are used as stimulant and to treatment of inflammations [1]. The present work describes a phytochemical study of aerial parts of *J. acuminatissima* (see Figure 1). As results, *β*-sitosterol (**1**), stigmasterol (**2**), lupeol (**3**), friedelin (**4**), *β*-friedelinol (**5**), *α*-amyrin (**6**), *β*-amyrin (**7**), betulin (**8**), erythrodiol (**9**), and betulinic acid (**10**) were isolated from the hexane and dichloromethane fractions. Glycosylated *β*-sitosterol (**11**), glycosylated stigmasterol (**12**), and 4-hydroxybenzoic acid (**13**) were isolated from the ethyl acetate fraction. *α*-Glucose (**14**), *β*-glucose (**15**), and sucrose (**16**) were isolated from the methanol fraction. The chemical identification of the isolated compounds was carried out by spectroscopic methods, mainly based on ^{1}H and ^{13}C NMR data.

Anti-inflammatory and antimicrobial activities of *J. acuminatissima* were evaluated for extracts, fractions, and isolated compounds of its aerial parts. Anti-inflammatory assay was performed by the transdermal drug application method using phonophoresis in rats, as previously described in the literature [2]. The antimicrobial assay was performed against *Gram*-positive (*Staphylococcus aureus* and *Bacillus cereus*) and *Gram*-negative (*Escherichia coli* and *Salmonella typhimurium*) bacteria and a fungi (*Candida albicans*), as previously described in the literature [3,4].

MATERIALS AND METHODS

General procedures

Uncorrected melting points were determined using Mettler equipment, model FP80 SNR H22439. IR spectra were taken on a Perkin Elmer - Spectrum One (ATR) spectrometer. ^{1}H and ^{13}C NMR spectra were taken on a Bruker DRX400 AVANCE spectrometer, using $CDCl_3$, Pyridine-d_5, DMSO-d_6, or D_2O as solvent. The chemical shifts were measured in parts per million (δ) relative to TMS, which was used as internal standard. The coupling constants (J) were recorded in Hertz.

Plant material

Aerial parts of *J. acuminatissima* were collected in February 2010 at 03° 08' 28.8" S and 58° 26' 54.3" W, in the City of Itacoatiara, State of Amazonas (Brazil). A voucher specimen of *J. acuminatissima* was deposited in the *herbarium* of the Instituto de Ciências Biológicas of the Universidade Federal do Amazonas, under code: 8,270.

Extraction and isolation of the phytoconstituents

Plant material was dried at 60 ºC until constant weight was achieved (about one week). A powdered sample (1370.00 g) was submitted to extraction in ethanol for a week. After solvent evaporation, the corresponding crude ethanol extract (**EE**; 146.00 g) and a residue of

the extraction were obtained. This residue was submitted to extraction using EtOH:water (7:3) for a week, providing the hydroalcoholic extract (**WE**; 70.00 g). **EE** was submitted to column chromatography using silica gel as stationary phase (CCS) and elution with hexane, dichloromethane, ethyl acetate, and methanol, in increasing polarity order, providing the corresponding fractions. The hexane fraction (**HF**; 2.00 g) was submitted to CCS and elution with hexane and dichloromethane, in increasing polarity order, providing a mixture of **1** and **2** (0.245 g) and compound **3**. The latter was recrystallized from MeOH, providing a pure white solid (0.120 g).

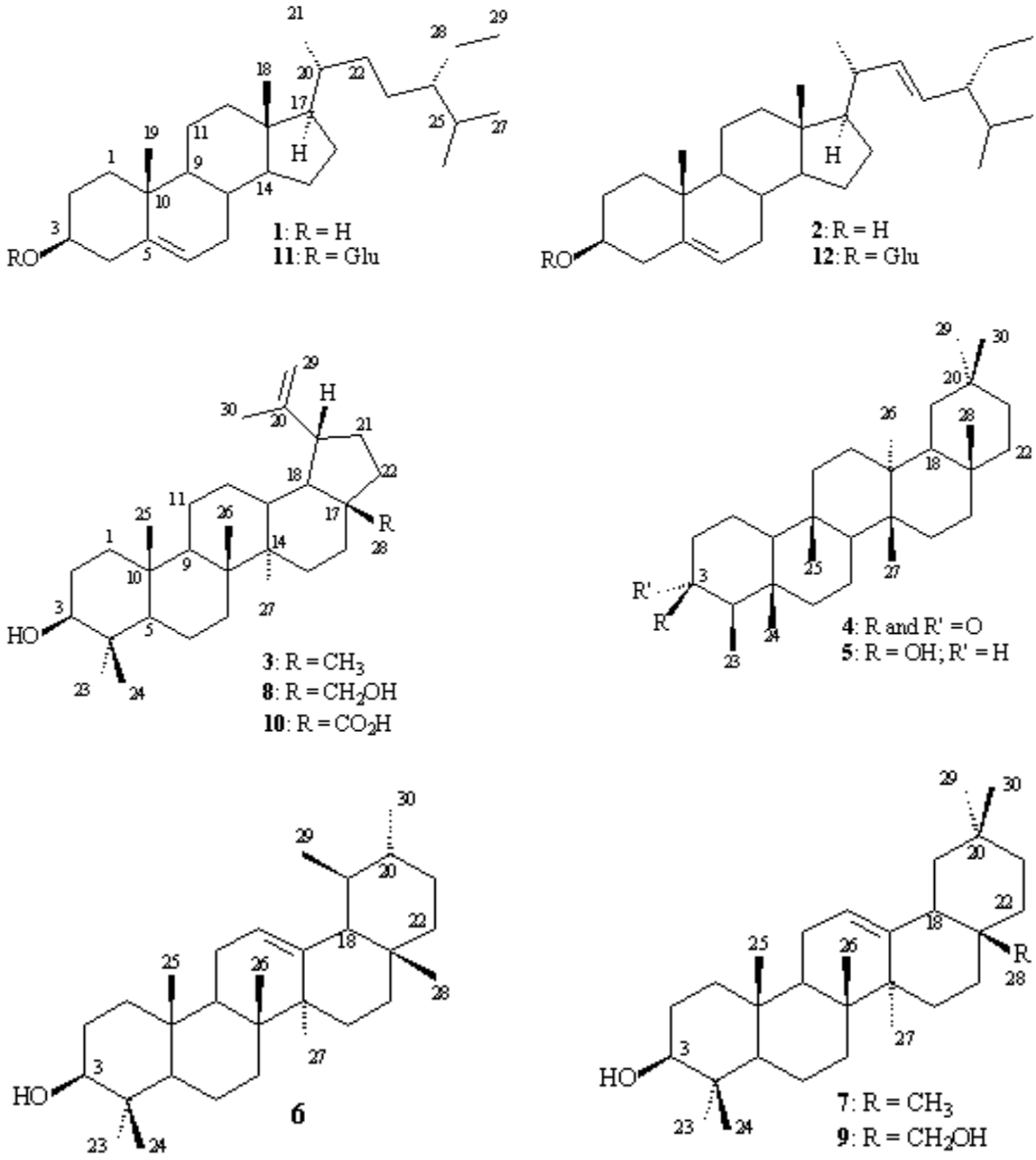

Fig. 1: Chemical structure of compounds isolated from aerial parts of *J. acuminatissima*: *β*-sitosterol (1), stigmasterol (2), lupeol (3), friedelin (4), *β*-friedelinol (5), *α*-amyrin (6), *β*-amyrin (7), betulin (8), erythrodiol (9), betulinic acid (10), glycosylated *β*-sitosterol (11), and glycolylated stigmasterol (12).

The dichloromethane fraction (**DF**; 6.00 g) was submitted to CCS and elution with hexane and dichloromethane, in increasing polarity order. The fraction eluted with hexane:CH_2Cl_2 (9:1) was recrystallized from MeOH, providing compound **4** (0.005 g) as a pure white solid. The fraction eluted with hexane: CH_2Cl_2 (3:1) was recrystallized from MeOH, providing compound **5** (0.006 g) as a pure white solid. The fraction eluted with hexane/CH_2Cl_2 (2:1) was recrystallized from MeOH, providing a mixture of **6** and **7** (0.060 g). The fraction eluted with hexane:CH_2Cl_2 (1:1) was recrystallized from MeOH, providing a mixture of **8** and **9** (0.085 g). The fraction eluted with hexane: CH_2Cl_2 (1:2) was recrystallized from MeOH, providing compound **10** (0.020 g) as a pure white solid.

The ethyl acetate fraction (**AF**; 10.00 g) was submitted to CCS and elution with CH_2Cl_2: EtOAc (1:1), providing a mixture of **11** and **12** (0.335 g). The fraction eluted with CH_2Cl_2: EtOAc (1:2) provided compound **13** (0.007 g) as a pure white solid. Finally, the methanol fraction (**MF**; 30.00 g) was submitted to CCS and elution with EtOAc and MeOH, in increasing polarity order, successively providing a mixture of **14** and **15** (0.130 g) and compound **16** (0.075 g) as a pure white solid.

Anti-inflammatory activity

Experiments were performed on male rat (200.0 to 300.0 g) purchased from Bioagri Laboratórios LTDA (City of Planaltina - DF, Brazil). The animals were kept in plastic cages at 22 ± 2 ºC on a 12 h light/dark cycle with free access to pellet food and water, according to International Guiding Principles for Biomedical Research Involving Animals [5]. The entire experiment was approved by the Ethics Committee on Animal Use of the Universidade Federal de Minas Gerais (CETEA/UFMG), under protocol number 222/11.

The animals were acclimatized for four days before beginning the experiments. Groups of rats (n = 21) were subjected to muscle injury in both the paws (right and left). The animals suffered muscle injury which was generated by the impact of a loose weight of 300.0 g at 30.0 cm high in the hamstring and calf backs.

After injury, the right paw of each animal was treated using therapeutic ultrasound in pulsed mode at a frequency of 1 MHz, with intensity 0.5 W/cm^2 for 9 min [6]. The treatment was performed once a day for three consecutive days. Animals were separated into groups: one negative control group (without treatment), one positive control group (with standard treatment using dexamethasone), and five treated groups (with treatment using extracts, fractions, and isolated compounds). Extracts (6.0% m/m), fractions (3.0% m/m), isolated compounds (0.5% m/m), and dexamethasone (0.1% m/m) were incorporated in the form of carbopol gel and used as couplant in the therapeutic ultrasound [2].

The trichotomy on bilateral gluteal region of all injured rat paws was performed by application of gels previously prepared. At 24 h after of the treatment, the animals were anesthetized and sacrificed for muscle collection. Other sacrifices were repeated at 48 and 72 h after of the treatment. Ketamine and xylazine (80.0 and 10.0 mg/kg body weight, respectively) were diluted in 1 mL of saline solution and employed to intraperitoneal (i.p.) anesthesia.

The paw tissues were removed,fixed in 10% formalin in Phosphate buffered saline (PBS) embedded in paraffin, and cut into 4 µm thickness sections. The sections were stained by hematoxylin-eosin for histological analysis. A representative area was selected for qualitative light microscopic analysis of the inflammatory cellular response with a 10x objective [7]. The results were expressed by the ability to decrease the inflammatory infiltrate.

Antimicrobial assays

Bioassays were conducted with *S. aureus* ATCC 29213, *B. cereus* ATCC 11779, *E. coli* ATCC 25723, *S. typhimurium* ATCC 14028, and *C. albicans* ATCC 18804. Microorganisms were individually inoculated in vials containing Brain Heart Infusion (BHI) broth [8]. The antimicrobial activity was performed using 96-wells sterile microplates. Extracts, fractions, and isolated compounds were dissolved in DMSO, yielding stock solutions. Subsequently, an aliquot of 40 µL of stock solutions was diluted in 960 µL of BHI culture medium, thus obtaining the stock solutions used in the bioassay. Samples were tested in triplicate in each plate. The microplates were prepared from the addition of 100 µL of the stock solution and 100 µL of inoculum of the microorganism in the wells, resulting in a sample solution with final concentration 250 µg/mL in each well. For control of microbial growth, the microorganisms were grown in the same conditions, except that the tested samples were not added to the wells. A control containing 100 mL of BHI culture medium and 100 mL of sterile distilled water was run in parallel to check the sterility of the culture medium. Ampicillin was used as positive control against bacteria and nystatin was used as positive control against *C. albicans*.

The prepared microplates were incubated at 37 °C for 48 h. The readings were performed on a Plate reader (Thermoplate, model PD-READER) in fixed wavelength of 492 nm, at 48 h for testing antibacterial and fungal inhibitions.

RESULTS AND DISCUSSION

Identification of the isolated compounds

IR spectrum of **1** and **2** shows strong absorptions at 3278, 1189, and 1094 cm^{-1}, which are characteristic of H–O and C–O stretches. ^{1}H NMR spectrum shows signals at δ_H 5.36 and 5.10, both signals are attributed to olefinic hydrogen atoms. The signal at δ_H 3.52 is attributed to carbinolic hydrogen. ^{13}C NMR spectrum shows signals at δ_C 140.8, 138.3, 129.3, and 121.7 (attributed to olefinic carbon atoms) and signal at δ_C 71.8 (characteristic of carbinolic carbon). The NMR data (see Table 1) are in agreement with the corresponding ones described in the literature for stigmasterol and *β*-sitosterol [9-10].

IR spectrum of **3** shows strong absorptions at 3276, 1192, and 1062 cm^{-1}, which are characteristic of H–O and C–O stretches. ^{1}H NMR spectrum shows signals at δ_H 4.62 (attributed to olefinic hydrogen atoms) and 3.19 (attributed to carbinolic hydrogen). ^{13}C NMR spectrum shows signals at δ_C 150.9 and 109.2 attributed to olefinic carbon atoms of lup-20(29)-ene-type skeleton. The signal at δ_C 78.9 is characteristic of carbinolic carbon. The NMR data (Table 1) are in agreement with the corresponding ones described in the literature for lupeol [11].

IR data of **4** show an intense absorption at 1730 cm^{-1} characteristic of C=O stretch. ^{1}H NMR spectrum shows signals at δ_H 2.50–1.22 characteristic of aliphatic hydrogen atoms. ^{13}C NMR spectrum shows a signal at δ_C 213.5 attributed to carbonyl carbon of ketone, and other signals characteristic of saturated carbon atoms. The NMR data (Table 1) are in agreement with the corresponding ones described in the literature for friedelin [9].

IR spectrum of **5** shows strong absorptions at 3305, 1211, and 1116 cm^{-1}, which are characteristic of H–O and C–O stretches. ^{1}H NMR spectrum shows signal at δ_H 3.75 attributed to carbinolic hydrogen, and overlapped signals at δ_H 2.01–1.37 characteristic of aliphatic hydrogen atoms. ^{13}C NMR spectrum shows signal at δ_C 72.3 attributed to carbinolic carbon, and other signals characteristic of aliphatic carbon atoms. The NMR data (Table 1) are in agreement with the corresponding ones described in the literature for *β*-friedelinol [9].

IR spectrum of **6** and **7** shows strong absorptions at 3421, 1172, and 1109 cm^{-1}, which are characteristic of H–O and C–O stretches. ^{1}H NMR spectrum shows overlapped signals at δ_H 5.15–5.10 attributed to olefinic hydrogen atoms, and signals at δ_H 3.21–3.16 attributed to carbinolic hydrogen atoms. ^{13}C NMR spectrum shows signals at δ_C 139.6 and 124.5 (attributed to olefinic carbon atoms of urs-12-ene-type skeleton), δ_C 145.1 and 121.8 (attributed to olefinic carbon atoms of olen-12-ene-type skeleton), and δ_C 79.1 and 79.0 (attributed to carbinolic carbon atoms). The NMR data (Table 1) are in agreement with the corresponding ones described in the literature for *α*-amyrin and *β*-amyrin [11].

IR spectrum of **8** and **9** shows strong absorptions at 3372, 3202, 1128, and 1043 cm^{-1}, which are characteristic of H–O and C–O stretches. ^{1}H NMR spectrum shows signals at δ_H 5.20, 4.69, and 4.58 characteristic of olefinic hydrogen atoms. The signals at δ_H 3.80, 3.34, and 3.19 are attributed to carbinolic hydrogen atoms. ^{13}C NMR spectrum shows signals at δ_C 150.5 and 109.6 (attributed to olefinic carbon atoms of lup-20(29)-ene-type skeleton), δ_C 144.2 and 122.3 (attributed to olefinic carbon atoms of olen-12-ene-type skeleton), and δ_C 79.0, 69.7, and 60.6 (attributed to carbinolic carbon atoms). The NMR data (Table 1) are in agreement with the corresponding ones described in the literature for betulin and erythrodiol [11].

IR spectrum of **10** shows strong absorptions at 3331, 1189, and 1042 cm^{-1}, which are characteristic of H–O and C–O stretches. The strong absorption at 1729 cm^{-1} is attributed to C=O stretch of carboxylic acid. ^{1}H NMR spectrum shows signals at δ_H 4.94 and 4.77, both signals attributed to olefinic hydrogen atoms. The signal at δ_H 3.53–3.47 is attributed to carbinolic hydrogen. ^{13}C NMR spectrum shows signal at δ_C 179.1 characteristic of carboxylic carbon. The signals at δ_C 150.9 and 109.2 are attributed to olefinic carbon atoms of lup-20(29)-ene-type skeleton. The signal at δ_C 78.3 is attributed to carbinolic carbon. The NMR data (Table 1) are in agreement with the corresponding ones described in the literature for betulinic acid [9].

Table 1: ^{13}C NMR data of steroids and triterpenes isolated from aerial parts of *J. acuminatissima*

Carbon	1	2	3	4	5	6	7	8	9	10
1	37.3	37.3	38.6	22.1	15.2	38.7	38.7	38.6	38.6	39.4
2	31.6	31.7	27.3	41.6	36.4	27.2	27.3	27.2	27.2	28.4
3	71.8	71.8	78.9	213.0	71.6	78.3	79.0	79.0	79.0	78.3
4	42.3	42.3	38.8	58.6	48.6	38.7	38.8	38.8	38.8	39.6
5	140.8	140.8	55.2	42.5	37.8	55.2	55.3	55.3	55.2	56.0
6	121.7	121.7	18.2	41.9	41.1	18.3	18.4	18.3	18.3	18.8
7	31.9	31.9	34.2	18.3	16.9	32.9	32.9	34.0	32.6	34.9
8	31.9	31.9	40.7	56.5	52.5	40.0	38.8	41.0	39.8	41.2
9	50.2	50.2	50.3	37.8	37.2	47.7	47.7	50.4	46.5	51.0
10	36.5	36.5	37.1	59.8	60.8	36.9	37.2	37.2	37.0	37.7
11	21.1	21.1	20.8	35.7	35.9	23.3	23.4	20.9	23.6	21.3
12	39.8	39.7	25.0	30.9	30.0	124.4	121.8	25.2	122.3	26.2
13	42.3	42.3	38.0	40.1	38.6	139.6	145.1	37.3	144.2	38.7
14	56.9	56.9	42.9	38.7	39.0	42.1	41.6	42.7	41.7	42.9
15	24.3	24.4	27.3	32.4	31.6	28.7	26.7	27.0	25.6	31.3
16	28.9	29.1	35.5	36.4	34.8	26.6	27.0	29.2	22.0	33.0
17	56.1	56.1	42.7	30.3	29.3	33.7	32.5	47.8	39.8	56.8
18	11.9	12.0	48.2	43.2	42.2	59.0	47.4	48.8	42.4	47.9
19	19.4	19.4	47.9	35.4	34.6	39.6	46.9	47.6	46.5	49.8
20	36.1	40.5	150.9	28.5	27.5	39.6	39.6	150.5	30.9	151.5
21	18.8	21.1	29.8	32.8	32.2	31.2	34.3	29.8	34.1	30.4
22	33.9	138.3	39.9	39.6	38.6	41.5	37.2	34.3	31.0	37.6
23	26.1	129.3	27.9	7.2	11.1	28.1	28.2	28.0	28.1	28.8
24	45.8	51.2	15.3	15.0	15.7	15.6	15.6	16.0	15.5	16.4
25	29.2	31.9	16.0	18.6	17.6	15.6	15.6	16.1	15.6	16.5
26	19.8	21.2	15.9	20.6	19.4	16.9	16.8	15.6	16.7	16.5
27	19.0	19.0	14.5	19.0	18.0	23.3	26.0	14.8	25.8	15.0
28	23.1	25.4	17.9	32.1	31.3	28.1	28.2	60.6	69.7	179.1
29	12.2	12.2	109.2	35.7	34.3	17.4	33.5	109.7	33.2	110.1
30			19.2	31.6	31.1	21.4	23.4	19.1	23.5	19.6

^{13}C NMR spectrum of **11** and **12** are similar to corresponding signals observed to **1** and **2**. Thus, IR spectrum of **11** and **12** shows strong absorption at 3449 cm^{-1}, which is characteristic of H–O stretch. Signals at δ_H 4.54–3.96 are attributed to carbinolic hydrogen atoms. ^{13}C NMR spectrum shows signals at δ_C 102.6, 78.6, 78.5, 78.2, 75.4, 71.7, and 62.9 characteristic of carbinolic carbon atoms of glycoside residues [12]. Finally, structural identification of compounds **13**–**16** were based on ^{13}C NMR data described in the literature for 4-hydroxybenzoic acid [13], *α*-glucose, *β*-glucose, and sucrose, respectively [14].

Antimicrobial activity

Table 2 shows antimicrobial activity of extracts, fractions, and steroids isolated from aerial parts of *J. acuminatissima* against different microorganisms. The ethanol extract (**EE**) exhibited higher activity than hydroalcoholic extract (**WE**). **EE** inhibited growth of *S. aureus*, *B. cereus*, *S. typhimurium*, and *E. coli*. Fraction **HF** contains the less polar compounds and exhibited low activity. Similar results were observed to the fraction containing polar compounds (**MF**), which exhibited low activity against all tested microorganisms.

DF fraction exhibited an excellent growth inhibition to all tested microorganism (94.4%, 76.0%, 75.2%, 65.4%, and 61.0% for *C. albicans*, *B. cereus*, *S. typhimurium*, *S. aureus*, and *E. coli*, respectively). As results, it can be proposed a broad spectrum of antimicrobial activity to this fraction. On the other hand, **AF** fraction showed outstanding inhibition towards *C. albicans* and *E. coli*, exhibiting an interesting narrow spectrum of activity. As **DF** and **AF** fractions exhibited significant activities against all microorganisms, their antimicrobial activities can be attributed to compounds of intermediary polarities, i.e. steroids and triterpenes. These class of phytoconstituents are known as antimicrobial agents [15-19].

Mixture of **1** and **2** (compounds of low polarities) relatively exhibited low antimicrobial activity, being in agreement with the observed results for **HF**. Similarly, mixture of **11** and **12** (compounds of high polarity) also exhibited low antimicrobial activity, indicating that the effect of the glycoside residue on the steroids is not significant in this case.

Anti-inflammatory activity

The anti-inflammatory activity of extracts, fractions, and isolated compounds of *J. acuminatissima* were based on histological analysis to characterize inflammatory infiltrates. Figure 2 shows the histological aspect of injured muscles of the rat paws without treatment (negative control). At 24 h after of the muscle injury the section exhibits inflammatory infiltrates (purple dots spread on the section), and edemas (white regions on the section). Muscle tissues are represented by pink spots. A normal pattern of muscle tissue should show pink continuous fibers.

However, the fragmentation of muscle tissue observed to negative control in Figure 2 indicates significant degeneration of muscle fibers [20]. At 48 and 72 h after of the muscle injury without treatment the section exhibits some regions of edemas and inflammatory infiltrates.

Dexamethasone is a potent anti-inflammatory. The rat paws treated with dexamethasone (see Figure 2) exhibits mild inflammatory infiltrate at 24 and 48 h after of the treatment. The histological section at 72 h after of the treatment shows an area without injury. Regions of edemas are moderate to mild and degeneration of muscle fibers is mild at 24, 48, and 72 h after of the treatment with dexamethasone. Compound **3** exhibits high anti-inflammatory activity. Mixture of **1** and **2** reduces inflammations and edemas in rat paws (see Figure 2). However, mixture of **11** and **12** exhibits the highest anti-inflammatory activities. Consequently, it can be proposed a significant effect of the glycosylation on the anti-inflammatory activity of *β*-sitosterol and stigmasterol. Tests using triterpenes as anti-inflammatory are very relevant because, according the literature triterpenes such as lupeol and betulin are potent anti-inflammatory agents [21].

Extracts **EE** and **WE** and fractions **HF** and **MF** exhibit low anti-inflammatory activity (see Figure 3). However, the histological sections analyzed of the fractions **AF** and **DF** show expressive reduction of inflammatory infiltrates and edemas. In fact, mixture of **1** and **2**, compound **3**, and mixture of **11** and **12** were isolated from these fractions.

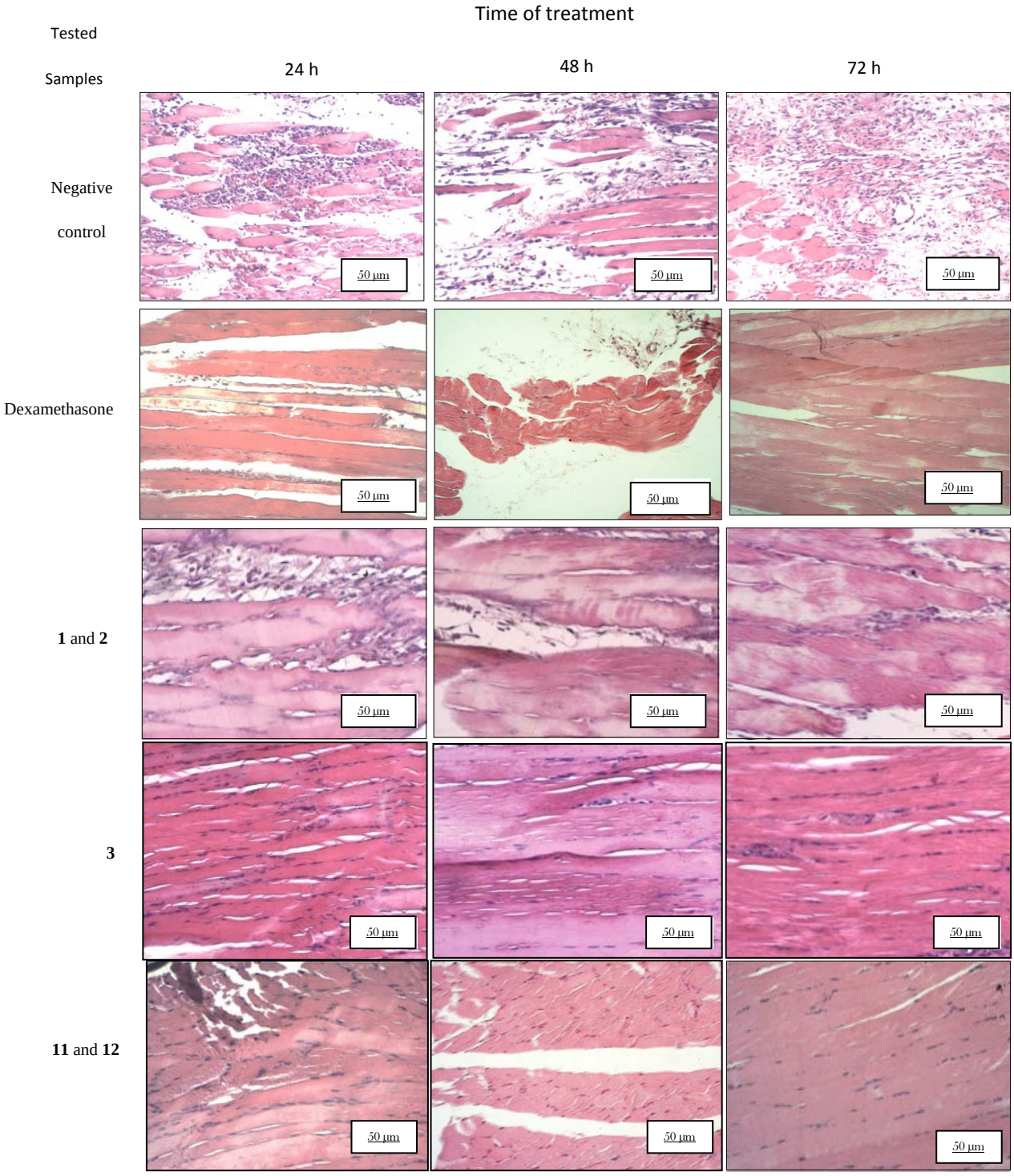

Fig. 2: Histological aspect of injured muscles of the rat paws without treatment (negative control) and treated with dexamethasone (positive control) and isolated compounds of *J. acuminatissima* at 24, 48, and 72 h after of the treatment.

Table 2: Antimicrobial activity of extracts, fractions, and steroids isolated from aerial parts of *J. acuminatissima* against *Gram*-positive and *Gram*-negative bacteria and a fungi (sample concentration: 250 µg/mL)

	% inihibition (± sd)								
Microorganism	**EE**	**WE**	**HF**	**DF**	**AF**	**MF**	**1 and 2**	**11 and 12**	**Positive control**
B. cereus	55.0 ± 1.1	0	0	76.0 ± 1.3	41.8 ± 1.0	2.2 ± 0.7	0	10.0 ± 1.1	95.46 ± 0.12
E. coli	67.5 ± 0.5	0	0	61.0 ± 1.2	81.2 ± 4.3	0	5.7 ± 1.6	4.5 ± 2.6	99.18 ± 0.57
S. aureus	57.7 ± 1.2	0	0	65.4 ± 3.0	38.5 ± 1.9	14.4 ± 2.5	9.5 ± 2.7	5.3 ± 1.4	94.88 ± 1.25
S. typhimurium	63.3 ± 0.5	0	0	75.2 ± 0.5	45.0 ± 1.2	1.2 ± 0.2	4.6 ± 1.5	3.7 ± 0.5	94.84 ± 1.72
C. albicans	14.0 ± 2.8	6.4 ± 1.5	7.8 ± 2.5	94.4 ± 1.6	75.8 ± 5.5	3.98 ± 0.3	3.5 ± 1.0	16.5 ± 1.0	98.46 ± 1.34

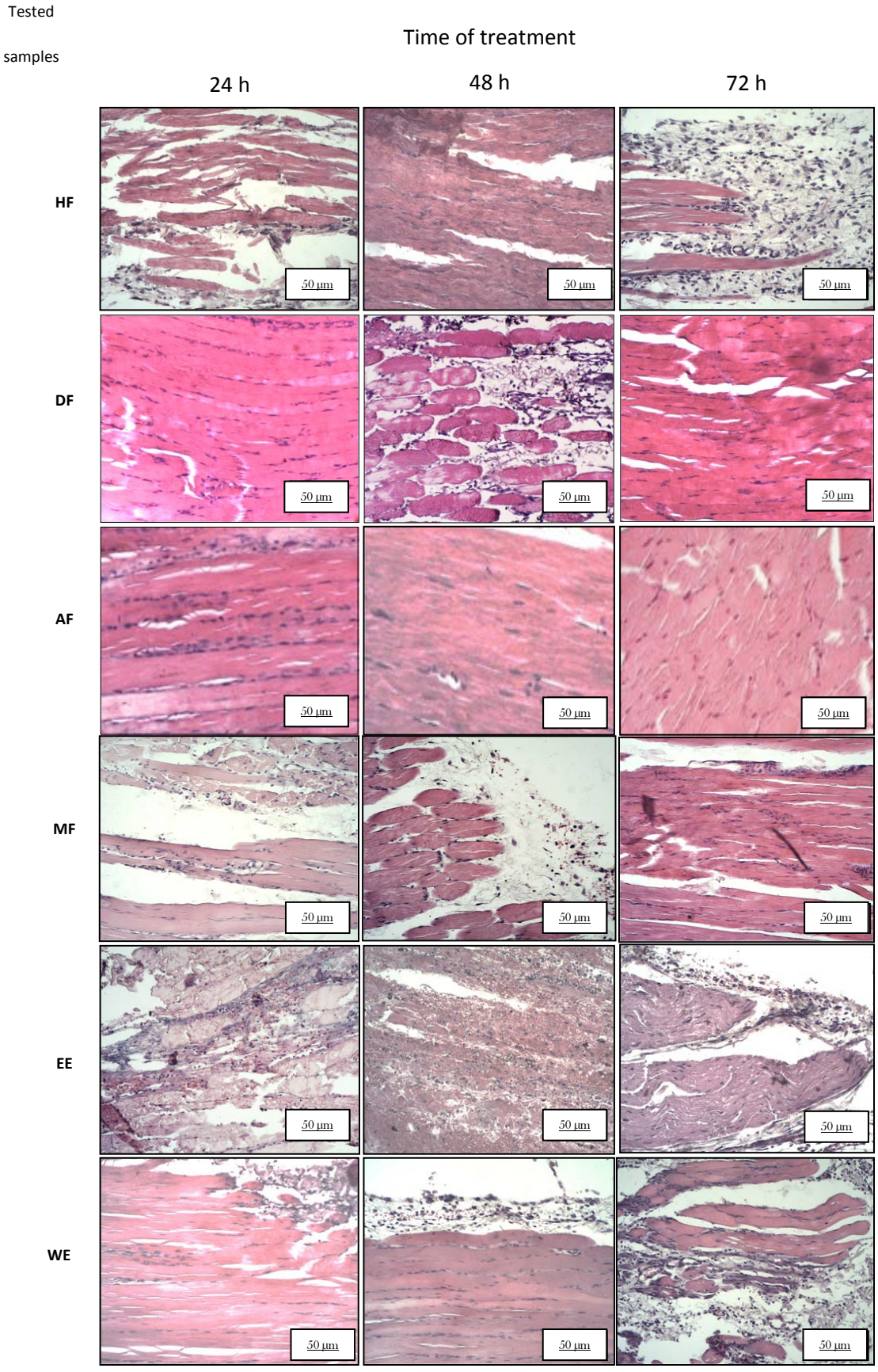

Fig. 3: Histological aspect of injured muscles of the rat paws treated at 24, 48, and 72 h after of the application of gels with extracts and fractions of *J. acuminatissima*.

Table 3: Anti-inflammatory activity of extracts, fraction, and some compounds isolated from aerial parts of *J. acuminatissima* (Legend: 0 = uninjured; 1 = small lesion; 2 = small to moderate injury lesion; 3 = moderate lesion; 4 = moderate to severe injury lesion; 5 = several lesion)

Sample	24 h after treatment	48 h after treatment	72 h after treatment
WE	2	3	5
EE	2	1	1
MF	2	2	0
AF	1	1	0
DF	2	0	0
HF	1	0	4
1 and **2**	2	1	0
11 and **12**	2	1	1
3	1	0	0
Negative control	3	2	2
Dexamethasone	1	1	0

Table 3 shows the relative scores of inflammatory infiltrate exhibited by extracts, fractions, and isolated compounds. These results of anti-inflammatory activity justify the use of *J. acuminatissima* by popular medicine of the Amazon region to the treatment of inflammatory diseases.

CONCLUSION

The phytochemical study of aerial parts of *J. acuminatissima* resulted in the isolations of compounds of different chemical classes, mainly steroids and triterpenes. Dichloromethane and ethyl acetate fractions exhibited high antimicrobial and anti-inflammatory activities, indicating higher biological activity of constituents presenting intermediate polarity. Mixture of *β*-sitosterol and stigmasterol and mixture of glycosylated *β*-sitosterol and glycosylated stigmasterol exhibited low antimicrobial activity. However, both the mixtures exhibited high anti-inflammatory activity, mainly the mixture of glycosylated *β*-sitosterol and glycosylated stigmasterol, indicating a significant effect of the glycosylated residue on their biological activities.

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REFERENCES

1. J. Corrêa GM, Alcântara AFC. Chemical constituents and biological activities of species of JusticiaA review Braz 2012;22:220-38.
2. Abreu VGC, Correa GM, Silva TM, Fontoura HS, Cara DC, Piló-Veloso D, et al. Anti-inflammatory effects in muscle injury by transdermal application of gel with Lychnophora pinaster aerial parts using phonophoresis in rats. BMC complementary and alternative medicine 2013;13:270.
3. Abreu VGC, Takahashi JA, Duarte LP, Veloso D, Junior PAS, Alves RO, et al. Piló- Alcântara AFC. Evaluation of the bactericidal and trypanocidal activities of triterpenes isolated from the leaves steams and flowers of Lychnophora pinaster Braz 2011;21:615-21.
4. Pacheco AG, Silva TM, Manfrini RM, Sallum WST, Duarte LP, Piló-Veloso D, Alcântara AFC, Knupp VF. Chemical study and antibacterial activity of steam of *Aristolochia esperanzae* Kuntze (Aristolochiaceae). Quim Nova. 2010;33:1649-52.
5. Cioms. from: http://www.ncbi.nlm.nih.gov/books/NBK25438.
6. Fontoura HS, Azevedo RB. Influence of anti-inflammatory properties of ultrasound therapy in muscle injuries treatment. Fisioter Bras 2008;9:17-22.
7. Vieira ELM, Leonel AJ, Sad AP, Beltrão NRM, Costa TF, Ferreira TMR, et al. Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. The Journal of nutritional biochemistry 2012;23(5):430-6.
8. Teles APC, Ataliba GS, Takahashi JA. Modulation of Paecilomyces lilacinus antimicrobial metabolite production by co-culturing with Salmonella typhimurium. Natural product research 2013;27(17):1598-601.
9. Sousa GF, Duarte LP, Alcântara AFC, Silva GDF, Vieira-Filho SA, Silva RR, et al. New triterpenes from Maytenus robusta: structural elucidation based on NMR experimental data and theoretical calculations. Molecules (Basel, Switzerland) 2012;17(11):13439-56.
10. Alcântara AF, Souza MR, Piló-Veloso D. Constituents of Brosimum potabile. Fitoterapia 2000;71(5):613-5.
11. Abreu VGC, Lagos IAS, Silva RR. Corrêa GM, Alcântara AFC. Pentacyclic triterpenes and steroids from the stem bark of uchi Sacoglottis uchi Humiriaceae Acta Amaz 2013;43:525-8.
12. Costa HNR, Santos MC, Alcântara AFC, Silva MC, França RC, Piló-Veloso D. Chemical constituents and antiedematogenic activity of *Peltodon radicans* (Lamiaceae). Quim Nova. 2008;31:744-50.
13. Yayli N, Yildirim N, Usta A, Ozkurt S, Akgun V, J. Chemical Constituents of Campanula lactiflora. Turk 2003;27:749-55.
14. Bubb WA, R. NM. in the Study of Carbohydrates: Characterizing the Structural Complexity. Concepts Magn Reson 19A 2003:1-19.
15. Fontanay S, Grare M, Mayer J, Finance C, Duval RE, J. Ursolic, oleanolic and betulinic acids: Antibacterial spectra and selectivity indexes. BMC complementary and alternative medicine 2006;120:272-6.
16. Mathabe MC, Hussein AA, Nikolova RV, Basson AE, Meyer JJM, Lall N, et al. Antibacterial activities and cytotoxicity of terpenoids isolated from Spirostachys africana. BMC complementary and alternative medicine 2006;116:194-7.
17. Popova M, Trusheva B, Gyosheva M, Tsvetkova I, Bankova V. Antibacterial triterpenes from the threatened wood-decay fungus Fomitopsis rosea. Fitoterapia 2009;80(5):263-6.
18. Katerere DR, Gray AI, Nash RJ, Waigh RD. Antimicrobial activity of pentacyclic triterpenes isolated from African Combretaceae. Phytochemistry 2003;63(1):81-8.
19. Pacheco AG, Alcântara AFC, Abreu VGC, Corrêa GM. Relationships Between Chemical Structure and Activity of Triterpenes Against *Gram*-Positive and *Gram*-Negative Bacteria. In: Varaprasad Bobbarala editor. A Search for Antibacterial Agents. Rijeka:In Tech;2012.
20. Huard J, Li Y, Fu FH. Muscle injuries and repair: current trends in research. The Journal of bone and joint surgery. American volume 2002;84-A(5):822-32.
21. Moraes WF, Galdino PM, Nascimento MVM, Vanderlinde FA, Bara MTF, Costa EA, et al. Triterpenes involved in the anti-inflammatory effect of ethanolic extract of Pterodon emarginatus Vogel stem bark. Med 2012;66:202-7.