Antinociceptive and edematogenic activity and chemical constituents of *Talinum paniculatum* Willd

Maria Perpétua Oliveira Ramos[a, b], Grácia Divina de Fátima Silva[a], Lucienir Pains Duarte[a], Valdir Peres[b], Roqueline Rodrigues Silva Miranda[a], Gustavo Henrique Bianco de Souza[c], Valdenir José Belinelo[d] and Sidney Augusto Vieira Filho[c*]

[a] *Núcleo de Estudo de Plantas Medicinais (NEPLAM), Depto de Química, Universidade Federal de Minas Gerais, Campus Pampulha. Av. Antônio Carlos, 6627. CEP 31270-901. Belo Horizonte, MG, Brazil*
[b] *Grupo de Estudos de Plantas Medicinais (GEPLAM), Centro Universitário de Patos de Minas. Rua Major Gote, 808. CEP 38700-000. Patos de Minas, MG, Brazil*
[c] *DEFAR, Escola de Farmácia, Universidade Federal de Ouro Preto. Rua Costa Sena, 171. Centro. CEP 35000-000. Ouro Preto, MG, Brazil*
[d] *Centro Universitário Norte do Espírito Santo. Universidade Federal do Espírito Santo. Rodovia BR 101 Norte, Km. 60. CEP 29932-540. São Mateus, ES, Brazil*

_____

ABSTRACT

*Talinum paniculatum (Portulacaceae) is popularly known in the region of Patos de Minas, Minas Gerais State, Brazil, as "Maria Gorda" or "Benção-de-Deus" and in traditional medicine it is used to treat inflammatory processes in general. After dried and fragmented, the plant was sequentially submitted to exhaustive percolation with hexane, ethyl acetate and methanol. The crude extracts were investigated for their anti-edematogenic and anti-nociceptive activities through formalin-induced paw edema model. In comparison with a non-steroidal anti-inflammatory drug (NSAID) indomethacin, the hexane and ethyl acetate extracts showed higher anti-edematogenic and anti-nociceptive activity. In addition, through phytochemical studies, from these extracts were isolated and identified potassium nitrate (**1**), the mixture of long chain hiodrocarbons hentriacontane (**2**), dotriacontane (**3**), tritriacontane (**4**) and pentatriacontane (**5**). heneicosanoic acid (**6**), the ester nonacosyl nonacosanoate (**7**), urea (**8**), 3-O-β-D-glucosyl-β-sitosterol (**9**); the mixture of β-sitosterol (**10**) and stigmasterol (**11**), and a pentaciclyc triterpene 3-O-acethyl-aleuritolic acid (**12**). X-Ray difratometry was used for the characterization of inorganic constituent. TLC, HR-CG and spectrometric methods (IR. ^1H and ^{13}C NMR) were used to identify the structures of organic compounds. To the best of our knowledge, it is the first time that compound **1** to **8** and **12** are cited as constituents of T. paniculatum.*

Keywords: *Talinum paniculatum,* Portulacaceae, anti-edematogenic activity, antinociceptive property, 3-*O*-acethyl-aleuritolic acid.

_____

INTRODUCTION

The family Portulacaceae has a world-wide distribution and is most prevalent in tropical and subtropical areas including North Africa, South America and East Asia, particularly in China. This family comprises about 20 genera and 500 species [1]. Many species of this family are wetland plants constituted by succulent herbs and, in some cases, by soft shrubs, with annual reproductive cycle and are chemically characterized by the presence of betalains and *p*-plastids [1]. The genus *Talinum* consists of about 23 species, among them *T. paniculatum* and *T. triangulare* have been traditionally used in Brazil.

In the Patos de Minas region, Minas Gerais, Brazil, *Talinum paniculatum* (Jacq.) Willd. [*T. patens* (Jacq,) Gaertn] is popularly known as "Maria gorda", "Benção-de-Deus" or "Beldroega" [2-4]. Throughout the Patos de Minas region, *T. paniculatum* is often cultivated as ornamental plant in house gardens and small farms where it can reach up to 30 cm height. In traditional medicine *T. paniculatum* is used to treat urine with bad smelling, gastrointestinal disorders, physical debility and other general body weaknesses. Due to its fleshly and succulent aspect this plant is also used as food, mainly in different kinds of salads [4]. The leaves are topically used in the treatment of edemas, inflammatory skin diseases and superficial skin lesions, such as minor cuts, scratches and scrapes. The oral administration of decoction of the powdered root is indicated to treat scurvy, nerve distention and arthritis [5]. In eastern China and some South America countries, the decoction of the roots has also been used in the oral treatment of stomach inflammations and pneumonia [2,5].

Mucilage, tannins, carotene, chlorophyll and folic acid, represent the main constituents of *T. paniculatum* [4]. The mixture of 1-hexacosanol, 1-octacosanol and 1-triacontanol, and its respective acetates, and the mixture of the steroids campesterol, stigmasterol, β-sitosterol and 3β-D-glucosyl-sitosterol were previously cited as constituents of the roots [6]. The alkaloids javaberine A and B were isolated from methanol root extract of *T. paniculatum* [7]. In accordance with the literature, javaberine A has been used in type 2 diabetes treatments [7].

In the present work is reported the phytochemical study of leaves, stems and roots of *T. paniculatum* with consequent isolation and identification of potassium nitrate and eleven organic compounds. The characterization of organic compounds was realized through high-resolution gas chromatography (HR-GC) and infrared (IR) and hydrogen and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectrometries. The identification of inorganic constituent isolated from *T. paniculatum* was realized by X-ray difratometry. Hexane and ethyl acetate extract from leaves were analyzed in relation to their potential antinociceptive and antiedematogenic activities. Compared with indomethacin, a NSAID derivative of indolacetic acid, these extracts showed higher anti-edematogenic and anti-nociceptive activity

EXPERIMENTAL SECTION

Plant material
The *Talinum paniculatum* Willd (Portulacaceae) was obtained from house gardens and in small farms at Patos de Minas Municipality, Minas Gerais, Brazil. The sample was authenticated and a voucher (No. OUP-17.465) was deposited at the Herbário José Badini of the Universidade Federal de Ouro Preto (UFOP), Minas Gerais State, Brazil. The leaves, stems and roots were separated and dried at room temperature (r.t.) and subjected to limestone grinding mill to obtain a fine powder.

General procedures

Silica gel (Kieselgel 60, 70–230 mesh, 0.063–0.200mm, Merck) was used in column chromatography (CC) and (Kieselgel 60, 230-400 Mesh) in flash CC processes. All of the fractions were combined based on thin layer chromatography (TLC) analysis and the chromatoplates were revealed with 1:1 (v/v) solution of vanillin (1% in ethanol) with perchloric acid (5% in water).

All solvents used for CC as well as for TLC were from analytical grade purchased from VETEC (Brazil). Melting points (m.p.) (^{0}C) were determined in Metler FP82 equipped with Metler FP800 processor. The infrared (IR) spectra were obtained on a Perkin Elmer SPECTRUM 1000 device (1 mg % in KBr solution), and the range of 500 a 4000 cm^{-1}. ^{1}H and ^{13}C NMR, including DEPT-135 and 2D (HMBC and HMQC) techniques also were employed in structure elucidation of the isolated organic compounds using a Brüker AVANCE DRX-400 or AVANCE DPX-200 spectrometer. The internal standard TMS ($\delta_H = \delta_C = 0$) and specific deuterated solvent were used in the experiments. Chemical shift displacement (δ) was expressed in ppm and coupling constant (*J*) in Hertz (Hz). High-resolution gas chromatography (HR-GC) was performed on a VARIAN Model CP-3380, with flame ionization detector. To identify the inorganic constituent X-ray diphratogram was obtained on a Rigaku Geigerflex diphratometer, using chromium tube, speed 4, time constant 1 and scale 1×10^4.

Extraction and isolation procedures

Powdered leaves (48.81 g) of *T. paniculatum* were submitted to sequential extraction at r.t. with hexane, ethyl acetate and finally ethanol. The hexane extract (3.14 g) was fractionated by CC using hexane, chloroform, ethyl acetate and methanol pure or in mixture. After solvent evaporation, fraction 1 eluted by hexane furnishes a white solid material (40.0 mg), m.p. 68-72 ^{0}C. This material was submitted to HR-GC using CP Sil SCB column (0.25 μm, 0.25 mm I.D., 30 m) with H_2 flow rate 20 mL/min, $T_{Inj} = T_{Col} = 300$ ^{0}C and $T_{Det} = 320$ ^{0}C. The compound hentriacontane (**2**) [C_{31}, $t_R = 10.59$', 47.3 %], tritriacontane (**4**) [C_{33}, $t_R = 15.45$', 38.7 %] and pentatriacontane (**5**) [C_{35}, $t_R = 22.38$', 3.9 %] were identified as the principal constituents of this solid. After the evaporation of hexane, fraction 3 was characterized as nonacosyl nonacosanoate (**7**) (270.2 mg, m.p. 72-74 ^{0}C). The ethyl acetate extract of leaves (2.93 g) was submitted to silica gel CC eluted with gradient of ethyl acetate and methanol. Fractions 2 and 3, eluted with ethyl acetate, were grouped and submitted to a further CC in similar elution conditions that permit the isolation of 3-*O*-β-D-glucosyl-β-sitosterol (**9**) (80.0 mg). Fraction 10 also eluted with ethyl acetate was purified by flash silica gel CC, eluted with ethyl acetate and methanol (8:2) to give a white solid material (400.0 mg) identified as urea (**8**).

Powdered stems (277.37 g) of *T. paniculatum* were subjected to exhaustive hexane extraction in a Soxhlet apparatus. The hexane extract (4.92 g) was submitted to a silica gel CC using mixtures of hexane, ethyl acetate and methanol. The group of fractions 1 to 14 (635.0 mg) was eluted with hexane to give a white solid, m.p. 58-63 ^{0}C. Through HR-GC [SE-54 column (0.25 μm, 0.25 mm I.D., 30 m), H_2 flow rate 20 mL/min, $T_{Inj} = T_{Col} = 280$ ^{0}C and $T_{Det} = 300$ ^{0}C] was possible to identify dotriacontane (**3**) [C_{32}, $t_R = 7.60$', 61.8 %] and tritriacontane (**4**) [C_{33}, $t_R = 8.70$', 21.0 %]. The solid material obtained from fraction 21, eluted with hexane-ethyl acetate (9:1), was washed with hexane, and dried. Submitted to IR, ^{1}H and ^{13}C quantitative NMR spectrometry, the solid material obtained was identified as heneicosanoic acid (**6**) (823.0 mg, m.p. 78-83 ^{0}C). Fractions 44 to 66 were grouped and submitted to flash silica gel CC eluted with gradient of ethyl acetate and methanol. Through this process, a white solid material (16.0 mg) was obtained (fractions 7-9). Through IR and NMR spectral data this solid was further identified as 3-*O*-acetyl-aleuritolic acid (**12**), m.p. 249-252 ^{0}C. Fraction 25-27 showed Liebermann-Burchard (LB) test positive for steroids [8,9], and was subjected to HR-GC analysis [SE 54 column, H_2

_____

flow rate 20 mL/min, $T_{Inj} = 280$ ^{0}C, $T_{Col} = 260$ ^{0}C, and $T_{Det} = 300$ ^{0}C]. By this process, stigmasterol (**11**) [$t_R = 10.32$', 30.7 %] and β-sitosterol (**10**) [$t_R = 11.39$', 32.3 %] were identified by comparison with standards and retention time. An unidentified constituent [$t_R = 12.83$', 32.1 %] also was observed in the chromatogram. Probably this constituent corresponds to campesterol that, like compounds **10** and **11**, was previously cited in literature as *T. paniculatum* constituents [6].

By pharmacognostic evaluation [8,9] performed for testing different chemical groups present in powdered extracts of *T. paniculatum* were detected alkaloids (Dragendorff test), coumarins (10 % KOH soln.), anthraquinone aglicons (Borntrager test), triterpenes/steroids (Liebermann-Burchard test), saponins (Vanilin-perchloric acid) and tanines/polyphenols (Ferric chloride). And by aluminum chloride 5 % solution test, flavonoids were not detected in these extracts.

After the ethanol extraction, during the solvent removal, a white solid precipitation was observed. The precipitate was collected by vacuum filtration on a Büchner funnel and dried in Abderhalden´s apparatus, at 60 ^{0}C/12 hours producing a white solid material (820.0 mg). At the IR spectra of this solid were observed a large and intense absorption band at 1380 cm^{-1} (ν_s N-O), and at 850 cm^{-1} (ν bond π in N-O) [11]. Due to its high melting point (> 300 ^{0}C), a sample (5.70 g) of this solid was submitted to X-ray difractometry. The diphratogram of this solid was compared with the data of Joint Committee on Powder Diffraction Standards (JCPDS) and identified as potassium nitrate (**1**).

Pharmacology
Animals and assay procedures
Experiments were carried out in 12-week old male Swiss mice (*Mus musculus*), weighing 25-30 g. The mice were acquired from the animal house of UNIPAM, Patos de Minas, Minas Gerais State, Brazil, kept in polyethylene boxes, in a climate-controlled ambient (25 ± 3 °C, 55 ± 5 % humidity) with a 12-h light/dark cycle. Standard rodent diet pellet (Nurvital®) and water were available *ad libitum*. The food was suspended 24 h before the test, maintaining the free access to water. Mice were randomized into experimental groups of six animals each (n = 6). Throughout the experiments, animals were manipulated according to the suggested ethical guidelines for the care of laboratory animals. The protocol for the study (2010/07) was approved by the Ethical Committee in the Use of Animals of Universidade Federal de Ouro Preto.

Preparation of extract for bioassay
Each extract was dissolved in a mixture of distilled water with 0.5 % sodium carboxymethyl cellulose (CMC), and doses (0.1, 1.0 and 10.0 mg/kg) were orally administered to animals. The control group was treated only with an adequate volume of the CMC solution. Indomethacin (20.0 mg/kg) in 0.5 % CMC was used as reference NSAID drug.

Antinociceptive and antiedematogenic evaluation
In animals of all the groups, chronic inflammation was produced by a single plantar injection of 0.02 mL of freshly prepared 2.0 % suspension of formalin in normal saline in right hind paw of mice used as the edematogenic agent. Sterile physiologic solution was administered subcutaneously in lateral paws [12]. Two hours after the subcutaneous administration of formalin solution, 0.5 mL/kg of CMC was orally administered to animals of the group 01 (control). The animals of group 02, 03 and 04 were respectively treated by intraperitoneal route with 0.1, 1.0 and 10.0 mg/kg of extract. The animals of the group 05 were treated with indomethacin (20.0 mg/kg) as positive NSAID control. The inflammatory phase response was observed during the first five minutes, and the second phase (nociceptive) during the 15 to 30 minutes after formalin

_____

administration. The time during which the animals lick their paws was determined according to method suggested by Debuisson and Dennis [12]. To evaluate the antiedematogenic effect induced by the extracts, at the end of the experiment the paw weight of the animals of each group was measured using an analytical balance. The antiedematogenic effect was evaluated through the weight difference of control and experimental groups.

Statistical analysis

Results are expressed as mean ± SEM (standard error of the mean). The statistical significance was estimated by one-way analyses of variance (ANOVA), complemented by Duncan test using the GraphPad Instat® software, version 3.01. Data were considered different with the level of significance set at $p \leq 0.5$ [10].

RESULTS AND DISCUSSION

Through usual phytochemical methods, from the extracts of *T. paniculatum* were isolated and identified potassium nitrate (**1**) and the following substances: a mixture of long chain hydrocarbons [hentriacontane (**2**), dotriacontane (**3**), tritriacontane (**4**) and pentatriacontane (**5**)]; heneicosanoic acid (**6**); nonacosyl nonacosanoate (**7**); urea (**8**); 3-*O*-β-D-glucosyl-β-sitosterol (**9**); a mixture of steroids [β-sitosterol (**10**) and stigmasterol (**11**)] and the triterpene 3-*O*-acethyl-aleuritolic acid (**12**) (Figure 1).

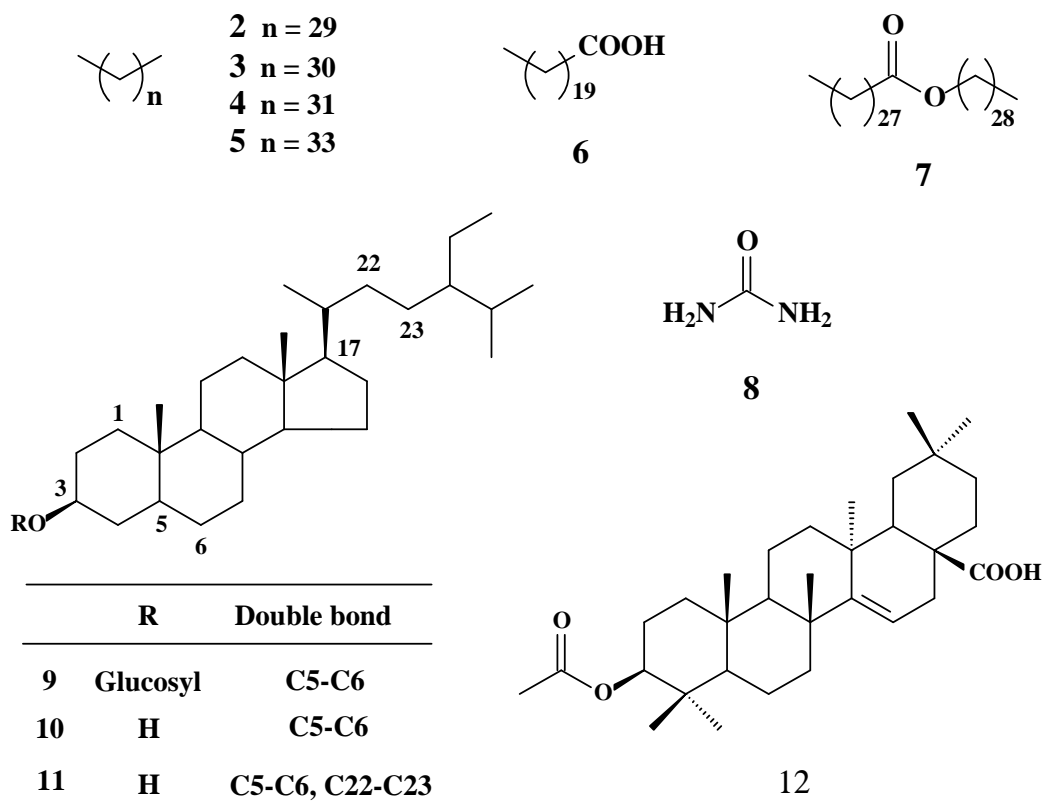|	R	Double bond
9	**Glucosyl**	**C5-C6**
10	**H**	**C5-C6**
11	**H**	**C5-C6, C22-C23**

Figure 1: Chemical structures of the constituents isolated from *T. paniculatum*

The IR spectra of compound **7** showed absorption bands at 2900 and 2800 cm^{-1} (ν CH), 1730 cm^{-1} (ν C=O of ester), 1460 and 1450 cm^{-1} (δCH) associated to double bond, and at 730 e 720 cm^{-1} correspondent to (CH$_2$)$_n$ (n ≥ 7) [11,13]. The ^{1}H NMR showed a triplet at δ_H 0.88 (3H, *J*=7.0 Hz)

_____

which was correlated to H-29 and H-29'. The triplet at δ_H 2.28 (J=7.6 Hz) was associated to H-2 alpha to carbonyl and a triplet (J=6.8 Hz) at δ_H 4.06 to H-1' adjacent to oxygen. The multiplet at δ_H 1.61 (J=7.3 Hz) was associated to 4 hydrogen and a singlet at δ_H 1.27 to methylene groups (102 H). The ^{13}C NMR spectra showed signals at δ_C 14.10, δ_C 34.45, δ_C 64.36 and at δ_C 173.83. Through DEPT-135 these signals were respectively associated to aliphatic methyl, methylene α to carbonyl group, methylene bonded to oxygen and ester carbonyl. The signals between δ_C 22.70 to δ_C 31.95 were attributed to methylene chain. Through HMBC and HMQC was possible to observe correlations among hydrogen and its respective carbon. The chemical structure of compound **7** was confirmed by the integration of the hydrogen signals.

The IR spectra of compound **8** showed absorption bands at 3430 cm^{-1} (ν_{as}) and 3320 cm^{-1} (ν_s) characteristics of axial deformation of primary amide bonded to hydrogen. Also were observed intense band at 1675 cm^{-1} (ν C=O), 1630 cm^{-1} (δN-H, amid II band) and at 1470 cm^{-1} correspondent to deformation of C-N axial. And, the signals at δ_H 5.58 and at δ_C 160.33 were respectively associated to NH$_2$ and to amide carbonyl [11,13].

Compound **9** gave LB positive test for steroid [8,14]. The IR spectra of **9** showed absorption bands at 3700-3100 cm^{-1} (OH) and at 2950 a 2800 cm^{-1} which demonstrate its aliphatic character [11,13]. The profile of the bands at 1640 cm^{-1} (C=C) and at 1250-1000 cm^{-1} suggested the presence of glycosylated sterol. By comparative analysis with standard samples, using TLC eluted with solvent of different polarities and IR spectrometry was possible to confirm **9** as been 3-*O-β*-glicosyl-β-sitosterol.

Compound **10** and **11** were isolated as a mixture, which also gave LB positive test for steroid [8,14]. The identification of each component of this mixture was carried out through HR-GC using steroids standards. β-Sitosterol (**10**) [(t$_r$ 11.39 min (32.27 %)] and stigmasterol (**11**) [t$_r$ 10.32 min (30.72 %)] were identified as the main constituents.

The infrared spectra of **6** showed absorption bands at 3600 and 2250 cm^{-1} (ν OH), 2850 cm^{-1} (ν CH), 1700 cm^{-1} (ν COOH), 1460 cm^{-1} (CH) and a double band at 720 e 730 cm^{-1} associated to (CH$_2$)$_n$ [11,13]. The ^{1}H NMR spectra showed a triplet at δ_H 0.88 (J=6.6 Hz) that was associated to methyl hydrogen, and other triplet at δ_H 2.33 (J=7.3 Hz) attributed to methylene hydrogen alpha to carbonyl. A multiplet at δ_H 1.62 (J=6.8 Hz, 2H) was associated to 2H and a singlet at δ_H 1.25 to 30H, attributed to methylene chain [13]. In the ^{13}C NMR spectra the signal at δ_C 14.13, δ_C 22.70, δ_C 24.70, δ_C 29.37, δ_C 31.93, δ_C 33.85, δ_C 77.02 and at δ_C 178.86 were observed. Through DEPT-135 was possible to identify methyl group (δ_C 14.13), alpha carbonyl methylene (δ_C 33.85) and the carbonyl of acid (δ_C 178.86) [13]. The other signals were attributed to methylene carbons. By these data was possible to identify heneicosanoic acid (**6**).

Compound **12** gave LB positive test for pentacyclic triterpenes [8,14]. The IR spectra of **12** showed a broad absorption band at 3600-3100 cm^{-1} (OH), and at 1730 and 1680-1690 cm^{-1} which were associated to carbonyl groups, suggesting the presence of carboxylic acid. The absorption band at 1730 and 1240 cm^{-1} were attributed to acethyl group [11,13]. The aliphatic nature of **12** was confirmed in the ^{1}H NMR spectra through the profile of signals between δ_H 2.00 and δ_H 0.80 correspondent to methyl and methylene hydrogen. This profile is commonly found in the NMR spectra of pentacyclic triterpenes [8,14]. It was also observed the presence of 8 signals correspondent to methyl group at δ_H 0.86, 0,88, 0.92, 0.93, 0.94, 0.95, 0.96 and at δ_H 2.09. The double duplet at δ_H 5.51-5.54 was attributed to C=C hydrogen, and the multiplet at δ_H 4.40-4.51

to carbinolic hydrogen [13,16]. The study of ^{13}C NMR spectra indicated the presence of 32 carbons which were classified in according to DEPT-135 as been 8CH$_3$, 10CH$_2$, 5CH and 9C. Signals of a double bond were observed at δ_C 160.65 (C) and δ_C 116.71 (CH). These signals are important to classify the pentacyclic triterpene like an olean-14-en type of the taraxane serie. Through the analyze of HSQC and HMBC contour maps and comparison of the carbon chemical shift assignments of **12** with the literature data was possible to identify this triterpene as 3-*O*-acethyl-aleuritolic acid [17].

The high concentration of potassium nitrate (**1**) in all parts of the plant, the presence of urea (**8**) in the extract of leaves, and the 3-*O*-acethyl-aleuritolic acid (**12**) isolated from stems, represent an important chemotaxonomic data for the genus *Talinun*. The 3-*O*-acethyl-aleuritolic acid is not frequently founded in plants and it is the first time that this triterpene is isolated from specie of the Portulacaceae family.

T. paniculatum is traditionally used by people of Patos de Minas, to treat inflammatory diseases. Then the extracts of this plant were tested for their antinociceptive and antiedematogenic activity using formalin induced paw edema. This experimental model is based upon the ability of the extract or to inhibit the edema induced in the hind paw of the mice after injection of formalin. Consistent with previous investigations with formalin model, the animals exhibited two periods of nocifensive grooming: (1) an acute phase that began immediately, peaked at 3 min and almost completely abated by 6 min, and (2) a tonic phase that began between 6 and 9 min, peaked at 21 min and slowly diminished over the ensuing 24 min [18-20]. The anti-inflammatory and analgesic activities of indomethacin occur *via* inhibition of cyclooxygenase 2 (COX 2), which reduces prostaglandins (PGE2) formation from arachidonic acid [21-24].

In this work was demonstrated that the hexane and ethyl acetate extracts of *T. paniculatum* exhibited significant antinociceptive and antiedematogenic activities A certain dose-response relationship was observed for both extracts; through the reduction of male Swiss mice sensibility in second phase of inflammatory process, i.e., 15-30 minutes after formalin injection (Figure 3 and 4).
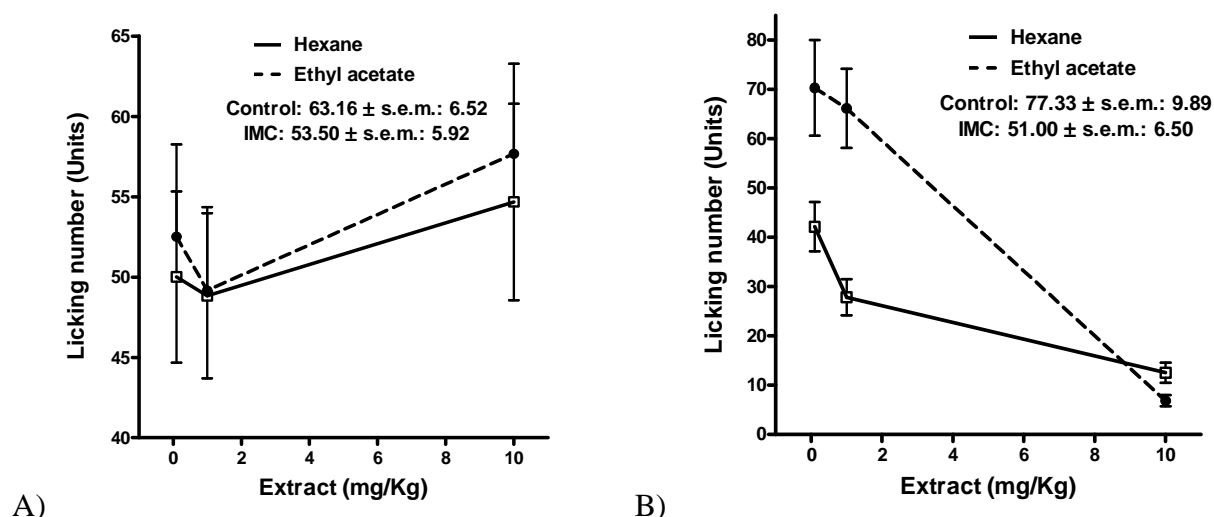

Figure 3: Effect of the intraperitoneal administration of hexane (HE) and ethyl acetate (EA) extracts of *T. paniculatum*, on mice paw formalin induced nociceptive (A) and inflammatory (B) phases, in comparison with control, and indomethacin (IMC) (20.0 mg/kg).
Results are reported as average ± SEM, n = 6, with P ≤ 0.05.

The ethyl acetate extract showed anti-inflammatory activity with a dose of 1.0 mg/kg and both hexane and ethyl acetate extract at dose of 10.0 mg/kg shown antinociceptive activity higher than indomethacin (20.0 mg/kg), observed mainly at the second phase of inflammatory process (Figure 3). Doses above 1.0 mg/kg of the hexane and ethyl acetate extracts induce higher edema reduction (Figure 4).
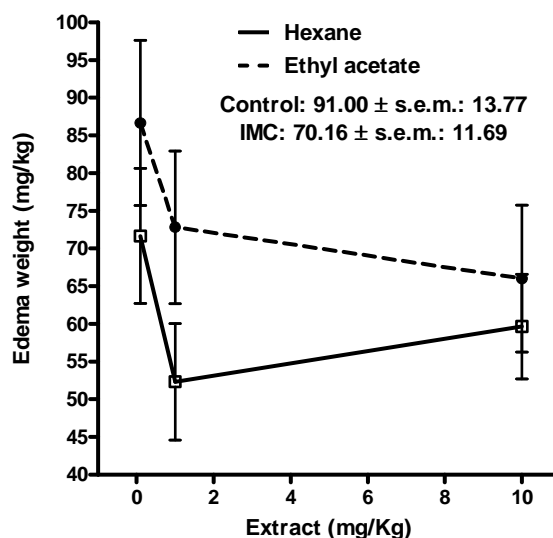

Figure 4: Effect of the intraperitoneal administration of hexane (HE) and ethyl acetate (EA) extracts of *T. paniculatum* on mice paw formalin induced edema, in comparison with control and indomethacin (IMC) (20.0 mg/kg). The weight of paw edema was taken at 3rd hour after formalin injection.
Results are reported as average ± SEM of n = 6, with P ≤ 0.05.

Studying analgesic constituents of *Croton urucurana* Peres et al (1998) [25] showed that β-sitosterol, β-sitosterol glucoside, stigmasterol and acethyl-aleuritolic acid have analgesic activity. These compounds also were isolated from *T. paniculatum.* This fact allied to the antinociceptive property observed for the hexane and acethyl acetate extracts may be suggest the existence of other analgesic compounds not yet determined due to its probably small concentration and/or the existence of some synergistic effect.

Chemical data of compounds

Compound **1**. **Potassium nitrate.** White needle solid, m.p. > 300 ^{0}C. IR (KBr, cm^{-1}): 1380 and 850 [10,25].

Compound **6. Heneicosanoic acid**. White amorphous solid, m.p. 78-83 ^{0}C. [Lit. 80-82 °C] [26]. IR (KBr, cm^{-1}): 3600-3000, 2850, 2800, 1700, 1460, 1410 and 720-730. ^{1}H NMR (CDCl$_3$, 200 MHz), δ_H: 0.88; 2.33; 7.26; 1.62 and 1.25. ^{13}C NMR (CDCl$_3$, 50 MHz), δ_C: 178.86; 77.08; 33.85; 31.93; 29.71-29.07; 24.70; 22.70 and 14.13.

Compound **7. Nonacosyl nonacosanoate.** White solid, m.p. 72-74 ^{0}C. IR (KBr, cm^{-1}): 2900, 2800, 1730, 1450-1460, 730-720. ^{1}H NMR (CDCl$_3$/py-d$_5$, 400 MHz), δ_H: 0.88; 1.27; 1.61; 2.28 and 4.06. ^{13}C NMR (CDCl$_3$, 100 MHz), δ_C: 173.83; 64.36; 31.95; 34.45; 28.76; 26.01; 25.10; 22.70 and 14.10.

_____

Compound **8. Urea.** White amorphous solid, m.p. 132–134 ºC. [Lit. 132-134 °C] [26]**.** IR (KBr, cm^{-1}): 3430, 3320, 1675, 1630, 1470. ^{1}H NMR (DMSO, 200 MHz), δ_H: 5.58. ^{13}C NMR (DMSO, 50 MHz), δ_C: 160.33.

Compound **9. 3-*O*-β-D-glucosyl-β-sitosterol.** White amorphous solid, m.p. 259-262 ^{0}C [Lit. 255-258 ^{0}C] [24]. IR (KBr, cm^{-1}): 3400–3300, 2910, 2800, 1730, 1640, 1470, 1370, 1230, 1210, 1090–1100.

Compound **12. 3-*O*-acethyl-aleuritolic acid.** White needles solid, m.p. 244-252 ^{0}C [Lit. 297-300 °C] [15,17]**.** IR (KBr, cm^{-1}): 3600-3500; 3400-3100; 2900-2800; 1730; 1690-1680; 1450 and 1240. ^{1}H NMR (CDCl$_3$, 400 MHz), δ_H: 0.86; 0.88; 0.92; 0.93; 0.94; 0.93; 0.96; 1.10; 1.16; 1.25; 1.29; 1.45; 1.47; 1.60; 1.63; 1.65; 1.68; 1.78; 1.98; 2.00; 2.05; 2.30; 4.40-4.51 and 5.51-5.57. ^{13}C NMR (CDCl$_3$, 100 MHz), δ_C: 15.61; 16.60; 17.33; 18.72; 21.29; 22.40; 23.49; 26.13; 27.98; 28.70; 29.30; 30.82; 31.45; 31.94; 33.36; 33.71; 35.40; 37.39; 37.44; 37.71; 37.96; 39.40; 40.87; 41.60; 49.10; 51.31; 55.66; 80.93; 116.71; 160.65; 170.97 and 184.20.

CONCLUSION

To the best of our knowledge, until this moment, it is the first time that compounds **1**, **2**, **3**, **4, 5, 6, 7, 8** and **12** are being cited as constituents of *T. paniculatum*. The detection of high potassium nitrate (**1**) content demonstrated the capacity of this plant to uptake and concentrate inorganic compounds. The present experimental protocols showed that, even at lower doses, the hexane and ethyl acetate extracts of *T. panuculatum* individually elicited a significant anti-edematogenic activity in formalin induced paw edema mice model.

REFERENCES

[1] M Pio-Correa. *Dicionário de Plantas Úteis do Brasil e das Exóticas Cultivadas*. Edited by Ministério da Agricultura do Brasil. **1978**.
[2] J Caribé & JM Campos. *Plantas que Ajudam o Homem*. Círculo do Livro Pensamento Ltda. São Paulo. **1991**.
[3] AB Joly. *Botânica: Introdução à Taxonomia Vegetal*, 12nd.Edition Companhia Editoral Nacional. São Paulo. **1998**.
[4] S Panizza. "*Plantas que Curam*". 17^{a} Ed. IBRASA. São Paulo. **1998**.
[5] ER Martins & DM Castro. *Plantas Medicinais*. Editora UFV. Viçosa. **2000**.
[6] M. Komatsu; I Yokoe; Y Shirataki; T. Tomimori. *J. Pharm. Soc. Jap.* **1982**, 102(5):499-502.
[7] H Shimoda; N Nishida; K Ninomiya; H Matsuda; M Yoshikawa. *Heterocycles* **2001,** 55(11): 2043-2050.
[8] H Wagner; S Bladt; GM Zganski. *Plant Drug Analysis*. Springer-Verlag. Berlim. **1984**.
[9] R Chulet; L Joseph; M George; P Pradhan. *J. Chem. Pharm. Res.,* **2010**, 2(1): 432-443.
[10] IBM Sampaio. *Estatística Aplicada à Experimentação Animal*. Fundação de Ensino e Pesquisa em Medicina Veterinária e Zootecnia. Belo Horizonte. **1998**.

[11] K Nakanishi & PH Solomon. *Infrared absorption spectroscopy*. 2nd Edition, Holden-Day, San Francisco. **1977**.

[12] D Debuisson & SG Dennis. *Pain*. **1997**, 4: 161-174.

[13] RM Silverstein; GC Bassler; TC Morrill. *Spectrometric identification of organic compounds*. 6th. Edition, John Wiley & Sons, New York. **1998**.

[14] FJA Matos. *Plantas Medicinais: guia de seleção e emprego de plantas usadas em fitoterapia no Nordeste do Brasil*. 2[md] Edition, Imprensa Universitária / UFC, Fortaleza **2000**. 171-172.

[15] SB Mahato & AP Jundi. Phytochemistry **1994**, 37: 1517-1573.

[16] E Breitmaier. *Structure elucidation by NMR in organic chemistry. A practical guide*. 3rd Edition, John Wiley & Sons, Chichester. **2002**.

[17] S McLean; M Perpick-Drumont; WF Reynolds; H Jacobs; SS Lachmansing. *Can. J. Chem.* **1987**, 65: 2519-2525.

[18] R Turner. *Screening Methods in Pharmacology. Anti-inflammatory agent*. Academic Press New York, London. **1965**. 13, 158.

[19] SD Gilbert; TM Clark; CM Flores. (). *Pain* **2001**, 89(2-3): 159-65.

[20] AA Suralkar. *In-Vivo Animal Models for Evaluation of Anti-Inflammatory Activity*. Latest Reviews, **2008**. Vol. 6 Issue 2.

[21] M Tramèr; J Williams; D Carroll; PJ Wiffen; HJ McQuay; RA Moore. *Acta Anaesth Scand* **1998**, 42: 71-79.

[22] GHB Souza; AA Silva Filho; VA De Souza; AC Pereira; VA Royo; MLA Silva; R Silva; PM Donate; JCT Carvalho; JK Bastos. *Il Farmaco* **2004**, 59: 55-61.

[23] C Su-Hui; W An-Bang; L Chia-Jung; C Fu-An. *Biol. Pharm. Bull*. **2005**, 28(12): 2206-2210.

[24] SJ Correia; JP David; JM David. *Quim. Nova* **2003**, 26 (1): 36-38.

[25] MTLP Peres; F Delle-Monache; MG Pizzolatti; ARS Santos; A Beirith; JB Calixto; RA Yunes. *Phytoth. Res.* **1998**, 12: 209-211.

[26] CRC Handbook of Chemistry and Physics: a Ready - Reference Book of Chemical and Physical Data. 76. CRC Press. New York. **1995**.