SUPPLEMENTARY MATERIAL

Quantification of polyphenols and flavonoid content and evaluation of anti-inflammatory and antimicrobial activities of *Stenocereus stellatus* extracts.

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Quantification of polyphenols and flavonoid content and evaluation of anti-inflammatory and antimicrobial activities of *Stenocereus stellatus* extracts.

The hexanic, ethyl acetate, and methanolic extracts from branches of *Stenocereus stellatus* were tested in both the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) - induced ear edema model and the antimicrobial activity assay. The % of edema inhibition, the Minimum Inhibitory Concentration (MIC), as well as the polyphenolic and flavonoid content were determined. Also, extracts were analysed by gas chromatography - mass spectrometry (GC-MS). In TPA model, the three extracts showed moderate edema inhibition. In the antimicrobial activity assay, the methanolic extract show better MIC against all strains. The lowest MICs were for *Candida albicans* (31 µg/mL) and *Rhizopus* sp. (15 µg/mL). Also, 50.78 mg eq. of gallic acid/g extract of polyphenol and 115.12 mg eq. of catechine/g extract of flavonoids content, were founded in ethyl acetate extract. In the chromatographic analysis β-sitosterol, β-amyrine, betulin, and some other molecules were identified. The results show that *S. stellatus* possess antimicrobial activities against some fungus species.

Keywords: *Stenocereus stellatus*, anti-inflammatory, antimicrobial, *Candida albicans*, *Rhizopus* sp., Betuline.

Experimental procedures

Reagents

12-*O*-tetradecanoylphorbol-13-acetate (TPA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Gallic acid, and Catequine, and all solvents were obtained of Sigma. Folin Ciocalteu reagent (FCR), Sodium Carbonate, Sodium Nitrate, Aluminium Chloride and Silica gel were obtained from J.T. Baker.

Plant material and obtention of organic extracts

The plant material was collected in 2013y from Cohetzala, Puebla (18°16'58''N, 98°43'18''W). A voucher specimen was deposited in the National Herbarium from Instituto de Biología, UNAM for further identification. The plant material was air-dried, cut into slides and macerated (r.t.) with hexane, ethyl acetate and methanol for 24 h each one. After this, the macerate was filtered and concentrated under vacuum (Büchi R- 410) to obtain the hexanic (St. HX), ethyl acetate (St. AO) and methanolic (St. MH) extracts.

Anti-inflammatory activity test

The test was conducted using the TPA as edemogenic agent as previously described for ear – induced model (Salazar et al., 2001). Groups of three male CD-1 mice weighing 20-25 g were used. Each extract was used at 1 mg/ear. Indomethacin was used as reference at 0.15 mg/ear. The percentage of Edema Inhibition was calculated with:

$$\text{Edema Inhibition (\%)} = 100 - \left(\frac{A}{B} \times 100 \right) \quad (1)$$

where *A* and *B* are the weights of group edema and control group edema. The averages $\pm$ SD were reported.

Microbial Cultures.

The strains used in this assay were the bacteria: *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 33862), *S. epidermidis* (ATCC 12228), *Salmonella enterica* (ATCC 13076), and *Klebsiella pneumonia* (ATCC 10031); or the fungi: *Candida albicans* (ATCC 33862), *Aspergillus niger*, *A. oryzae*, *Penicillium notatum*, *Rhizopus* sp.

Antimicrobial activity.

The tests were performed using the macro dilution assay (NCCLS) with Mueller Hinton or Saboreaud broths. Each extract were at 500, 250, 125, 62, 31, and 15 $\mu\text{g/mL}$. The strains were standardized to 0.5 of the McFarland scale for the bacteria and *C. albicans*. The fungi were inoculated with a solution of spores. As negative control was used the solvents and as positive controls were used Ofloxacin (5 $\mu\text{g/mL}$) or Clotrimazol (100 $\mu\text{g/mL}$) were used. The Minimum Inhibitory Concentration (MIC) was determined. (Nurmahani et al., 2012)

Total Phenolic Content (TPC)

For TPC of the extracts the Folin-Ciocalteu method was used. The ethanolic solutions of the extracts were prepared (100 mg/mL), 10 μL of the solutions of the extracts were placed in the wells of the microplate. The extracts were mixed with FCR (0.2 N) and with sodium carbonate (0.7 M). The absorbance was measured with a microplate reader (Biotec, type Epoch SN252829) at 760 nm using Software Gen 5 (Biotec). The calibration curve was prepared with gallic acid. Then TPC were calculated with the calibration equation:

$$y = 2.0982x + 0.125 \quad (2)$$

With an $R = 0.9871$. The results were expressed in milligrams equivalents of gallic acid per gram of organic material (Chew et al., 2011). The average of three measures $\pm$ SD were reported. One Way Anova was conducted ($p=0.05$).

Total flavonoids content (TFC)

For the flavonoids determination we used the spectrophotometric aluminum chloride method. The ethanolic solutions of the extracts (100mg/mL), 10 μL of the solutions of the extracts were placed in the wells of the microplate. Then the solutions were mixed with sodium nitrate (1.5 %), aluminum chloride (3%) and ethanol. Finally the absorbance was measured with a microplate reader (Biotec, type Epoch SN252829) at 490 nm using Software Gen 5 (Biotec). The calibration curve was prepared with a known flavonoid catequine. The total flavonoids content were determined using the calibration equation:

$$y = 1.2185x + 0.0697 \quad (3)$$

With an $R = 0.9934$. The results were expressed in milligrams equivalents of catechine per gram of organic material (Tanaka & Takahashi, 2103). The average of three measures $\pm$ SD were reported. All data were analyzed by ANOVA, the values of $p \leq 0.05$ are considered as statistically different.

GC-MS Conditions

The chromatographic analysis was developed in a 6890N (Agilent Technologies) gas chromatograph equipped with a 5973nN (Agilent Technologies) mass spectrometer fitted with 60m x 0.25mm DB-1 Agilent 122-1061 capillary column at carrier gas flow (Helium) of 1.5 mL/min with splitless 10 min and sample size of 0.2 μ L. the column temperature was programmed at 80°C for 3 min. with 10°C/min. Remained at 320°C for 80 min. While the detector and injector temperatures were maintained at 280°C and 300°C respectively. The mass spectrometer was at 70 eV with a source and a quadrupole of 240 and 180 eV respectively, with a mass range (m/z) of 40-500 Da. The samples were compared with the NIST library. The solutions of each extract were prepared previously at 1% with acetone. The most abundant compounds found in the extracts of *S. stellatus* were β -amiryn, β -sitosterol, and betuline (Tables S1 and S2).

References

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Table S1. GC-MS of the hexanolic extract from *S. stellatus*.

Retention time (min)	Compound	% of total spectrum	% of correlation
27.63	β -amyrin	3.14	19.74
32.87	β -sitosterol	12.58	19.19
34.22	Betuline	15.89	100

Table S2. GC-MS of the ethyl acetate extract from *S. stellatus*.

Retention time (min)	Compound	% of total spectrum	% of correlation
17.09	1,4-eicosadiene	1.57	18.35
32.87	β -sitosterol	8.57	100
34.20	Betuline	8.09	94.37