Original Article

Evaluation of the Anti-inflammatory and Cytotoxic Activity of Methanol Extracts of *Psacalium decompositum* and *Scopulophyla parryi* in VERO and Detroit Cell Lines

Patricia García-Palencia[1], Catalina Rivas-Morales[1], Azucena Oranday-Cárdenas[1], Myriam Angélica de la Garza-Ramos[2], Catalina Leos-Rivas[1] and Ernesto Torres López[3*]

[1]*School of Biological Sciencies, Universidad Autonoma de Nuevo Leon, Pedro de Alba s/n, Ciudad Universitaria San Nicolas de Los Garza, Mexico*
[2]*School of Dentistry, "Dr. Jose Eleuterio Gonzalez" University Hospital, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico*
[3]*School of Medicine, "Dr. Jose Eleuterio Gonzalez" University Hospital, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico*

Abstract

Periodontal diseases are characterized by inflammation and destruction of tooth-supporting tissues. There is interest in anti-inflammatory substances of plant origin because of their advantage in relation to classic anti-inflammatory drugs. The cytotoxic and anti-inflammatory effect of extracts of *Scopulophila parryi* and *Psacalium decompositum* (A.Gray) H.Rob. & Brettell were evaluated with the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium (MTT) method and their pro-inflammatory cytokine profile was determined in a human peripheral blood monocyte culture model. The plant was collected and identified and the methanol extract was prepared by maceration. Cytotoxicity was evaluated *in vitro* with the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium) assay in two cell lines, VERO and Detroit 551 human fibroblasts. The anti-inflammatory activity of human peripheral blood monocytes and gene expression of the proinflammatory cytokines, IL-1, IL-6 and IL-8, were evaluated The extract of *P. decompositum* was less toxic on the VERO and Detroit 551 cell lines with a DL_{50} of 77.30 µg/mL and the *S. parryi* extract had 150.58 µg/mL. Both extracts significantly reduced expression of IL-1 , IL-6, and IL-8 at 300 µg/mL for *S. parryi* and at 400 µg/mL for *P. decompositum*. The extract of *P. decompositum* was less toxic for both cell lines and the two methanol extracts are good alternatives as immunomodulators of proinflammatory cytokines.

Keywords: Cytokines, Toxicity, Cell lines, Periodontal.

Introduction

Periodontal diseases, which are characterized by inflammation and destruction of tooth-supporting tissues that can cause loosening or loss of teeth, because of their magnitude and transcendence, represent one of the main problems of oral health [1,2]. Periodontitis, which is an inflammatory disease of tooth-supporting tissues, is also caused by specific microorganisms that cause progressive destruction of the periodontal ligament and alveolar bone with the formation of pockets, gum recession or both [3,4].

Medicinal plants are used in dentistry because of their background in traditional medicine. They are an effective method to cure stomatological conditions such as canker sores, candidiasis, gingivitis, inflammatory processes, tooth pain, and as antibiotics and antifungals in diverse preparations such as toothpaste, mouthwash, gels, and topical paste, among others [5,6]. Currently, and despite advances in pharmacology, people continue to turn to medicinal plants to cure different conditions that occur in the oral cavity. Some of the plants used by the Mayas to

*Corresponding author: School of Medicine and "Dr. Jose Eleuterio Gonzalez" University Hospital, Universidad Autonoma de Nuevo Leon, Av. Madero y Calle Dr. Aguirre Pequeño, Colonia Mitras Centro, Monterrey, Mexico C.P. 64460
Email Address: ernestotorreslopez57@gmail.com

treat gingivitis were the Nance (*Brysonima crassifolia*), the physic nut (*Jatropha curcas*), and the pond apple (*Annona glabra*); for tooth pain, they used the nosegay (*Plumeria rubra*) and the pagoda tree (*Plumeria obtusa*) [7]. Studies have focused on the bacteria that participate in the etiology of oral and dental diseases, clearly establishing a series of substances with potential use in the dental industry, given their biological activity against cariogenic bacteria and those associated with periodontal disease [8]. Some plants of traditional medicine present other properties, such as anti-inflammatory activity. Therefore, it is important to evaluate the biological properties of extracts of *Scopulophila parryi (Sp)* and *Pscalium decompositum (Pd)*; this will help determine which of these plants possess anti-inflammatory and non-cytotoxic action and which can be a potential candidate for periodontal or gingival therapy to improve the recovery of affected tissues.

In recent years, anti-inflammatory activity has awakened scientific interest in pharmacology, mainly in the property that certain compounds possess in interfering in the evolution of diseases that course with inflammation. Within the context of anti-inflammatory products, traditional medicine has been used to treat patients with inflammatory diseases; for example, in Africa, its use is greater than 80%, in China around 40%, while in Asia and Latin America, its use is linked to cultural beliefs and historical circumstances. In developed countries, instead of traditional medicine, complementary and alternative medicine is used: 75% in France, 70% in Canada, and 42% in the United States [9].

The incorporation and use of medicinal plants in the treatment of inflammatory reactions are common practice in traditional medicine. Current interest in anti-inflammatory substances of plant origin is evident because of the advantages they offer in relation to classic anti-inflammatory drugs, such as a low incidence of side effects. Some of the drugs traditionally used in the treatment of rheumatic disease and recently studied are found in willow bark (*Salix alba*), the root of the grapple plant (*Harpagohytum procumbens DC*), and eucalyptus leaves (*Eucalyptus globulus*). Some anti-inflammatory drugs have been obtained from natural sources; for example, acetylsalicylic acid, commonly known as aspirin. This is classified as a nonsteroidal anti-inflammatory drug derived from salicin, a glycoside from the bark of the willow tree. On the other hand, pimecrolimus (*Streptomyces hygroscopicus*), which is ascomycin FK520, is a drug that selectively inhibits the release of cytokines and is used to treat inflammatory diseases of the skin [9].

The evaluation of anti-inflammatory activity can be carried out using *in vivo* and *in vitro* models; among the former, there are acute inflammation models and among these, there are three methods, each one with special characteristics depending on the conditions in which the study is performed. The first is carrageenan-induced paw edema, first described by Winter and Porter in 1957 and modified by Sughisita *et al*. in 1981 [10]. It is one of the most useful methods for the discrimination of anti-inflammatory drugs because of its simplicity and reproducibility. The second is the experimental protocol of Croton oil; this consists of topical administration of Croton oil, a mixture of esters and other components that are irritants that are used to induce localized inflammation in the ears of laboratory animals. The third is ear edema in rats induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), a technique described by De Young *et al*. in 1989 [11] and modified by Payá *et al*. in 1993 [12]; of the Croton oils (*Croton tiglium L*), TPA is the most potent. It possesses irritant, pro-inflammatory, and tumor promotion properties [13].

In *in vitro* evaluation models, phagocytic cells such as polymorphonuclear (PMN) leukocytes and modular macrophages respond to a variety of membrane stimuli by the production and extracellular release of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Macrophages play a fundamental role in modulating the start and conservation of the inflammatory response. It is characterized by their ability to synthesize biological mediators such as prostaglandins and nitric oxide which activate cardiovascular and inflammatory effects. Lipopolysaccharide (LPS) is another important element. It is a polymer that is present in the cell wall of gram-negative bacteria and it stimulates macrophages so they release cytokines and nitric oxide making LPS a potent stimulator of acute inflammation. This is why the *in vitro* stimulation of macrophage cell lines by LPS provides an estimate of anti-inflammatory effect. Other assays used to evaluate *in vitro* anti-inflammatory activity are directed at quantifying enzyme expression and/or anti-inflammatory mediators, such as the enzyme beta-secretase (BACE-1), C-reactive protein (CRP), connective tissue growth factor (CTGF), and other bioassays [9].

The basic objective of toxicological experimentation is to contribute to the knowledge of the dangers or intrinsic toxicity of substances; the toxic effects caused and their reversibility; the molecular mechanisms and the biological target cells on which they act; the different toxic susceptibility of species, sex, and population groups; the kinetics and metabolism in organisms and in the environment; the sensitivity and specificity of diagnostic tests; the effectiveness of therapeutic and prophylactic measures; and finally to integrate all the available information to estimate the risk involved in its use in different applications, establishing levels of safety to their exposure. In contrast to most of the experimental disciplines, there are strict regulations that must be followed in toxicological experimentation, which demand the evaluation of substances with different requirements

according to their intended use applying standardized experimental protocols, as well as complying with good laboratory practices and established standards. Among the parameters that standards demand to evaluate toxicity, there is the mean lethal dose (DL_{50}). It is relevant to emphasize that toxicity assays are not designed to demonstrate that a compound is safe but to characterize the effects that it can produce.

According to the aim of the study, there are three types of experimental toxicological research: regulated, non-regulated, and academic and formative experimentation. Regulated experimentation is generally called "toxicity evaluation." It is intended to classify substances and evaluate their risk. Non-regulated toxicological research is not limited by regulations and can be divided into basic and applied. The former has as its essential aim the search for knowledge without direct practical implications; it includes the study of mechanisms of action, interactions, effects, and others. Applied research is aimed at answering questions with the practical objective of selecting or screening new substances, comparing their biological activity [14].

Cell cytotoxicity is defined as an alteration of basic cell functions that lead to damage that can be detected. Different authors have developed several *in vitro* tests that predict the toxic effects of drugs and chemical compounds using primary experimental culture models and organs isolated from established cell lines. Among these *in vitro* assays, there are the neutral red uptake assay, kenacid blue binding, and the MTT method, which determines the possible cytotoxic effect of an agent based on the metabolic reduction of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) [15,16].

The cytotoxic and anti-inflammatory effect of active extracts of *Scopulophila parryi* and *Psacalium decompositum* were evaluated with the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium (MTT) method and their pro-inflammatory cytokine profile was determined in a human peripheral blood monocyte culture model.

Material and Methods

Plant Material

The plants, *S. parryi* and *P. decompositum*, were collected in Real de Catorce in the state of San Luis Potosi in Mexico and later identified taxonomically in the Botany Department of the School of Biologogical Sciences of the Universidad Autonoma de Nuevo Leon.

The root was separated from each plant; they were washed with water, cut into slices, dried in the shade for 7 days, crushed in a mill, and stored in refrigeration until use.

Preparation of the Methanol Extract

A 200 g portion of the root was taken from each plant, *S. parryi* and *P. decompositum*, pretreated and deposited in a 500 mL Erlenmeyer flask. A total of 120 mL of 99.9% methanol was added and the flask was hermetically sealed and shaken constantly for 7 days in a Dual Action Lab Line Shaker. The solvent was separated in each flask from the plant material by filtration with Whatman No 1 filter paper; afterward, the same plant material was extracted again twice using the same method. The methanol extract was concentrated in a Büchi rotavapor at a temperature lower than 60 °C; it was then totally dried at room temperature and stored in amber colored vials at 4 °C until use.

The yield of the extract was calculated with the following formula:

$$\%Yield = \frac{Weight\,obtained}{Initial\,weight} \times 100$$

Where weight obtained refers to the weight obtained from the extract and the initial weight is the weight of the plant material for extraction.

Determination of the Cytotoxic Effect of the Methanol Extract with the MTT Method

Cytotoxicity of the active methanol extracts were evaluated *in vitro* with the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium) assay in two cell lines: VERO (ATCC®CCL-81™) and Detroit 551 human fibroblasts (ATCC®CCL-110™). A vial of the cells was thawed using a beaker with water at 37° C submerging the vial for approximately 2 minutes. The cells were then suspended in a Falcon centrifuge tube with 9 mL DMEM-10% FSC medium and centrifuged at 1200 rpm for 5 min. The culture medium was then decanted and the pellet was resuspended in 5 mL PBS and centrifuged again at 1200 rpm for 5 min. This procedure was performed twice to subsequently resuspend the pellet in 5 mL DMEM-10% FSC medium and the content was transfered to a T-25 culture bottle (25 cm2), incubated at 37 °C, with 5% CO2 and 100% humidity. Once a 100% cell confluence was obtained, a cell count was performed in a Neubauer chamber and 100 µL of a cell suspension (20,000 D551 cells and 10,000 VERO cells per well) was inoculated into a 96-well microplate and incubated for 24 h at 37 °C with 5% CO2 and 100% humidity. A total of 100 µL of each of the extracts was applied at different concentrations, with their respective controls and incubated again for 24 hours in the same conditions. The medium was subsequently removed and 10 µL of MTT at 0.5 mg/mL was added and the content was incubated for 4h h at 37 °C, with 5% CO_2 and 100% humidity. Finally, 100 µL of DMSO was added to lyse the cells, which were then shaken for 5 min, and a reading was taken at 570 nm in an Epoch Microplate

spectrophotometer (BioTek Instruments, Inc., Winooski, VT).

Evaluation of the Antiinflammatory Activity of Active Extracts on Peripheral Blood Monocytes

Obtention and Cell Culture of Human Peripheral Blood Monocytes

The evaluation of antiinflammatory activity was performed with human peripheral blood monocytes. An intravenous puncture was performed in the median cephalic vein to draw venous blood from a voluntary donor. Once the vein was identified, asepsis was performed with 70% ethyl alcohol, allowing the skin to dry, and then a Vacutainer® system adapter was inserted to obtain 25 mL of venous blood. The vein was immobilized by placing the thumb beneath the puncture site. The skin was punctured with the bevel facing upwards with a smooth and rapid movement. A tube with anticoagulant (BD®EDTA-K2) was placed on the end and was left to fill. The tube was shaken gently and inverted several times. After filling all the tubes, the needle was quickly removed and pressure was applied with cotton.

Isolation of the peripheral blood mononuclear cells (PBMC) was performed with the gradient of the Ficoll-Hypaque™ PLUS (GE Healthcare Life Sciences). In sterile conditions, the blood was transferred to a 50 mL tube with a conical bottom and was resuspended in sterile PBS (pH 7.4) at a ratio of 1:1 and gently homogenized. Five 15 mL tubes with a conical bottom were prepared with 5 mL of Ficoll-Hypaque™ PLUS and with a sterile 10 mL pipette, 10 mL of the sterile blood and PBS mixture was slowly dispersed on the wall of the tube without mixing it with Ficoll-Hypaque™ PLUS; two phases remained in sterile conditions: the lower one, Ficoll-Hypaque™ PLUS and the upper one, PBS-diluted blood. The tubes were centifuged at 1,400 rpm for 30 minutes at 20 °C, without the handbrake. The tubes were then carefully removed from the centrifuge without disturbing the layers formed. With a sterile 1 mL pipette, the cell interphase was carefully aspirated and transferred to a 15 mL conical bottom tube; the cells were then homogenized by inversion in 10 mL sterile PBS. They were then centifuged at 900 rpm for 10 min at 20 °C. This procedure was repeated twice; finally, the supernatant was eliminated by decanting and the cells were resuspended in 1 mL of RPMI-10% FSC cculture medium supplemented with 100 IU/mL of penicillin, 100 µg/mL of streptomycin, and 0.5mL mL of glutamine 100X (GlutaMAX™ Supplement); the cells were resuspended by digital tapping [17,18].

Later, a cell count was performed using a Neubauer chamber. In an Eppendorf® tube, 10 µL of the PBMC in suspension and 10 µL of 4% trypan blue were diluted. Ten microliters of the solution was placed in each Neubauer chamber and was read under a microscope at 40X to determine the average of the four large corner quadrants. The formula for the calculation of the cell concentration was used to obtain an approximate cellular vision of 1×10^6 cell/mL:

$$Cell concentration/n/mL = \frac{Total\ cells\ counted \times 10,000}{Number\ of\ quadrants}$$

RNA Extraction from Peripheral Blood Mononuclear Cells (PBMC)

For RNA extraction, the Direct-zol™ RNA MiniPrep (ZYMO RESEARCH) kit was used. Initially, supplemented RPMI medium was removed from the wells of the microplates by aspiration with a sterile 5 mL pipette. To lyse the samples, cells were resuspended with 300 µL of TRI-Regent in each well and were incubated for 5 min and with a spatula the cells were removed from the bottom of the wells for transfer to 1.5 mL Eppendorf® tubes labelled with the concentrations for evaluation. They were then centrifuged at 12,000 rpm/30s and the supernatant was transferred to a new Eppendorf® tube. Later, ethanol was added at a a proportion of 1:1 to the supernatant, homogenized, and transferred again to a IIC Zymo-Spin™ column in a collection tube and centrifuged at 12,000 for 30 s; afterward the medium was discarded. A total of 400 µL of RNA Wash Buffer was added to the tube and it was centrifuged at 10,000 rpm for 30 s; in another sterile tube 5 µL DNasa I, and 75 µL of DNA Digestión Buffer were added. This mixture was added directly to the column matrix. The mixture was incubated at room temperature for 15 minutes. After this period, 400 µL of Direct-zol™ RNA Prewash was added to the column and centrifuged at 12,000 rpm for 30 s; the medium was discarded and the procedure was repeated again. A total of 700 µL of RNA wash buffer was added to the column and was centrifuged at 12,000 rpm for 2 minutes to ensure complete elimination of the wash buffer and was then carefully transferred to the column to another Eppendorf® tube free of RNase. DNase/Rnase-free water, 50 µL, was added to the column and centrifuged at 12,000 rpm for 30 s [19].

Synthesis of Complementary DNA (cDNA) from the mRNA of PBMC

For the synthesis of cDNA the Promega ImProm-II™ Reverse Transcription System was used. The reaction mixture was prepared by combining the following ImProm-II™ components according to the specifications reommended by the manufacturer. In a sterile 1.5 mL Eppendorf® tube on ice, a sufficient mixture of 15 µL was prepared for each cDNA synthesis reaction that was performed. The volumes needed for each component were determined: nuclease-free water, ImProm-II™ 5X buffer, MgCl₂ at 1.5 mM, dNTP mix at 0.5mM and ImProm-II™Reverse Transcription, gently stirred with a vortex to

mix, and kept on ice before the reaction. A total of 15 µL of the reverse transcription was added to each Eppendorf® tube with 5 µL of extracted RNA (10 ng) of the different samples, in addition to the negative and positive controls, for a final volume of 20 µL. The tubes were placed in a block at a temperature of 25 °C and were incubated for 5 minutes. Afterward, the tubes were incubated in a block at a temperature of 42 °C for 1 hour. Finally, they were incubated in the Thermomixer comfort at 70 °C for 5 minutes to stop synthesis [20].

Polymerase Chain Reaction (PCR) of the PBMC

The reaction mixture was prepared with the following elements: Maxima SYBER Green qPCR Master Mix (2X), no-ROX 12.5 µL, forward primer 0.4 µM, reverse primer 0.4 µM, DNA template 1 µL (10 ng) nuclease-free water 10.3 µL to obtain a final volume of 25 µL for each reaction. The thermocycler (LightCycler®480II) was programmed under the following conditions with four programs: denaturation with a cycle of 95 °C for 10 min and a ramp 4.4 °C/s; amplification with 40 cycles; each cycle under the following conditions: 95 °C for 15s and a ramp of 4.4 °C/s, 52 °C for 22s and a ramp of 2.2 °C/s and 72 °C for 25s and a ramp of 4.4 °C/s. The melting curve program with the following conditions: first, 95°C x 5s and a ramp of 4.4 °C/s; second, 65 °C x 1 min and a ramp of 2.2 °C/s; third, 95 °C continuous and a ramp of 0.11 °C/s, and a cooling program (Cooling) at 40 °C x 5s and a ramp of 2.2 °C/s. After the results of the expression of the proinflammatory cytokine genes, Il-6, IL-8, IL-1, INF, and TNF of the PBMC were obtained, a statistical analysis was performed with the REST 2009 program to determine the number of times each gene analyzed was expressed, using the 2-^{CT} method [21].

Results

Cytoxicity of the Active Extracts with the MTT Method

The methanol extracts *S. parryi* and *P. decompositum* reduced the viability of VERO cells after 24 hours of treatment, with the lowest viability of 17.25% for *S. parryi* at 60 µg/mL as shown in Figure 1 and of 32.38% at 120 µg/mL for *P. decompositum* as shown in Figure 2. The DL$_{50}$ was determined with a probit regression analysis using the PASW Statistics program 18.0. The DL$_{50}$ value of the *S parryi* extract was 52.70 µg/mL and for *P. decompositum* 77.30 µg/mL.

The methanol extracts of *S. parryi* and *P. decompositum* on the cell line Detroit 551 (skin fibroblasts) reduced the viability of the cells under treatment for 24 h with the percentage of viability less than 13.51% at 400 µg/mL for *S. parryi* as shown in Figure 3 and of 20.82% for *P. decompositum* at 400 µg/mL as shown in Figure 4.
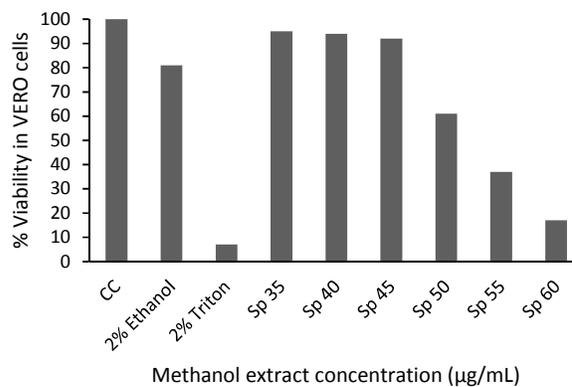

Fig. 1 Viability of VERO cells treated with different concentrations of methanol extracts of *S. parryi*. Sp 35 – 60 = *S. parryi* 35-60 µg/mL, CC = cell culture medium with VERO cells without treatment; negative control = 2% ethanol, positive control = 2% Triton.
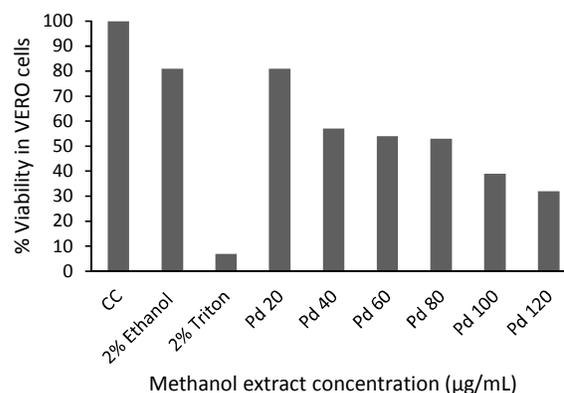

Fig. 2 Viability of VERO cells treated with different concentrations of methanol extracts of *Psacalium decompositum* (A.Gray) H.Rob. & Brettell. Sp 35 – 60 = *P. decompositum* 35-60 µg/mL, CC = cell culture medium with VERO cells without treatment; negative control = 2% ethanol, positive control = 2% Triton.

The DL$_{50}$ was determined with a probit regression analysis using the PASW Statistics program 18.0. The DL$_{50}$ of the methanol extracts of *S. parryi* and *P. decompositum* on the Detroit 551 cell line at 24 h was 203.63 µg/mL and 150.58 µg/mL, respectively.

Antiinflammatory activity of the methanol extracts of *S. parryi and P. decompositum* on peripheral blood monoclonal cells (PBMC)

The antiinflammatory activity on PBMC of the methanol extracts obtained from *S. parryi* and *P. decompositum* were evaluated and the statistical interpretation of the results was performed with the Rest 2009 program using the 2-^{ct} method with a confidence interval of 95%.
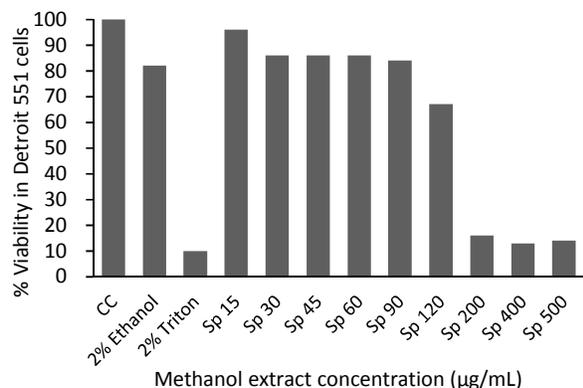
Fig. 3 Cytotoxicity of the methanol extracts of *S. parryi* on Detroit 551 cells. *Sp* 15 – 500 = *S. parryi* 15-500 µg/mL*; CC* = cell culture medium with Detroit 551 cells without treatment; negative control = 2% ethanol, positive control = 2%Triton.
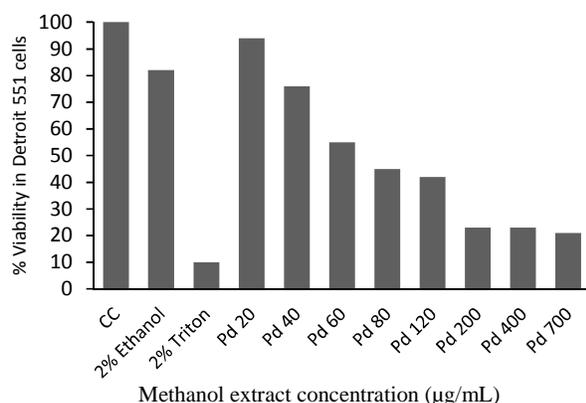

Fig. 4 Cytotoxicity of the methanol extract of *Psacalium decompositum* (A.Gray) H.Rob. & Brettell on Detroit 551 cells. *Pd* 20 – 700 = *P. decompositum* 20-120 µg/mL; CC = cell culture medium with Detroit 551 cells without treatment; negative control = 2% ethanol, positive control = 2% Triton.

The gene expression of interleukin-1 (IL-1) on PBMC treated with the methanol extract *P. decompositum* at a concentration of 500 and 700 µg/mL is shown in Figure 5a. IL-1 was normalized with the expression of the endogenous gene -actin; a significant difference exists in the three concentrations evaluated with regard to the control LPS.

The gene expression of IL-1 in PBMC treated with the methanol extract of *S. parryi* at concentrations of 300, 400, and 500 µg/mL is shown in Figure 5b. IL-1 was normalized with the expression of the endogenous gene -actin; a significant difference exists in the three concentrations evaluated with regard to the control LPS.

The gene expression of IL-6 in PBMC treated with the methanol extract *P. decompositum* at concentrations of 400, 500, and 700 µg/mL is shown in Figure 6a. IL-6 was normalized with the expression of the endogenous gene -actin; a significant difference exists in the 400 µg/mL concentration with regard to the control LPS.

The gene expression of IL-6 in PBMC treated with the methanol extract *S. parryi* at concentrations of 300, 400, and 500 µg/mL is shown in Figure 6b. IL-6 was normalized with the expression of the endogenous gene -actin; a significant difference exists in the three concentrations evaluated with regard to the control LPS.
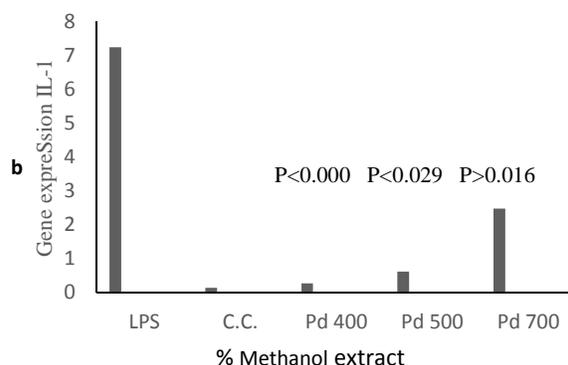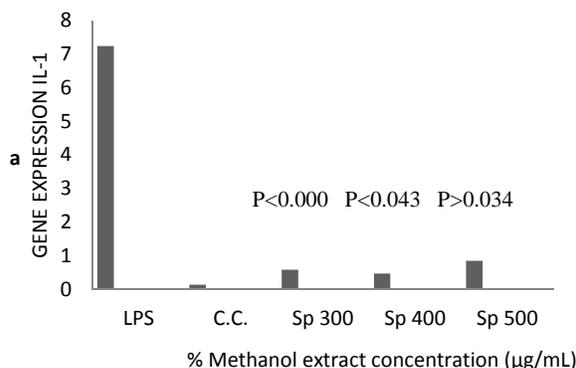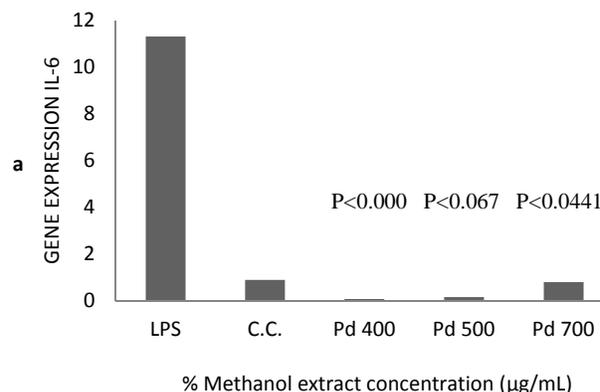

Fig. 5 a. Gene expression of IL-1 in PBMC treated with *Psacalium decompositum* (A.Gray) H.Rob. & Brettell. *Pd* 400–700 = *P. decompositum* 400-700 µg/mL; **b.** Gene expression of IL-1 in PBMC treated with *S. parryi*. *Sp* 300 – 500 = *S. parryi*. 300-500 µg/mL; CC= PBMC without treatment; positive control = LPS.

Fig. 6 a. Gene expression of IL-6 in PBMC treated with *Psacalium decompositum Psacalium decompositum* (A.Gray) H.Rob. & Brettell . *Pd* 400–700 = *P. decompositum* 400-700 µg/mL; **b.** Gene expression of IL-6 in PBMC treated with *S. parryi*. Sp 300 – 500 = *S. parryi* 300-500 µg/mL; CC= PBMC without treatment; positive control = LPS.
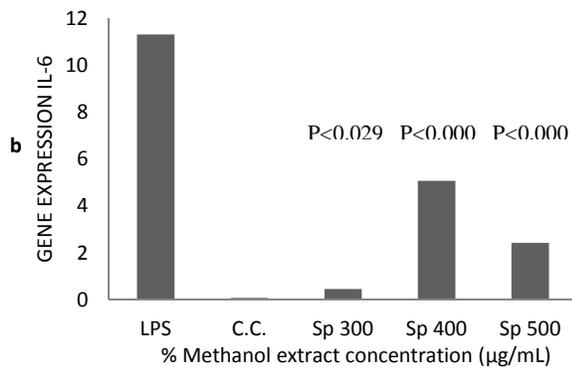

Fig. 7 a. Gene expression of IL-8 in PBMC treated with *Psacalium decompositum* (A.Gray) H.Rob. & Brettell. *Pd* 400–700 = *P. decompositum* 400-700 µg/mL; **b.** Gene expression of IL-6 in PBMC treated with *S. parryi*. *Sp* 300 – 500 = *S. parryi* 300-500 µg/mL; CC= PBMC without treatment; positive control = LPS.
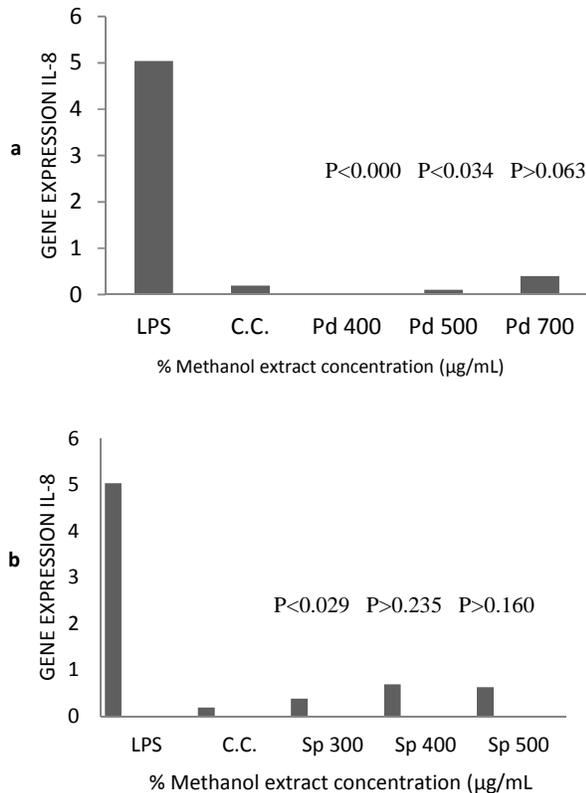
The gene expression of IL-8 in PBMC treated with the methanol extract *P. decompositum* at concentrations of 400, 500, and 700 µg/mL is shown in Figure 7a. IL-8 was normalized with the expression of the endogenous gene -actin; a significant difference exists in the 400 and 500 µg/mL concentration with regard to the control LPS.

The gene expression of IL-8 in PBMC treated with the methanol extract *S. parryi* at concentrations of 300, 400, and 500 µg/mL is shown in Figure 7b. IL-8 was normalized with the expression of the endogenous gene -actin; a significant difference exists in the 300 µg/mL concentration with regard to the control LPS.

Discussion

The results of our *S. parryi* and *P. decompositum* methanol extract cytotoxicity evaluations showed that cell viability with the MTT technique, demonstrated in different cell lines, is reduced. In the case of *P. decompositum* with VERO cells, it was 32.38% at a concentration of 120 µg/mL and with Detroit 551 cells it was 20.82% at a concentration of 700 µg/mL. This was greater than the viability reflected by *S. parryi* since with VERO cells it was 17.25% at a concentration of 60 µg/mL and with Detroit 551 cells it was 13.51% at a concentration of 500 µg/mL. Our results of *P. decompositum* differ from those of García *et al*. [22] with cumaric acid who obtained an aqueous extract from *P. decompositum* that was used to treat the HepG2 cell line where viability was 97% at a concentration of 5.2 µg/mL. On the other hand, there are few reports on the biological activities of *S. parryi*, and regarding its toxicity, there are no reports in the literature to date. The most important point mentioned is about the family *Caryophyllaceae Juss* to which the plant *S. parryi* belongs in the publication "The plants of Extracts. Basis for a sector development plan" by the Alonso Martín Escudero Foundation [23]. In this publication the plant *Saponaria officinalis* is described as toxic as a result of its saponin content; therefore, the recommended dose should not be exceeded since it can cause, by overdose or by individual hypersensitization, irritation of the digestive mucosa and depression with decreased activity of the respiratory and cardiac nerve centers. Also, in the book "Toxic Plants of North America" [24], four genders of the family *Caryophyllaceae Juss* are mentioned: *Agrostemma, Drymaria, Saponaria*, and *Vaccaria*, which can cause intoxication because they contain steroidal saponins that are glycosides of pentacyclic oleananes. However, some saponins that are similar to these that are known toxic genders are also found in some other genders, for example, *Doanthus* and *Gypsophila* [25]. Although the species of these taxons have not been reported as toxic, they can, in unusual circumstances, cause adverse effects when ingested in large quantities; also, type 1 ribosome-inactivating proteins (RIPs) are present in some genders [26].

With regard to the results of antiinflammatory activity, the methanol extract *S. parryi* significantly represses, in contrast with the control, expression of the cytokines IL-1 , IL-6, both at the concentrations tested, 300, 400, and 500 µg/mL, and in IL-8, only at the concentration of 300

µg/mL. It is relevant to mention that there are no previous reports about this activity in the literature. The methanol extract *P. decompositum* significantly represses, in contrast to the control (LPS), expression of the cytokine IL-1 at the three concentrations tested, 400, 500 and 700 µg/mL; in IL-6, it represses expression only in the concentration of 400 µg/mL and in IL-8, it represses expression at the concentration of 400 and 500 µg/mL. Although there are few reports on this activity, in an *in vivo* study, "the Effect of isolated fructooligosaccharides of *Psacalium decompositum* on the inflammatory profile of obese rats" researchers tested an inulin-type carbohydrate fraction (fructooligosaccharides) where, in addition to significantly reducing the body weight of the experimental specimens (Wistar rats), they generated important changes in the inflammatory profile, affecting the serum concentrations of IL-6, and IL-1, which coincide with the cytokines that were studied in our research; also, the serum concentrations of IFN-, MCP-1, and VEGF were affected [27]. Likewise, in the work carried out to evaluate the antiinflammatory activity of compounds isolated from *Psacalium decompositum* in vivo and *in vitro* (macrophages), the same cells used in the present study in the *in vivo* assay both cacalol and cacalol acetate (substances isolated from the root of *P. decompositum*) significantly inhibited the development of ear edema by up to 40% with respect to the control, and cultures treated with the compounds significantly decreased the concentrations of TNF-, IL-6 and IL -1, which is concluded with the reduction of inflammation through the modulation of inflammatory cytokines. These cytokines coincide with those evaluated in our study where their expression is also significantly reduced when treated with the methanol extract of *P. decompositum* and not only with a specific compound of the plant [28].

Conclusion

Regarding the cytotoxicity of the methanol extracts of *S. parryi* and *P. decompositum* on the VERO cell line and the Detroit 551 cell line (skin fibroblasts) *S. parryi* was more toxic in both cell lines than *P. decompositum* with a LD_{50} of 203.63 µg/mL for *S. parryi* and 77.30 µg/mL for *P. decompositum* on VERO cells and an LD_{50} of 150.58 for *S. parryi* and of 52.70 for *P. decompositum* on skin fibroblasts.

The anti-inflammatory activity of the extracts under study reduced the expression of the cytokines IL-1, IL-6 and IL-8 at concentrations of 300 µg/mL for *S. parryi* and of 400 µg/mL for *P. decompositum*, respectively. These results suggest significant changes in the inflammatory profile when testing these extracts indicating that they may be a good alternative as cytokine immunomodulators by decreasing their expression.

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