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Antioxidant, antimicrobial and immunostimulant properties of saline extract from *Caesalpinia pulcherrima* (L.) Swartz (Fabaceae) leaves

Lethícia Maria de Souza Aguiar[1], Dayane Kelly Dias do Nascimento[1], Bárbara de Azevedo Ramos[2], Maiara Celine de Moura[2], Luana Cassandra Breitenbach Barroso Coelho[2],Thiago Henrique Napoleão[2], Maria Tereza dos Santos Correia[2], Iranildo José da Cruz Filho[1], Ana Maria Souto Maior[1], Túlio Diego da Silva[3], Cláudia Sampaio de Andrade Lima[4], Virgínia Maria Barros de Lorena[5] & Cristiane Moutinho Lagos de Melo*[,1,+]

[1]Department of Antibiotics, Biosciences Centre, Federal University of Pernambuco, Pernambuco, Brazil 50740 525
[2]Department of Biochemistry, Biosciences Centre, Federal University of Pernambuco, Pernambuco, Brazil 50740 525
[3]Center for Strategic Technologies of the Northeast, Pernambuco, Brazil 50740 525
[4]Department of Biophysics, Biosciences Centre, Federal University of Pernambuco, Pernambuco, Brazil 50740 525
[5]Aggeu Magalhães Research Centre, FIOCRUZ/PE, Pernambuco, Brazil 50740 525
E-mail: [+]cristianemout@gmail.com

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Caesalpinia pulcherrima (L.) Swartz is a species that originates in India and Central America and is widely distributed in the world. This plant presents important biological properties, due to the presence of some secondary medicinal metabolites, such as tannins, glycosides, flavonoids and diterpenes. In folk medicine, *C. pulcherrima* is used in different diseases as bronchitis, asthma, infections, wounds and eye irritations. The aim of this study was to perform a phytochemical screening and evaluate which biological properties the saline extract of leaves from *C. pulcherrima* have against microorganisms and animal cells. Results showed that 15 majority compounds could be found in saline extract distributed among phenol, coumarin and quercetin groups.Saline extract of leaves from *C. pulcherrima* also showed good results in antioxidant test and showed significant antifungal property against *Candida* strains. Moreover, the saline extract did not showed cytotoxicity against mice splenocytes and promoted proliferation in these cells. These results may be predictive for future studies using the saline extract from *C. pulcherrima* leaves as cell stimulant agent in a pharmaceutical phytotherapic formulation used in cicatricial and *in vitro* immunostimulant assays.

Keywords: Antifungal, Antioxidant, *Caesalpinia pulcherrima*, Cell stimulation, Extract
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The use of medicinal plants by the population has been reported by literature over many years[1-3]. These plants are considered an alternative means used by communities for the relief of symptoms and cure of diseases inside of alternative medicine[2-5].

Caesalpinia genus[6-8]. This species is found in India[9], Central America[9,10], tropical and subtropical regions of Africa, Asia, Australia, America and the Caribbean[11]. It is also commonly found in Brazil, popularly known as flamboyan or cheap beard, being widely used as an ornamental plant due to flowers with yellow, red or orange colors.

According to Figueiredo and Sáber, 2016 and Kumbhare et al, 2012[9,12], *Caesalpinia pulcherrima* also has medicinal uses. In popular medicine, leaves, pods, flowers and bark are commonly used in the treatment of different diseases as rheumatism, ulcers[13], fevers, infections, wounds, eye irritations[14] bronchitis, asthma and tumors[8,15,16]. Specifically, in China it is used as tonic that stimulates blood flow and its bark is [17]ndia, its dried seeds are e population.[18] Other properties such as anticonvulsant[7] and antioxidant[19] were also reported for this species.

Here we performed assays with the saline extract from *Caesalpinia pulcherrima* leaves aiming to investigate its phytochemical composition, antioxidant and antimicrobial activities and discover if this extract is dangerous to mice spleen cells *in vitro*.

Material and methods

Botanical material collection and extract preparation

Leaves from *Caesalpinia pulcherrima* (L.) Swartz were collected in Federal University of Pernambuco -

*Corresponding author

Brazil. The botanical authentication was done at the Geraldo Mariz Herbarium in the Department of botany, at the Center of Biological Sciences (Federal University of Pernambuco), where exsiccate was deposited with registration number 82.702. The leaves were kept at room temperature for 2 days to remove humidity. The dried leaves were crushed in reverse black oster and the pulverized material (20 g) was diluted in 0.15 M NaCl (200 mL) in the proportion of 10% (w/v). Therefore, using an orbital and alternative incubator shaker (Lab Companion™ IS-971) the material was kept under agitation (400 rpm) in constant temperature (28 ºC) for 16 h. After the stirring time, the material was filtered using paper filter and centrifuged (15 min, 10,000 g). The obtained supernatant was collected and called saline extract from *Caesalpinia pulcherrima* leaves. After this, the extract was lyophilized and 9.2 g of crude material was obtained. Material was stored at -20 °C until use.

Phytochemical characterization

Total phenolic compounds measurement

The contents of total phenols were determined using the method of Folin-Ciocalteu, described by Li et al, 2008 and Rocha et al, 2011[20,21] with some modifications. The extract diluted 1 mg/mL was dissolved in distilled water. In 0.02 mL of diluted extract was added 0.1 mL of solution Folin (1:10 v/v). After incubation, protected from light, for 3 min, was added 0.08 mL of sodium carbonate (Na_2CO_3) (7.5%). The samples were incubated protected from light, at room temperature, for 120 min. After incubation time, the absorbance was measured at 765 nm against a blank (distilled water). A calibration curve of the graphical representation of the absorbance as a function of the concentration of gallic acid (0 - 500 µg/mL) was prepared and the linear equation ($y = 0.0048x + 0.0016$ $R^2 = 0.9999$). Phenols are expressed in gallic acid equivalent (mg EAG/g of extract).

Determination of total flavonoids

The measurement of total flavonoid content was determined according to the methodology described by Woisky and Salatino, 1998[22] with some modifications. Using test tubes, 1 mL of the extract, previously diluted (1 mg/mL) was added. After this, 1 mL of the 3% solution of aluminum chloride ($AlCl_3$) prepared with methanol was added in tubes. After 30 min of incubation, at room temperature and protected from light, the absorbance was measured at 425 nm. A standard curve with quercetin (0 - 500 µg/mL) was performed to obtain the equation $Y = 0.023x + 0.1509$, $R^2 = 0.9956$. The assays were performed in quintuplicate. Flavonoids are expressed in quercetin equivalent (mg QE/g of extract).

Ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS)

Ultra-performance liquid chromatograph (UPLC) was performed with an Acquity H-Class (Waters) employing a 2.1 x 100 mm BEH column with a particle size 1.7 µm. The column was maintained under a constant temperature of 40ºC and the auto injector at 10ºC. We use an aqueous solution (eluent A) containing 2% methanol (MeOH), 5 mM ammonium formate and 0.1% formic acid and methanolic solution (eluent B) containing 0.1% formic acid, which were pumped at a flow rate of 0.3 mL/min. Ten microliters of the *C. pulcherrima* extract was injected. Elution was performed in gradient mode and the initial condition (98% A/2% B) was maintained for 0.25 min. The B ratio increased linearly to 99% in 8.5 min, remaining at 99% B for one minute, followed by immediate decrease to 2% B, where it was maintained for up to 11 min. The UPLC system was coupled to a single quadrupole mass spectrometer SQ Detector 2 (Waters). The data acquisition was done in full scan mode, searching for masses between 100 and 1000 Da, in negative ionization. The acquisition of the chromatograms and mass spectra were acquired through MassLynx™ software (Waters).

Antioxidant analysis

Total Antioxidant Activity

The total antioxidant activity of saline extract was determined as a function of ascorbic acid, according to Pietro et al., 1999 and Balestrin et al., 2008[23,24]. Saline extract (100 µL at 500 µg/mL) was mixture with ascorbic acid (1 mg/mL) and with 1 mL of phosphomolybdenum solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). This mixture was incubated in water at 95°C for 90 min. After returning to room temperature, the absorbance was measured at 695 nm against a blank (1 mL solution with 0.1 mL of water). A standard curve with ascorbic acid (0 - 500 µg/mL) was performed to obtain the equation $Y = 0.019x + 0.0723$, $R^2 = 0.9937$. The total antioxidant activity calculated by the formula ATT (%) = [(As - Ac) / (Aaa - Ac)] 100, where: Ac = Control Absorbance, As = Sample Absorbance and Aaa = Ascorbic acid absorbance.

Free radical sequestration by DPPH•

The antioxidant activity of saline extract was measured by the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•), as described by Blois, 1958[25]. Using 0.04 mL of different concentrations of the saline extract (3.9; 7.8; 15.6; 31.3; 62.5; 125; 250 and 500 µg/mL) it was added 0.25 mL of the DPPH solution (1 mM and OD_{517} = 0.650±0.50). After incubation time (25 min) at room temperature, protected from light, the absorbance was measured at 517 nm. We used as control the DPPH solution added to water. The sequestration of DPPH radicals was calculated by the formula: SRL [DPPH•] (%) = [(As - Ac) / Ac] x 100. When: As = sample Absorbance and Ac = control Absorbance.

Reduction of Ferric Ion (FRAP assay)

The stock solution of the FRAP assay was prepared using 300 mM acetate buffer (3.1 g of CH_3COONa and 16 mL of CH_3COOH) at 3.6 pH, 10 mM TPTZ (2,4,6-tripyridyl-triazine) solubilized in 40 mM HCl, and 20 mM $FeCl_3$ solution as described by Benzie and Strain, 1996[26]. The working solution was prepared by mixing the acetate buffer, TPTZ and $FeCl_3$ in a ratio of 10:1:1 (v/v/v) and incubated for 5 min at 37ºC. Saline extract (25 µL in 1 mg/mL) was added in 0.180 mL of FRAP reagent. After stirring, and resting for 30 minutes at 37°C, protected from light. Subsequently the absorbance was measured at 593 nm. A standard curve with $FeSO_4$ (0 - 1000 µg/mL) was performed to obtain the equation Y = 0.0024x+0.0019, R^2 = 0.9953. The results were expressed as mg EFeSO4 (II)/g of extract.

Antimicrobial assays

Bacterial and fungal strains, culture conditions and preparation of samples

Fungal strains were obtained from the culture collections at Mycology Department from Federal University of Pernambuco (URM) and bacteria isolates were provided by the culture collection of the Antibiotics Department from Federal University of Pernambuco (UFPEDA). Stock cultures were kept under refrigeration (-20°C) in sterilized skim milk containing 10% (v/v) glycerol. The bacterial and fungal pathogens used in this study are *Candida albicans* (URM 5901), *Candida krusei* (URM 6391), *Candida tropicalis* (URM 6551), *Candida parapsilosis* (URM 6951), *Candida glabrata* (URM 4246), *Escherichia coli* (UFPEDA 224), *Klebsiella pneumoniae* (UFPEDA 396), *Pseudomonas aeruginosa* (UFPEDA 416), *Salmonella enteritidis* (UFPEDA 414), *Staphylococcus aureus* (UFPEDA 02) and *Staphylococcus saprophyticus* (UFPEDA 833). For antimicrobial activity assay, bacterial and yeast species were cultured in Mueller Hinton and Sabourand Dextrose agar medium, respectively, overnight at 36°C, and subsequently the colonies were resuspended in sterile saline solution (0.15M NaCl) and turbidimetrically adjusted at a wavelength of 600 nm (DO_{600}) to obtain the suspension equivalent to 10^6 colony forming units (CFU) per mL. For the assay, the samples were filtered on the sterile polyvinylidene difluoride (PVDF) syringe filter (pore size: 0.22 µm; diameter: 13 mm).

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The minimum inhibitory concentration (MIC) promoted by saline extract from *Caesalpinia pulcherrima* leaves was determined by the microtiter test based on CLSI, 2012[27] criteria. In 96-well microtiter plates, the extract (12 mg/mL) was added (80 µL) into the fourth well from which a serial dilution in sterile Milli-Q water was performed to the twelfth well of the same row. Subsequently, 40 µL of Mueller Hinton (bacteria) or Sabourand Dextrose (yeast) broths were added to all wells, but to the first one was filled with 200 µL of the culture medium, corresponding thus to the sterility medium control. Antibiotics ampicillin and tetracycline (8 µg/mL) and antifungal fluconazole (64 µg/mL), obtained from Sigma-Aldrich (USA), were used as positive controls in the second well. Finally, the bacterial or yeast suspensions (80 µL, 10^6 CFU/mL) were added in the second well to the last well in the row. The third well (containing microorganisms in the absence of the sample) corresponded to the 100% growth control or negative control. The plates were incubated at 36ºC and the optical density was measured at time zero and after 24 h of incubation. The MIC90 and MIC50 corresponded to the lowest concentration of the sample capable of promoting a reduction of ≥90% or ≥50%, respectively, in optical density, as compared to the 100% growth control. For determination of the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC), aliquots of the wells containing concentrations of ≥MIC50 samples were inoculated into petri dishes containing Mueller Hinton or Sabourand Dextrose agar medium, which were subsequently incubated at 36°C for 24 h. The MBC and MFC corresponded to the lower

concentration of the sample able to reduce the number of CFU in 99.9% in relation to the initial inoculum. Each assay was performed in triplicate and three independent experiments were performed.

Viability assay against mice splenocytes

Animals

Female BALB/c mice (6–8 weeks old) were raised and maintained at the animal facilities of the Keizo Asami Immunopathology Laboratory – LIKA located in Federal University of Pernambuco, Brazil. Mice were kept under standard laboratory conditions (20–22°C and 12 h day and night cycle) with free access to a standard diet (Labina/Purina, Campinas, Brazil) and water. All experimental procedures were performed with accordance of Ethics Committee of Animal Use (CEUA) of Federal University of Pernambuco (protocol number: 0048/2016).

Preparation of splenocytes

This procedure was performed in accordance to Melo et al., 2011[28]. After animal's euthanasia, the spleen of each mouse was removed aseptically and placed in a Falcon tube containing RPMI 1640 with fetal calf serum (complete medium). In a vertical flow, each spleen was transferred to a petri dish where they were soaked. The cell suspensions obtained from each spleen were transferred to Falcon tubes containing approximately 10 mL of incomplete medium. Spleen homogenates were overlaid onto a Ficoll-PaqueTM PLUS layer, with the density adjusted to 1.076 g/mL, and centrifuged at 1000 *x* g at room temperature for 25 min. The interface cell layer containing immune cells was recovered by Pasteur pipette, washed twice in phosphate-buffered saline (PBS) and centrifuged twice at 500 g for 10 min. Cells were counted in a Neubauer chamber, and cell viability was determined by the trypan blue exclusion method. Cells were only used when viability was >98%.

Analysis of cell viability by annexin V-FITC and propidium iodide-PE staining

Mice splenocytes (10^6 cells) were treated with *C. pulcherrima* saline extract in 50, 25, 12.5, 6 and 3 µg/mL and were maintained in 24-well plates for 24 h to analyze the cytotoxicity power of extract. Untreated cells, only in RPMI 1640 medium, were used as negative control. Following this, lymphocytes were centrifuged at 26°C, 450 g for 10 min. After discarding the supernatant, 1 mL of PBS 1X was added to the precipitate and this was then centrifuged at 26°C, 450 g for 10 min. After discarding the supernatant, the pellet was resuspended in a binding buffer of cell viability kit (Becton Dickinson Biosciences) and annexin V conjugated with fluorescein isothiocyanate (FITC) (1:500) and propidium iodide (PI, 20 µg/mL) were added to each labeled cytometer tube. Flow cytometry was performed in a FACS Calibur flow cytometer (Becton Dickinson Biosciences) and analyzed using CELL QUEST PRO software (Becton Dickinson). Result analysis was performed in graphs by dot plot. Annexin-FITC negative/PI positive cells were considered necrotic cells and Annexin-FITC positive/PI negative represented splenocytes in the early stage of apoptosis. Double negatives were considered viable cells.

Cell proliferation analysis using CFSE staining

Same protocol to obtained splenocytes was used for proliferation assay. After splenocytes obtainment, cell solution was centrifuged at 300*x* g at room temperature for 5 min with sterile PBS 1X added with SFB 5% (pH 7.2). After this, the cell solution was adjusted to$10x10^6$ cels/mL and received 5 mM of 5(6)-carboxyfluorescein diacetate N-succinimidyl ester (CFSE). Cells were incubated for 10 min at room temperature in the dark. Cells were centrifuged twice at 300*x* g/5 min with sterile PBS 1X. Cells stained were cultured for 24 and 48 h with 12.5 µg/mL of saline extract from *C. pulcherrima* leaves and only culture complete RPMI 1640 medium (negative control). After culture time cells were centrifuged (300*x* g/5 min), were carried to acquisition on FACSCalibur platform (Becton Dickinson Biosciences) and results were analyzed using Cell Quest Pro software (Becton Dickinson).

Statistical analysis

To test the normal hypothesis on the variable involved in this study the Shapiro–Wilke test was applied. Means of samples were analyzed using non-parametric tests. The statistical difference between two groups was analyzed by Wilcoxon test and that among more than three groups by one-way analysis of variance (ANOVA). All the conclusions were considered with a significance level of 5%. For statistical analysis was used GraphPad Prim 5.01 software.

Results

Prevalence of phenolic acids, phenol and flavonoids in saline extract from C. pulcherrima leaves

This study investigated the phytochemical profile of extract from *C. pulcherrima* leaves made using saline

solution (NaCl 0.15M) as a vehicle. This solution is routinely used in some medical applications and is named as physiological solution in health field. Results of analysis in UPLC-MS showed that was possible identify many compounds in saline extract from *C.pulcherrima* leaves (see Fig. 1a and b), especially phenolic and flavonoid compounds (Table 1).

The phytochemical composition showed a greater number of phenolic compounds, being these distributed among flavonoids, phenolic acids and polyphenols. In fact, we found mostly 15 compounds in saline extract (summarized on Table 2) in accordance with area, retention time, molecular weight, molecular formula and negative mass.

Antioxidantefficacy of *C. pulcherrima* leaves

Results by the antioxidant tests showed that saline extract present higher antioxidant power being superior to butylated hydroxytoluene (BHT) standard, in relation to all assays evaluated, i.e., free radical

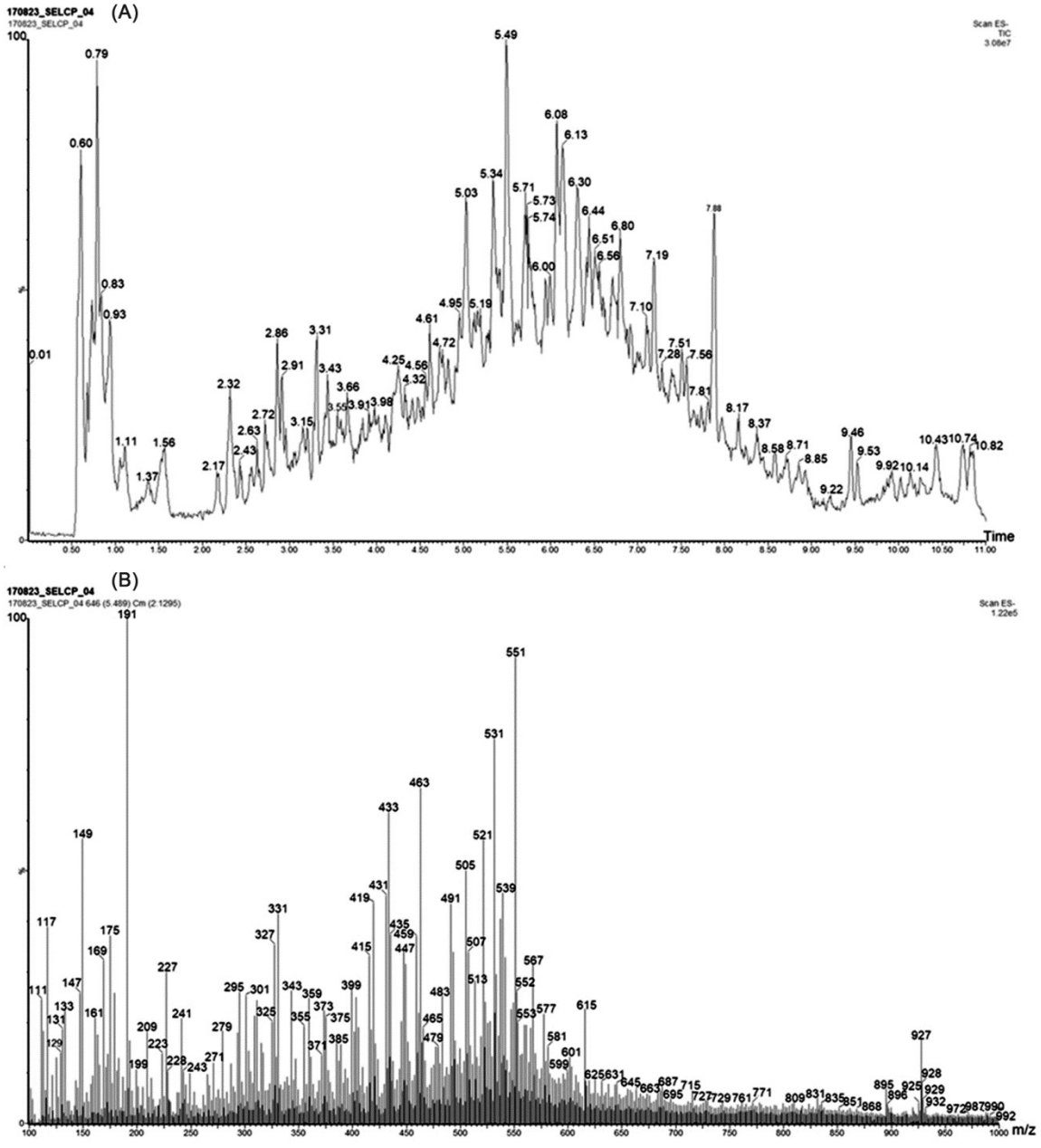

Fig. 1 — UPLC-MS Chromatogram of saline extract from *C. pulcherrima*leaves (A). Mass spectrum of same saline extract (B).

Table 1 — Result of antioxidant activities and phenol amounts found on saline extract from *C. pulcherrima* leaves

	Unites	Saline extract	BHT	Ascorbic acid
Total phenolic compounds	(mg GAE/g)	67.813 ± 0.101	-	-
Total of flavonoids	(mg QE/g)	11.868 ± 0.061	-	-
Free radical sequestration by DPPH	(% mg/mL)	97.94 ± 3.09	93.13 ± 0.58	90.04 ± 0.16
Total antioxidant capacity	(mg AAE/g)	6.782 ± 0.023	4.12 ± 0.10	100
Reduction of Ferric Ion	(mg $EFeSO_4$(II)/g)	973.19 ± 19.69	679.17 ± 25.98	1215.00 ± 48.55

Table 2 — UPLC-MS spectral analysis of compounds found in saline extract from *C. pulcherrima* leaves.

Identified tentative	Retention time (min)	Area	Molecular formula	Mass	[M-H]-
Isoferulicacid	0.71	71688.734	$C_{10}H_{10}O_4$	194.186	193.0501
CaffeicAcid	0.76	80650.695	$C_9H_8O_4$	180.0423	179.0344
Scopoletin	0.93	888721.250	$C_{10}H_8O_4$	192.0423	191.0344
Quinicacid	0.93	89235.788	$C_7H_{12}O_6$	192.0634	191.0556
Gallicacid	2.32	372184.250	$C_7H_6O_5$	170.0215	169.0137
Quercetin 3-O-rhamnoside	3.31	166493.219	$C_{21}H_{22}O_{11}$	448.38	449.1084
Ellagicacid	3.45	104292.820	$C_{14}H_6O_8$	302.0063	300.9984
p-coumaricacid	3.68	38649.922	$C_9H_8O_3$	164.0473	163.0395
Quercetin 3-O-xylosyl(1→2) rhamnoside	5.63	13349.838	$C_{26}H_{28}O_{16}$	596.138	595.1299
4-Hydroxybenzoic acid	5.7	18288.523	$C_7H_6O_3$	138.0317	137.0239
Quercetolglucoside	5.74	517174.281	$C_{21}H_{20}O_{12}$	464.0955	463.0877
Quercitrin	6.32	190709.328	$C_{21}H_{20}O_{11}$	448.1006	447.0927
Rosmarinicacid	7.51	161730.375	$C_{18}H_{16}O_8$	360.318	359.0767
Quercetin	7.55	84838.086	$C_{15}H_{10}O_7$	302.0427	301.0348
1. 3,4',5-stilbenetriol	7.88	295351.156	$C_{14}H_{12}O_3$	228.0786	227.0708

Table 3 — Results of antifungal activity against *Candida* spp. promoted by saline extract from *C. pulcherrima* leaves

Fungalstrains	Fluconazole (µg/mL)	Saline extract from *C. pulcherrima*(L.) Swartz leaves(µg/mL)		
	MIC_{50}	MIC_{50}	MIC_{90}	MFC
C. albicans	0.25	7.6×10^{-7}	62	ND
C. parapsilosis	4	0.8	0.8	ND
C. krusei	32	0.012	ND	-
C. tropicalis	ND	0.0004	0.0008	0.003
C. glabrata	64	ND	-	-

MIC_{50}, MIC_{90} and MFC expressed as $\mu g \cdot mL^{-1}$ of the saline extract from *C. pulcherrima* (L.) Swleaves. Saline extract and fluconazole initial concentrations: 12 mg/mL and 160 µg/mL, respectively.

sequestration, total antioxidant capacity and reduction of ferric ion. Moreover, this same extract also present values to free radical sequestration and reduction of ferric ion very next to ascorbic acid standard (see Table 1).

Antimicrobial efficacy of *C. pulcherrima* leaves

In relation to bacterial assays, none results were found to saline extract from *C. pulcherrima* leaves. However, among the standard strains of *Candida* spp. used in this study, the saline extract showed significant inhibitory capacity against *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis* species. Moreover, some results were better than Fluconazole in relation to MIC50 parameter (see Table 3).

C. pulcherrima leaves as immunostimulant

After investigation about antimicrobial activity we performed a cell viability assay to evaluate if saline extract can also promote damage in animal cells. Results showed that mice spleen cells challenged with distinct concentrations (50, 25, 12.5, 6 and 3 µg/mL) of saline extract did not show significant damage in relation to control cells (cells with medium). Although in 50 µg/mL the saline extract promoted higher necrosis, this value not was statistical (Fig. 2) and present associated with apoptosis index more that 93% of cell viability. In addition, we performed another test to evaluate if saline extract is able to activate mice immunological cells and induces these cells to proliferation mechanism. For this, we choose

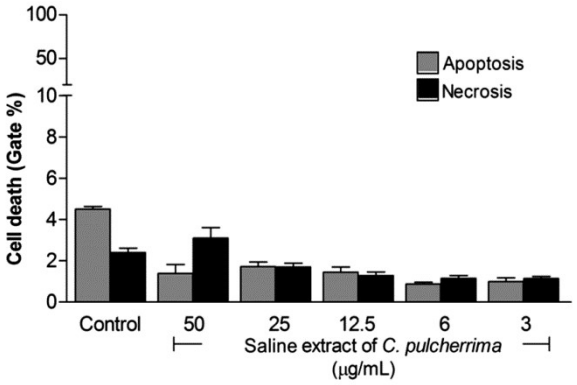

Fig. 2 — Mice splenocytes viability evaluated using annexin V and propidium iodide staining. Saline extract from *C.pulcherrima* leaves did not promoted cell death in none concentration.Horizontal bars represent the average of three independent experiments performed in duplicate.

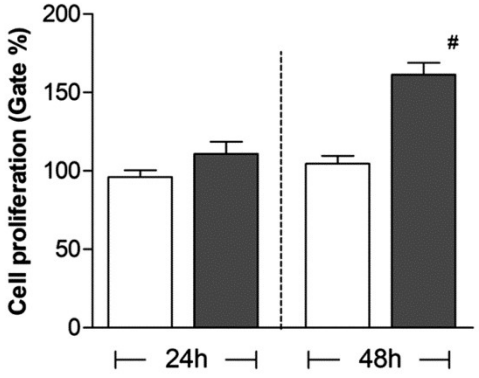

Fig. 3 — Mice splenocytes proliferation index evaluated using CFSE staining. Saline extract from *C. pulcherrima*leaves promoted proliferation in mice splenocytes only in 48 hours of assay. Vertical white bars represent negative control cells (cells+culture medium) and vertical gray bars represent saline extract from*C. pulcherrima* leaves at 12.5 µg/mL concentration. This assay was performed using three independent experiments performed in duplicate. #*p*= 0.003.

one concentration of saline extract (12.5 µg/mL) for measure this immunostimulatory parameter. Results showed that saline extract from *C. pulcherrima* leaves promoted higher proliferation in 48 h of assay (Fig. 3).

Discussion

The presence of flavonoids, tannins, triterpenes, cardiac glycosides, alkaloids, saponins, steroids and proanthocyanins from hexane, chloroform, acetone, methanol and aqueous extracts of *C. pulcherrima* seeds and bark were previously described. In addition, Chew et. al., 2011[29] showed the presence of flavonoids, tannins, terpenoids, and steroids in aqueous, methanol and dichloromethane extract of leaves and flowers from *C. pulcherrima*. According to the studies of Kumbhare et al., 2012[12] investigating extracts of pods from *Caesalpinia pulcherrima*, the major compounds founds were flavonoids, tannins, steroids and alkaloids. Beyond, methanolic extract was good scavenger of DPPH$^{\bullet}$ radical.

Also similar to our study, Sivasankari et al., 2010[30] performed a study with aqueous extract from *C. pulcherrima* leaves and they found important classes of compounds of pharmacological interest, such as phenolic compounds (flavonoids, phenols, anthocyanins and coumarins), quinones and tannins. Chiang et al., 2003[15] found one flavonoid (quercetin) in their aqueous extract composition. These results about phytochemical compounds present in saline extract from *C. pulcherrima* leaves are promisors because some studies have revealed that isolated flavonoid, for example, can exhibit anti-inflammatory properties[31].

Free radicals are produced in spontaneous oxidation process inside of animal organism[32,33]and these radicals exert important functions in the body acting on the transfer of electrons that occur in the biochemical reactions[33]. However, the excess of these mediators can promote a variety of side effects, causing various degenerative and neurological diseases as well as diabetes and cancer[32,35,36]. Results of our saline extract showed higher antioxidant performance.

Studies in the literature of *C. pulcherrima* confirm their action against antioxidant potential. Experiments by Chew et al., 2011[29]showed that the methanol extracts of leaves from *C. pulcherrima* present a high content of total phenols and a moderate capacity to eliminate free radicals (DPPH$^{\bullet}$). Krishnaveni et al., 2014[37]investigating aqueous extract of fresh leaves of *C. pulcherrima* found a low content of flavonoid and total phenols. The extracts of n-hexane, chloroform, acetone, methanol and aqueous seeds from *C. pulcherrima* fruit showed higher total phenol content than flavonoid and a good DPPH$^{\bullet}$ result[18].

Recent study with extracts of leaves and flowers from *Caesalpinia pulcherrima* showed antimicrobial activity for Gram positive bacteria, but not against Gram negative[29]. Dhaked et al., 2011 and Figueiredo & Saber, 2016[8,9] reinforced these findings testing ethanol and aqueous extracts. Here, we did not find antibacterial activity, but significant antifungal property could be observed for the saline extract.

Figueiredo & Sáber, 2016[9] show that the seeds of *C. pulcherrima* presented antifungal potential against

Trichosporonbeigelii, *Cryptococcus*, *Candida tropicalis*, *Candida albicans*. Similar to them, our results confirm the inhibition for these two latter fungi. In Srinivas et al., 2003[38] isolated flavonoids from *C pulcherrima* showed moderate antifungal activity against *Aspergillusniger* and *Candida albicans*. Ethanol extracts from *C. pulcherrima* again exhibited antifungal potential against *Candida albicans, Aspergillus niger and Rhizopus oligisporus*[13].

Caesalpinia pulcherrima saline extract did not show significant damage in animal cells at different concentrations in this study. In addition, present a potential induction to proliferation in mice immune cells in 48 h of culture. We did not found studies using this plant species in cytotoxicity assays against animal cells. Only two studies investigated cytotoxic potential *in vitro*. Erharuyi et al., 2017[11] conducted studies with some diterpenoids isolated from the chloroform extract of *C. pulcherrima* roots by evaluating the cytotoxicity of these compounds against three cancer cell lineages (MCF-7, HeLa and PC-3), showing that the compounds decreased the cell viability. Moreover, studies by Islam et al., 2003[17] also showed the cytotoxic potential of a terpenoid isolated from the shell of *C. pulcherrima* but against brine shrimp.

Conclusion

Saline extract from *Caesalpinia pulcherrima* leaves have higher amounts of phenols and flavonoids compounds. This phytochemical characteristic is beside to higher antioxidant efficacy and antimicrobial property. Moreover, saline extract did not show cytotoxicity profile against mice splenocytes inducing these cells to proliferation status. These results may be predictive for future studies using the saline extract from *C. pulcherrima* leaves as cell stimulant agent in a pharmaceutical phytotherapic formulation used in cicatricial and immunostimulatory assays.

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Conflict of interest disclosure

The authors declare no conflict of interest.

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