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MICROBIOLOGY

# Combining *UFLC-QTOF-MS* analysis with biological evaluation of *Centrosema coriaceum* (Fabaceae) leaves

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**Abstract:** *Centrosema coriaceum* Benth belongs to Fabaceae family and have few studies of biological activity and chemical composition. Thus, the aims of this work were to determine chemical profile of the ethanolic extract of *C. coriaceum* leaves (CCE) by *UFLC-QTOF-MS* and to evaluate its *in vitro* biological potential. CCE showed MIC value of 1000 µg/mL against *Candida glabrata* (fungistatic effect) and high affinity in cell envelope by increasing cell permeability in nucleotide leakage, sorbitol and ergosterol assays. CCE showed antioxidant activity in all assays performed. For the anti-inflammatory and cytotoxicity activities, CCE, at all tested concentrations, significantly inhibited the production of nitric oxide and did not decrease J774A.1 cell viability below 70%. Finally, rutin, kaempferol-3*O*-rutinoside, caffeic acid, and sucrose were identified in CCE by *UFLC-QTOF-MS*. These results suggest, for the first time, that *C. coriaceum* has interesting antifungal, antioxidant, and anti-inflammatory activities.

**Key words:** anti-inflammatory, antioxidant, *Candida glabrata*, bioactivities, phenols.

## INTRODUCTION

Fungal diseases are responsible for much of morbidity and mortality in public health systems (Parente-Rocha et al. 2017), causing at least 1.4 million human deaths per year worldwide (Sanglard 2016). The invasive ability of fungi difficults the disease diagnosis and limits the treatment with the available drugs (Souza & Amaral 2017). Moreover, the presence of pathogenic *Candida* spp. fungi in the body may lead to candidiasis conditions that are accompanied by inflammatory processes (Roselletti et al. 2017). This process releases chemical mediators in the affected tissue, including excitatory amino acids, oxygen-reactive species (ROS) and other free radicals, peptides, lipids and cytokines (Chen et al. 2016). In addition, the presence of resistance genes (Candida Drug Resistance - CDR and Multidrug Resistance - MDR) in yeasts of the genus *Candida* (Khosravi et al. 2016) associated with side effects, toxicity, and inefficiency from current candidiasis treatment, have become challenging antifungal therapy through available drugs (Bhattacharjee 2016, Perlin et al. 2017).

The medicinal plants used to treat many diseases are associated with folk medicine from different parts of the world (Moreira et al. 2014). Different cultures know and use the therapeutic potential of plants in the treatment of disease, and it is a practice that has evolved over centuries (Sofowora et al. 2013). Fabaceae is an important plant family used in

traditional medicine, which has a wide variety of properties already described, as antifungal, anti-inflammatory, antimicrobial, anticancer, antileishmanial and antibacterial properties (Ahmad et al. 2016, Rahman & Parvin 2014). Nevertheless, some species of this family have few studies of biological activity and chemical composition, such as *Centrosema coriaceum* Benth, a sub-shrub native to South America present in Cerrado, forest and rupestrian field of Southeast Brazil (Mendonça et al. 1998).

Based on these considerations, this study was undertaken to evaluate the *in vitro* antifungal, antioxidant and anti-inflammatory activities of ethanolic extract from *C. coriaceum* leaves and to identify some of its compounds by *UFLC-QTOF-MS* analysis.

## MATERIALS AND METHODS

### Plant material

The leaves of *Centrosema coriaceum* Benth were collected in the district of Toledos, Juiz de Fora, State of Minas Gerais, Brazil, on December 4, 2014, at 13:00h. A voucher was deposited in the Herbarium Leopoldo Krieger, Federal University of Juiz de Fora under the number CESJ 49986 and according to the license number A032F41-SISGEN/BRAZIL.

### Preparation of the *C. coriaceum* ethanolic extract (CCE)

The leaves of *C. coriaceum* were dried in the shade at room temperature for 15 days, and then pulverized to obtain the dry and homogeneous plant material. The dried material was extracted by static maceration for 24h with the ethanol solvent (5 x 500 mL) and concentrated under reduced pressure using the rotatory evaporator (Heidolph - Laborota 4000), obtaining a yield of 28.7% in relation to the dried plant material, and kept under refrigeration until the time of the chemical and biological tests.

### Evaluation of antifungal activity

#### *Determination of minimum inhibitory concentration (MIC)*

The broth microdilution susceptibility assay was performed using the method described by CLSI (2014) for determination of MIC. All tests were performed using Sabouraud dextrose broth (SDB) and Sabouraud dextrose agar (SDA). Strains of five *Candida* species (*Candida albicans* ATCC® 24433, *Candida albicans* ATCC® 10231, *Candida glabrata* ATCC® 2001, *Candida krusei* ATCC® 6258 and *Candida tropicalis* ATCC® 750) were grown at 35°C for 24h up to 48h on Sabouraud Dextrose Agar. Successive dilutions of 4000 to 31.3 µg/mL of CCE were prepared in 96-well microplates. For this, 10000 µg/mL stock solutions in 1% dimethylsulfoxide (DMSO) were used. 80 µL of this solution was transferred to the microplates, which already contained 100 µL of SDB. To complete the final volume of 200 µL, 20 µL of inoculum ($10^4$ colony forming units (CFU/mL) according to McFarland's standard turbidimetric scale reaching 2 x $10^3$ CFU/mL per well) was added. The plates were incubated at 35 ºC for 24h to visualize fungal growth, except for *Candida tropicalis*, which was evaluated after 48h. The same tests were performed simultaneously for growth control (SDB + fungus + CCE vehicle) and sterility control (SDB + CCE vehicle). The positive control used was fluconazole at concentrations of 1000 to 8 µg/mL. The MIC was calculated as the lowest dilution showing complete inhibition of the tested strain. The experiment was performed in duplicate.

#### *Minimum fungicidal concentration (MFC)*

To determine the MFC, 20 µL from each well that showed no visible fungus growth in the MIC assay was plated on freshly prepared SDA plates and later incubated at 35 °C for 24h up to 48h, according to Spencer & Spencer (2004). Minimum fungicidal concentrations (MFC) were taken as the concentrations that showed no growth of fungal colonies on agar plates. The assay was performed in duplicate.

### *Effect on cell envelope*

Cell envelope assays consisted of assessing the ability of CCE to disrupt cell envelope integrity (nucleotide leakage), cause fungal cell wall damage (sorbitol protection assay) and membrane steroid binding capacity (exogenous ergosterol binding assay) against the species *C. glabrata*. For the assays on membranes, nystatin was used as a positive control. For this, the MIC value of nystatin for *C. glabrata* (concentrations ranging from 25 to 0.19 µg/mL) was identified under the same conditions of the MIC assay.

### *Nucleotide leakage*

The experiment was performed according to Tang et al. (2008) with some modifications. The strains (*C. glabrata*) were incubated in SDB at 35°C for 24h. The culture was then centrifuged at 10,000g for 10 min, washed and resuspended in 10 mM phosphate buffered saline (PBS) (pH 7.4), reaching the final density of about $10^6$ cells/mL. The fungal cells were incubated with CCE and nystatin (MIC values) at different times (0, 1, 2, 3, 4 and 5h); and also incubated only with 10 mM PBS (pH 7.4) (negative control). After incubation, cell suspensions were centrifuged at 10,000g for 15 min and the optical density (OD) at 260 nm was recorded in a spectrophotometer (Multiskan Go, Thermo Scientific, Waltham, MA, United States) at room temperature (2 °C). The experiment was performed in triplicate.

### *Sorbitol protection assay*

After determining MIC value for CCE against *C. glabrata*, the wall assay was performed using the osmotic protector sorbitol, according to the methodology of Frost et al. (1995). A serial microdilution was performed, in a 96-well sterile microplate, using SDB enriched with 0.8 M Sorbitol. The CCE stock solution was diluted in concentrations ranging from 4000 to 500 µg/mL. For this assay, fluconazole was used as antifungal positive control at concentrations of 250 to 15.6 µg/mL. New MIC values were determined after incubation at 35°C for 24h. The experiment was performed in duplicate.

### *Exogenous ergosterol binding assay*

For the ergosterol-binding assay, the change in MIC value from CCE to *C. glabrata* was determined according to the methodology of Leite et al. (2014). A serial microdilution was performed in a 96-well microplate using SDB enriched with ergosterol (400 µg/mL). The CCE stock solution was diluted in concentrations ranging from 4000 to 500 µg/mL. New MIC values were determined after 24h of incubation at 35°C. Nystatin was used as positive control. The experiment was performed in duplicate.

## Evaluation of antioxidant activity

### *2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH•) radical-scavenging method*

The free radical-scavenging activity of DPPH• was determined by the method described by Brand-Williams et al. (1995). Successive dilutions in methanol of CCE were made in 96-well plates and the 20 µg/mL of 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) solution in methanol was added, thus obtaining CCE concentrations from 250 to 0.49 µg/mL. The

plate was incubated in the dark and after 30 min the absorbance was measured at 517 nm. The percentage (%) of CCE oxidation inhibition was calculated as follows: % Inhibition = [($Abs_{DPPH}$ - $Abs_{sample}$)/ $Abs_{DPPH}$] ×100. Where: $Abs_{DPPH}$: measured absorbance for DPPH radical in methanol; $Abs_{sample}$: measured absorbance of the samples. The positive control used was rutin, whose concentration ranged from 250 to 0.0005 µg/mL. The results were expressed in the form of 50% inhibitory concentration ($IC_{50}$), which was defined as the concentration (in µg/mL) of sample required to inhibit the formation of DPPH radicals by 50%. The experiment was performed in triplicate.

### *Total antioxidant capacity by phosphomolybdenum assay*

The total antioxidant capacity (TAC) was determined by phosphomolybdenum assay based on the method proposed by Prieto et al. (1999). Test tubes containing 300 µL of the sample/ positives control (quercetin and rutin) in ethanolic solution (500 µg/mL) and 2 mL reagent solution (1.12 mL sodium phosphate solution, 0.48 mL ammonium molybdate and 0.40 mL $H_2SO_4$) were heated at 95°C for 90 min. The reading was performed by UV spectrophotometer at 695 nm. Results were expressed as a percentage of relative antioxidant activity (RAA%) in relation to the positive controls. The equation for calculating RAA% is shown below (Negri et al. 2009, Zocoler et al. 2009). RAA% = [($Abs_{sample}$ - $Abs_{blank\ of\ sample}$)/ $Abs_{positive\ control}$ – $Abs_{blank\ of\ positive\ control}$] × 100. Where: $Abs_{sample}$: absorbance measured for the samples; $Abs_{positive\ control}$: absorbance measured for the positive controls; $Abs_{blank\ of\ sample}$: absorbance measured for the sample blank; $Abs_{blank\ of\ positive\ control}$: absorbance measured for the positive control blank. The experiment was performed in triplicate.

### *β-carotene/linoleic acid system*

Lipid peroxidation inhibition activity was evaluated by the β-carotene/linoleic acid method described by Melo & Mancini Filho (1989) with some modifications. An emulsion containing β-carotene and linoleic acid was prepared and adjusted for a reading between 0.6 and 0.7 of absorbance at 470 nm. 250 µL of emulsion and 10 µL of CCE and rutin (positive control) solutions (Final concentration of 38.46 µg/mL) were added. Negative control was performed with 250 µL of the emulsion and 10 µL of methanol (100% oxidation). The plate was incubated at 45°C to accelerate oxidation reactions and initiate β-carotene discoloration. The absorbance of the samples was measured at 470 nm. Readings were taken at zero time and intervals of 15 min until completing 120 min of reaction.

The decrease in sample absorbance ($Abs_{sample}$ (time 0 min to time 120 min)) was related to the decrease in absorbance of the negative control ($Abs_{control}$ (time 0 min to time 120 min)), obtaining the percentage of lipid peroxidation inhibition (% I) by the formula: % I = ($Abs_{control}$ – $Abs_{sample}$) x 100/ $Abs_{control}$. The oxidation curve between the negative control and CCE/rutin was evaluated to find the values of F1 (blocking capacity of peroxide formation) between 15 and 45 min after the beginning of the reaction and F2 (capacity of inhibiting other reactions during the oxidative process, which produces radical species) between 75 and 90 min after the beginning of the reaction by the following formulas: F1 = tg $Abs_{sample}$ / tg $Abs_{control}$; F2 = tg $Abs_{sample}$ / tg $Abs_{control}$ (Duarte-Almeida et al. 2006). Where: $Abs_{control}$: absorbance measured for negative control; $Abs_{sample}$: absorbance

measured for the sample; %I: percentage of lipoperoxidation inhibition; tg: tangent. The experiment was performed in triplicate.

### *Malondialdehyde quantification*

The lipid peroxidation assay determines the presence of malonaldehyde (MDA), from a colored complex which is measured by spectrophotometric method, after its extraction in acid-aqueous extract (Osawa et al. 2005). The concentration of thiobarbituric acid (TBA) - Malondialdehyde (MDA) complex was established from the MDA standard curve using butanol as a blank. Rutin was used as positive control. The preparation of ground beef homogenates containing CCE and rutin in their different concentrations (10, 20 and 40 µg/mL) were performed. Homogenates were kept in amber flasks and refrigerated at 5°C for the 5 days of the assay. 0.5 g of homogenate, 50 µL of BHT 4% in ethanol, 2.5 mL of 1% phosphoric acid and 1.25 mL of TBA 1% were added using test tubes. The solutions were heated at 95°C for 15 min in a water bath and after that process they were cooled in ice bath for 10 min. After cooling, 3 mL of butanol was added to the tubes and centrifuged at 4000 rpm for 5 min. Readings of the supernatant were taken at 535 nm on days 0, 2 and 4. Samples vehicle was used as negative control (100% oxidation). The experiment was performed in triplicate.

## Evaluation of anti-inflammatory activity

### *Cell line*

J774A.1 cells (murine macrophages) were grown in culture bottles with Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 2mM L-glutamine, 100 µg/mL antibiotic (streptomycin and penicillin), 5% fetal bovine serum (FBS) and kept in an incubator at 5% $CO_2$ at 37°C until the day of the test.

### *NO dosage*

Macrophage cells of the J774A.1 cells were transferred to 96-well microplates ($2x10^4$ cell $well^{-1}$) and treated with CCE at concentrations ranging from 60 to 3.75 µg/mL. Subsequently, *Escherichia coli* LPS (lipopolysaccharide) at 1 µg/mL and IFN-γ at 0.9 ng/mL were added and the volume made up to 200 µL with RPMI-1640 medium. Negative controls were stimulated with LPS and IFN-γ and cells treated only with 0.06% DMSO (vehicle). The cells were incubated for 48h in a 5% $CO_2$ at 37°C. Subsequently, nitric oxide (NO) production was evaluated by the Griess method, an indirect NO measurement by nitrite dosing, in recovered culture supernatant after 48h stimuli and treatment as described by Sun et al. (2003). Briefly, 50 µL of Griess reagent in phosphoric acid 2.5% (v/v) were added to 50 µL of culture supernatant. The quantity of NO (µM) was determined by comparison with a standard sodium nitrite curve. The spectrophotometry reading was performed at a 540 nm wavelength. The experiment was performed in triplicate.

## Cell viability assessment

### *MTT assay*

J774A.1 cells at $2x10^4$ cell/well were transferred to 96-well microplates, and 24h later, treated with CCE at concentrations of 60 to 3.75 µg/mL. For the negative control, cells treated only with 0.06% DMSO (vehicle) were used. The cells were incubated for 48h in a 5% $CO_2$ and 37ºC. Cytotoxicity was assessed by cell viability by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Mosmann 1983). The assay for viability assessment is based on the metabolic reduction of MTT to

formazan (violet coloration). After 48h of the treatment, MTT solution (500 µg/mL) was added to each well, and the plate was incubated for additional 2.30h at 37°C, 5% $CO_2$. Absorbance (Abs) was read at 570 nm and cell viability (%) of macrophages after treatment obtained by the following formula: % viable cells = [($Abs_{sample}$)/ $Abs_{0.06\%\ DMSO}$] × 100. Where: $Abs_{sample}$: absorbance measured after treatment of cells with the sample; $Abs_{0.06\%\ DMSO}$: absorbance measured after treatment of cells with 0.06% DMSO (vehicle). The experiment was performed in triplicate.

### *Propidium iodide using flow cytometry*

The evaluation of CCE influence on J774A.1 murine macrophages viability was performed by flow cytometry following the methodology of Crowley et al. (2016) with some modifications. $2x10^4$ cells per well were transferred to 96-well plates and incubated for 48h at 37°C and 5% $CO_2$ with CCE (60 and 30 µg/mL). At the end of the incubation time, the supernatants were discarded and 200 µL of flow cytometry staining buffer (FACS) buffer solution (PBS with 1% SFB) was added. The cells were placed on ice and treated with trypsin, stained with propidium iodide (10 µg/ mL) 15 min before reading and collected into FACS (CORNING) tubes. Samples reading were performed in a FACS canto II flow cytometer, where 3000 events were acquired. Analyses were performed using FlowJo® software where the percentage of viable cells (without propidium iodide staining) was determined. Cells treated with 0.06% DMSO were used as negative control. The experiment was performed in triplicate.

### Chromatographic conditions

CCE was analyzed by ultra-fast liquid chromatography coupled with mass spectrometry (*UFLC-QTOF-MS*) in positive $[M + H]^+$ mode using a Shimadzu *UFLC* (Nexera model) and a Bruker mass spectrometer (*QTOF* Compact model) with electrospray ionization source. The mobile phase used was acidified water with formic acid, with pH = 3 (phase A) and methanol (phase B), the injection flow was 0.4 mL $min^{-1}$ and the running time was 12 min. The column used was the Kinetex 2.6 µm - C18 - 100A, length 100 mm X 3.0 mm. The chromatographic running began with 40% of phase B in 0.01 min time, reaching up to 70% of B in 8.20 min time and 95% of B from 8.20 to 9.70 min, subsequently the mobile phase returned to 40% B in the time of 10.20 min, following up to 11.50 min, the running ended in 12 min. The ionization conditions were set as follows: ion source electrospray voltage of 40 V, a capillary voltage of 4500 V, and a capillary temperature of 220 °C. The full scan mass acquisition was performed by scanning from 100 up to 1000 m/z range. The experiment was performed in duplicate.

### Statistical analysis

Statistical analysis was performed by ANOVA test followed by Bonferroni using the software GraphPrism 5.0. In some cases, the Student's *t* test was applied. Values of $p<0.05$ were considered significant. Results were expressed as mean ± standard deviation.

## RESULTS

### Evaluation of antifungal activity

The antifungal activity of CCE was evaluated by the MIC on different species of *Candida* spp. The results are shown in Table I. At the MIC value, a growth inhibition was observed in both yeasts (fungistatic effect). The MFC was not able to be determined at the concentrations tested.

*C. glabrata* was more susceptible to CCE and, therefore, the mechanism of action for this yeast was investigated. The action assay over plasma membrane showed a significant

**Table I. Minimal Inhibitory Concentration (MIC) of ethanolic extract of *Centrosema coriaceum* leaves (CCE) against *Candida* genus.**

| Microorganisms | CCE MIC[a] | Fluconazole MIC[a] |
|---|---|---|
| *Candida albicans* ATCC® 24433 | >4000 | 31.25 |
| *Candida albicans* ATCC® 10231 | >4000 | >1000 |
| *Candida grablata* ATCC® 2001 | 1000 | 31.25 |
| *Candida krusei* ATCC® 6258 | >4000 | 250 |
| *Candida tropicalis* ATCC® 750 | 4000 | 15.62 |

[a]**Values expressed in µg/mL of sample.**

increase ($p<0.05$) by nucleotide extravasation was performed in absorbance at 260 nm after the cells treatment with CCE and nystatin at MIC values when compared to the growth control (Figure 1). The action on the cell wall and the ability to bind to fungal membrane steroids using sorbitol and exogenous ergosterol were investigated, respectively. The results found in both assays demonstrated an increase in MIC value for CCE (greater than 4000 µg/mL), evidencing its action on *C. glabrata* cell envelope (Table II).

## Antioxidant activity

Antioxidant activity of CCE was determined using DPPH radical scavenging, phosphomolybdenum and β-carotene/linoleic acid system assays and malondialdehyde quantification. The results are shown in Table III and Figure 2. Rutin and quercetin were used as positive controls.

## Anti-inflammatory activity

CCE anti-inflammatory activity was assessed by NO dosage (µM) in the J774A.1 macrophage supernatant stimulated with LPS and with INF-γ (Figure 3). CCE reduced NO production in all tested concentrations ($p<0.05$). It is noteworthy that CCE, at 60 and 30 µg/mL, inhibited 81 and 62% of NO production, respectively.

## Cell viability assessment

Cells treated with the tested CCE concentrations did not reduce J774A.1 cells viability ($p> 0.05$) in MTT assay (Figure 4a). Its low cytotoxicity for the J774A.1 was confirmed by flow cytometry, using propidium iodide (PI) staining, indicating 73 and 84% viable cells at 60 and 30 µg/mL, respectively (Figure 4b).

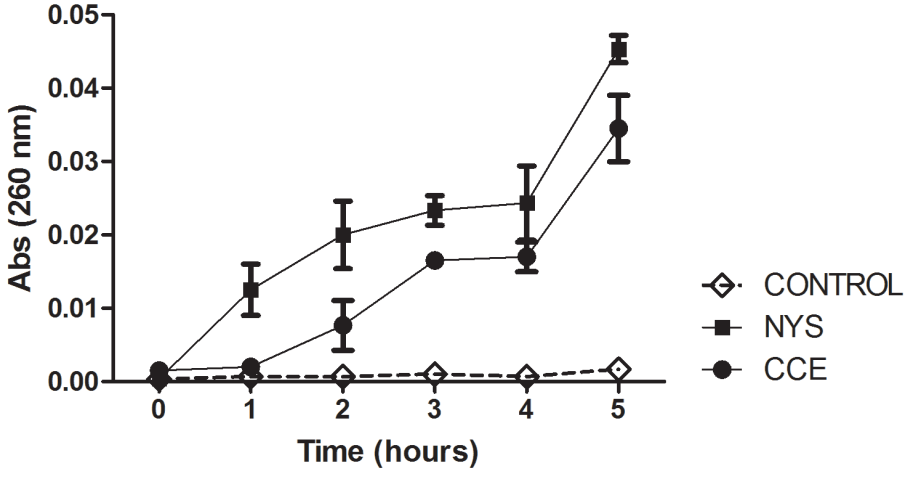


**Figure 1. Nucleotide leakage assay showing an increase in absorbance at 260 nm for a period of 5h, at 1h time intervals. Cells were treated with the ethanolic extract of *Centrosema coriaceum* leaves (CCE) and nystatin (NYS). CONTROL - Negative control (Culture without antimicrobial compounds). Results expressed as mean ± standard deviation of three independent experiments (ANOVA followed by Bonferroni, $p<0.05$).**

**Table II.** Minimal Inhibitory Concentration (MIC) of ethanolic extract of *Centrosema coriaceum* leaves (CCE) against *Candida glabrata* ATCC® 2001 during the sorbitol protection and ergosterol binding assay.

| Samples | MIC without treatment[a] | MIC with sorbitol[a] | MIC with ergosterol[a] |
|---|---|---|---|
| CCE | 1000 | >4000 | >4000 |
| Fluconazole | 31.25 | 31.25 | - |
| Nystatin | 0.78 | - | 3.12 |

[a]Values expressed in µg/mL of sample.

**Table III.** Antioxidant activity of ethanolic extract of *Centrosema coriaceum* leaves (CCE) by DPPH, total antioxidant capacity by phosphomolybdenum (TAC) and inhibition of the β-carotene/linoleic acid system assays.

| Samples | DPPH $IC_{50}$ (µg/mL) | TAC (%RAA) | β-Carotene / linoleic acid system | | | |
|---|---|---|---|---|---|---|
| | | Quercetin | Rutin | Inhibition (%) | F1 | F2 |
| CCE | 1.25 ± 0.06 [a] | 79.9 ± 2.7 | 128.3 ±4.3 | 39.2 ± 3.8 | 0.42± 0.094 | 1.14 ± 0.36 |
| Rutin | 0.037 ± 0.01[a] | - | - | 25.2 ± 0.9 | 0.59 ± 0.01 | 1.63 ± 0.32 |

DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl. $IC_{50}$, inhibitory concentration of 50%. RAA, relative antioxidant activity. F1, phase of initiation of lipoperoxidation. F2, phase of propagation of lipoperoxidation. [a]Statistical difference between CCE and rutin. Results expressed as mean ± standard deviation of three independent experiments. Statistical analysis performed with Student's *t* test.

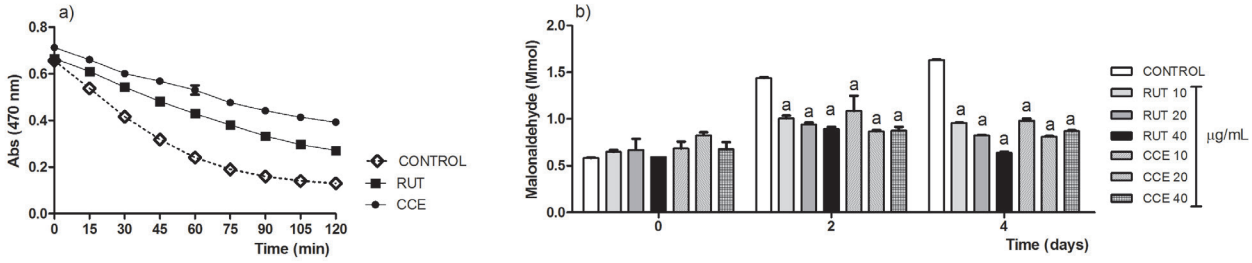


**Figure 2.** Antioxidant activity of the ethanolic extract of *Centrosema coriaceum* leaves (CCE). a) Oxidation curve of β-carotene absorbance evaluated at 470 nm, demonstrating the preservation of its coloration during the 120 min of reaction when using CCE and rutin. b) Inhibition of malondialdehyde production after treatment with CCE and rutin. [a]Statistical difference considering the negative control. Results expressed as mean ± standard deviation of three independent experiments. CONTROL - Negative control. RUT - rutin.

## Chemical profile of CCE by *UFLC-QTOF-MS*

According to the chromatographic profile performed by *UFLC-QTOF-MS*, 4 substances (Figure 5) were identified comparing with UV spectra and mass fragmentation profiles already described in literature (Farias & Mendez 2014, Santos et al. 2013, Stobiecki 2000, Salman et al. 2014, Zhu & Cole 2001).

Compound 1 was identified as sucrose and showed retention time at 1.2 min, UV with $\lambda_{max}$ at 195 nm and mass fragments at *m/z* 365.1051 $[M+Na]^+$, *m/z* 203.0525 $[M+Na-162]^+$ and *m/z* 138.0551 [*m/z* 203- 65]$^+$ by ESI+ .

Compound 2 was identified as rutin and showed retention time of 3.8 min and UV with $\lambda_{max}$ at 204, 256 and 355 nm, mass fragments in

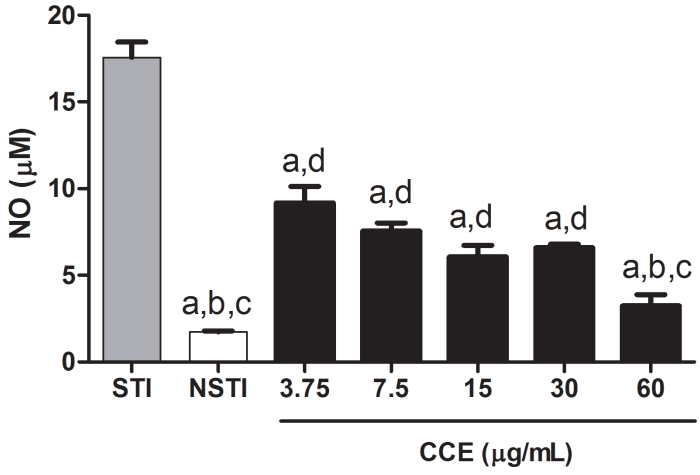


**Figure 3. Anti-inflammatory activity of different concentrations of the ethanolic extract of *Centrosema coriaceum* leaves (CCE) by the method of nitric oxide (NO) reduction. [a]Statistical difference when compared to cells stimulated with INF-γ and LPS and cultured with RPMI + 0.06% DMSO (STI). [b]Statistical difference when compared to cells treated with CCE (30; 15; 7.5 and 3.75 μg/mL). [c]No statistical difference between cells without stimulation (NSTI) and cells treated with CCE (60 μg/mL). [d]No statistical difference between the concentrations of 30; 15; 7.5 and 3.75 μg/mL of CCE. Results expressed as mean ± standard deviation of two independent experiments. (ANOVA followed by Bonferroni, p<0.05).**

*m/z* 611.1592 $[M+H]^+$, *m/z* 465.1013 $[M+H-146]^+$ and *m/z* 303.0491 $[M+H-308]^+$ by ESI+.

Compound 3 was identified as kaempferol-3*O*-rutinoside and presented retention time at 4.7 min, UV with $\lambda_{max}$ at 195, 265 and 348 nm and mass fragments in *m/z* 595.1643 $[M+H]^+$, *m/z* 449.1060 $[M+H-146]^+$ and *m/z* 287.0584 $[M+H-308]^+$ by ESI+.

Compound 4 was identified as caffeic acid and presented retention time at 5.3 min, UV with $\lambda_{max}$ at 220 and 325 nm and mass fragments in *m/z* 383.2158 $[2M+Na]^+$, *m/z* 203. 1497 $[M+Na]^+$ and *m/z* 181.1217 $[M+H]^+$ by ESI+.

## DISCUSSION

Brazilian flora is one of the most importants and diverses in the world. Despite plant richness, only 15% of species have been investigated for chemical and pharmacological properties (Wurtzel & Kutchan 2016). In the other hand, the high morbidity and mortality rates caused by fungal diseases and the current antifungal arsenal reduced to high toxicity drugs make it important to discover new therapies that may have fewer adverse effects (Scorzoni et al. 2017). Thus, our research group conducted this work, with *C. coriaceum* ethanolic extract looking for its biological activities and studying its chemical composition, hoping that it could be a new source of bioactive molecules in the treatment of fungal diseases, oxidative stresses and inflammatory process.

According to Simões et al. (2009), extracts from plants with the minimum inhibitory concentration (MIC) ≤ 1000 μg/mL are considered promising antimicrobial agents. Therefore, our present results demonstrate that CCE possesses a promising inhibitory activity for *C. glabrata* (MIC values =1000 μg/mL), with fungistatic effect. This fungi species has shown increased resistance in recent years (Whaley & Rogers 2016). The possible action mechanism of CCE in *C. glabrata* was investigated by cell envelope assays (nucleotide leakage, sorbitol and binding to exogenous ergosterol). Nucleotides are molecules that have a peak in the absorbance of light at the wavelength of 260 nm. So, an increase in absorbance at this wavelength considering the extracellular medium is due to the presence of these molecules (Khan et al. 2013). Since the nucleotides are located in intracellular compartments, their extracellular presence is a sign of cell damage due to membrane rupture (Campos et al. 2018). The result found for CCE nucleotide leakage assay was similar to

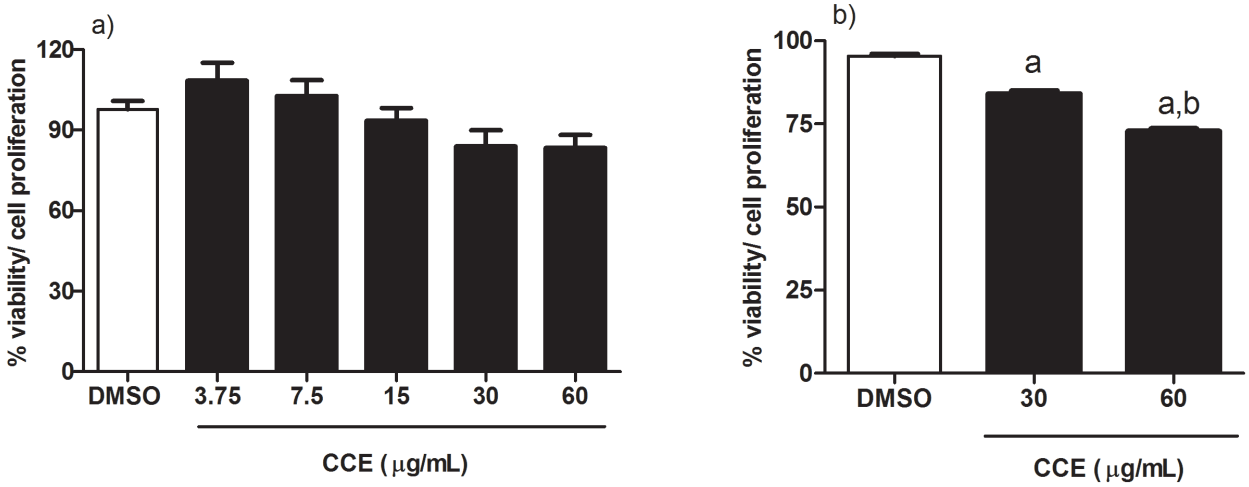


**Figure 4. Evaluation of cell viability in J774A.1 macrophages cell line treated with ethanolic extract of *Centrosema coriaceum* leaves (CCE). a) MTT method. b) Flow cytometry using propidium iodide (PI) as stain in the concentrations of 30 and 60 µg/mL. Cells treated with 0.06% DMSO was used as negative control. [a]Statistical difference when compared to negative control. [b]Statistical difference when compared to CCE (30 µg/mL). Results expressed as mean ± standard deviation of two independent experiments (ANOVA followed by Bonferroni, p<0.05).**

nystatin result (Figure 1). Both led to an increase of nucleotides in the extracellular medium, measured by the increased absorbance. The nystatin mechanism of action is already well known by membrane sterols binding, which leads to a cell membrane permeability alteration and consequent leakage of the cytoplasmic content. This information allows us to infer that CCE acts in the *C. glabrata* membrane.

Deepening studies on this mechanism of action was carried out using the tests with sorbitol and ergosterol. Sorbitol has an osmoprotective function and is essential for fungal growth (Frost et al. 1995). Fungal cells with cell wall damage cannot grow in the absence of sorbitol due to loss of their osmotic regulation. However, their growth is still possible if sorbitol is supplemented to the culture medium. Therefore, fungal cell wall inhibitors can be identified when MIC values obtained in the presence of sorbitol are higher than those in its absence, since the osmolality of the cells is conserved by sorbitol and this permits their growth even in presence of a cell wall inhibitor drug (Frost et al. 1995). The MIC values of CCE for *C. glabrata* increased when the assay was performed in the presence of 0.8M sorbitol in the culture medium (Table II). This result indicates a possible action of the CCE on the fungal cell wall inhibition.

Ergosterol is an anchored lipid in fungal membrane whose absence/lesion causes changes in membrane permeability (Iwaki et al. 2008). If the antifungal agent has ergosterol binding activity, the presence of exogenous ergosterol will prevent binding to membrane ergosterol, increasing the MIC of the substance (Leite et al. 2014). Our test to evaluate this ergosterol action resulted also in an increase in MIC value on the ergosterol presence (Table II), suggesting the ability of constituents present in CCE to bind to fungal ergosterol.

Once antifungal activity was detected and the mode of action was reached, others biological properties were researched. The infection process is always linked with the inflammatory process. So, considering that the production of free radicals, including reactive oxygen species, is crucial inflammation mediators that can cause cell damage (Conner & Grisham 1996), different antioxidant tests were performed.

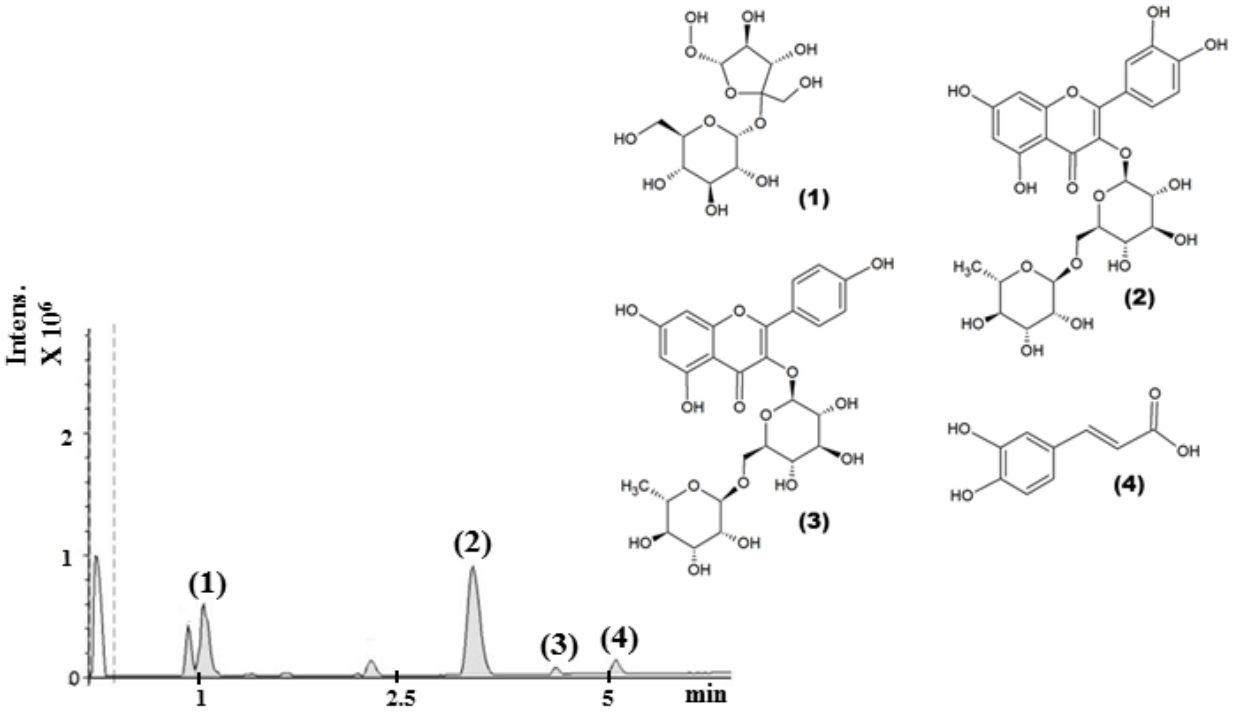


**Figure 5. Chromatogram and chemical structures of the compounds identified in the ethanolic extract of *Centrosema coriaceum* leaves (CCE) analyzed by *UFLC-QTOF-MS*. The assignments for identified compounds (time of retention and MS/MS spectra) are based on comparisons with the literature. (1) Sucrose, (2) Rutin, (3) Kaempferol-3*O*-rutinoside and (4) Caffeic acid. Intens. – Intensity.**

CCE was evaluated by different antioxidant tests and showed promising results in all assays (Table III). The free radicals are atoms or molecules containing one or more unpaired electrons in their valence shell (Phaniendra et al. 2015) which are produced by different biological and environmental sources (Arulselvan et al. 2016). When in presence of a hydrogen donor substance, the DPPH free radical (violet coloration) is reduced (yellow coloration) and its absorption intensity decreases (Duarte-Almeida et al. 2006, Kedare & Singh 2011). In this study, it was observed that CCE shows hopeful findings by inhibiting concentration of 50% DPPH radical, and this activity is related to the presence of phenolic compounds in CCE, such as rutin, used as positive control, a flavonoid widely known for its antioxidant property (Koval'skii et al. 2014). The data obtained by phosphomolybdenum assay corroborate the DPPH assay. The principle to assess the antioxidant capacity by phosphomolybdenum assay includes a green phosphate/molybdenum complex formed due to the reduction of molybdate VI to molybdate V by antioxidant substances (Mendes et al. 2017). CCE presented a relevant activity for this assay, demonstrating that it possesses reducing substances in its composition (Table III).

The presence of free radicals due to pathological conditions increases the rates of lipid peroxidation tissues and leads to the formation of the peroxyl lipid radical (LOO·), which may posteriorly give rise to peroxides (LOOH), a second lipid radical. Peroxides are decomposable and give rise to aldehydes, reactive substances that are also responsible for causing cellular damage (Yadav & Ramana 2013). The results found by the inhibition of

linoleic acid peroxidation are promising, being CCE considered significantly better than the rutin (Figure 2).

The $\beta$-carotene/linoleic acid test still allows us to calculate F1 and F2 values that can evaluate the antioxidant capacity of CCE to act on the blockage of peroxide radicals formed in the propagation stage or to act on other radical species in the terminal stage of lipid peroxidation (peroxide decomposition products) (Duarte-Almeida et al. 2006). From the values of F1 and F2, we can suggest that CCE possesses a greater ability to block the formation of peroxide radicals than other radical species formed in the terminal phase of lipid peroxidation.

The lipoperoxidation process generates, as one of the main aldehydes, malondialdehyde (Ayala et al. 2014). The blockage of malondialdehyde formation was verified by complexing this substance with thiobarbituric acid. As can be observed in Figure 3, CCE was able to block malondialdehyde formation during the oxidation process from the second day of this assay.

Since the free radicals are mediators of the inflammatory process, from the positive results in the tests of antioxidant activity, tests were made to evaluate the anti-inflammatory activity. Regarding the anti-inflammatory activity, a statistical difference was observed between cells treated with CCE at the concentration of 60 µg/mL and cells without stimulation. These results are encouraging since NO is a signaling molecule that plays a key role in the pathogenesis of inflammation (Sharma et al. 2007). Thus, the discovery of new medicinal plants that can be used in anti-inflammatory therapies is of great relevance, since some of the commercially available anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), may increase the risk of gastrointestinal and cardiovascular complications (Sostres et al. 2010).

Ideal pharmacotherapies are those that are effective and have low toxicity to human body cells (Piccolo et al. 2015). Therefore, in order to evaluate the cytotoxicity of CCE, a cell viability assay was performed, and the viable percentage determined according to ISO 10993-5 (2009) (cell viability > 70 %). Thus, it was possible to observe that CCE does not reduce the viability of normal cells for all concentrations tested by MTT and propidium iodide assays.

Considering that CCE presents promising biological activities, it is important to know its chemical constituents. LC-MS is one of the major analytical techniques to determine global metabolite profiles. This tool allows you to analyze and differentiate several compounds from a plant (e.g., alkaloids, glycosides, phenyl propanoids, flavonoids, isoprenes, glucosinolates, terpenes, benzoids) with medium and high polarity and/or higher molecular weight (Kumar et al. 2016). Therefore, we use *UFLC-QTOF-MS* to identify the compounds present in CCE. The results suggested the presence of two flavonoids, rutin and kaempferol-3*O*-rutinoside, one phenolic acid, caffeic acid, and one disaccharide, sucrose. Thereby, rutin and kaempferol-3*O*-rutinoside may be related to the results obtained in this work. Rutin is a flavonoid well known in the literature for its ability to neutralize free radicals and anti-inflammatory activity due to inhibition of nitric oxide synthase enzymes, phospholipase and suppression of inflammatory genes (Koval'skii et al. 2014), besides presenting antifungal properties (Han 2009). Kaempferol-3*O*-rutinoside is another flavonoid that also has antioxidant and antifungal activities already reported (Nascimento et al. 2018). The caffeic acid has also been identified in other species of the Fabaceae (Hanganu et al. 2016) family and is known for its antifungal (Teodoro et al. 2015),

anti-inflammatory and antioxidant properties (Genaro-Mattos et al. 2015).

## CONCLUSION

In summary, this study demonstrates that the ethanolic extract of *Centrosema coriaceum* leaves (CCE) have antifungal activity against *C. glabrata* with high affinity in cell membrane. Moreover, CCE showed promising antioxidant and anti-inflammatory activities, mainly in blocking/reducing free radicals and decreasing of lipid peroxidation. Rutin, kaempferol-3*O*-rutinoside, caffeic acid and sucrose were identified in CCE by *UFLC-QTOF-MS*. This is the first study describing the *in vitro* biological activity and chemical composition of *C. coriaceum* leaves, which demonstrate the therapeutic potential of the species. Finally, studies focusing on the isolation and structure elucidation of the bioactive compounds from *C. coriaceum* are in progress.

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## REFERENCES

AHMAD F, ANWAR F & HIRA S. 2016. Review on medicinal importance of Fabaceae family. Pharmacologyonline 3: 151-156.

ARULSELVAN P, FARD MT, TAN WS, GOTHAI S, FAKURAZI S, NORHAIZAN ME & KUMAR SS. 2016. Role of antioxidants and natural products in inflammation. Oxid Med Cell Longev 2016: 527613.

AYALA A, MUÑOZ MF & ARGÜELLES S. 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev 2014: 360438.

BHATTACHARJEE P. 2016. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Curr Med Mycol 2: 20-27.

BRAND-WILLIAMS W, CUVELIER ME & BERSET C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebens Wiss Technol 28: 25-30.

CAMPOS LM ET AL. 2018. *Mitracarpus frigidus*: A promising antifungal in the treatment of vulvovaginal candidiasis. Ind Crops Prod 123: 731-739.

CHEN H, PU J, LIU D, YU W, SHAO Y, YANG G, XIANG Z & HE N. 2016. Anti-Inflammatory and Antinociceptive Properties of Flavonoids from the Fruits of Black Mulberry (*Morus nigra* L.). PLoS ONE 11(4): e0153080.

CLSI – CLINICAL STANDARD LABORATORY INSTITUTE. 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement M100-S24. CLSI. 34.

CONNER EM & GRISHAM MB. 1996. Inflammation, Free Radicals, and Antioxidants. Nutrition 12: 274-277.

CROWLEY LC, MARFELL BJ, SCOTT AP & WATERHOUSE NJ. 2016. Triggering apoptosis in hematopoietic cells with cytotoxic drugs. Cold Spring Harb Protoc 2016: 635-638.

DUARTE-ALMEIDA JM, SANTOS RJ, GENOVESE MI & LAJOLO FM. 2006. Avaliação da atividade antioxidante utilizando sistema $\beta$-caroteno/ácido linoleico e método de sequestro de radicais DPPH·. Cienc Tecnol Aliment 26: 446-452.

FARIAS LS & MENDEZ ASL. 2014. LC/ESI-MS method applied to characterization of flavonoids glycosides in *B. forficata* subsp. *pruinosa*. Quim Nova 37: 483-486.

FROST DJ, BRANDT KD, CUGIER D & GOLDMAN R. 1995. A whole-cell *Candida albicans* assay for the detection of inhibitors towards fungal cell wall synthesis and assembly. J Antibiot 48: 306-310.

GENARO-MATTOS TC, MAURÍCIO ÂQ, RETTORI D, ALONSO A & HERMES-LIMA M. 2015. Antioxidant activity of caffeic acid against iron-induced free radical generation - A chemical approach. PLoS ONE 10: 1-12.

HAN Y. 2009. Rutin has therapeutic effect on septic arthritis caused by *Candida albicans*. Int Immunopharmacol 9: 207-211.

HANGANU D, OLAH NK, BENEDEC D, MOCAN A, CRISAN G, VLASE L, POPICA I & ONIGA I. 2016. Comparative polyphenolic

content and antioxidant activities of *Genista tinctoria* L. and *Genistella sagittalis* (L.) Gams (Fabaceae). Pak J Pharm Sci 29: 301-307.

ISO 10993-5. 2009. Biological Evaluation of Medical Devices. Part 5: Tests for *In vitro* Cytotoxicity. International Organization for Standardization, 3rd ed, Geneva, Switzerland, 34 p.

IWAKI T, IEFUJI H, HIRAGA Y, HOSOMI A, MORITA T, GIGA-HAMA Y & TAKEGAWA K. 2008. Multiple functions of ergosterol in the fission yeast *Schizosaccharomyces pombe*. Microbiology 154: 830-841.

KEDARE SB & SINGH RP. 2011. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48: 412-422.

KHAN MSA, AHMAD I & CAMEOTRA SS. 2013. Phenyl aldehyde and propanoids exert multiple sites of action towards cell membrane and cell wall targeting ergosterol in *Candida albicans*. AMB Express 3: 1-16.

KHOSRAVI RK, FALAHATI M, ROUDBARY M, FARAHYAR S & NAMI S. 2016. Over expression of MDR-1 and CDR-2 genes in fluconazole resistance of *Candida albicans* isolated from patients with vulvovaginal candidiasis. Curr Med Mycol 2: 24-29.

KOVAL'SKII IV, KRASNYUK II, KRASNYUK JR II, NIKULINA OI, BELYATSKAYA AV, KHARITONOV YY, FELDMAN NB & LUTSENKO SV. 2014. Mechanisms of rutin pharmacological action (review). Pharm Chem J 48: 73-76.

KUMAR PR, DINESH SR & RINI R. 2016. LCMS- A review and a recent update. World J Pharm Pharm Sci 5: 377-391.

LEITE MC, BEZERRA AP, DE SOUSA JP, GUERRA FQS & LIMA EO. 2014. Evaluation of antifungal activity and mechanism of action of citral against *Candida albicans*. Evid Based Complement and Alternat Med 2014: 378280.

MELO MSOM & MANCINI FILHO J. 1989. Antioxidantes naturais do fruto do dendezeiro (*Elaeis guineensis*, Jacq). Rev Farm Bioquim Univ São Paulo 25: 147-157.

MENDES RF, PINTO NCC, SILVA JM, SILVA JB, HERMISDORF RCS, FABRI RL, CHEDIER LM & SCIO E. 2017. The essential oil from the fruits of the Brazilian spice *Xylopia sericea* A. St.-Hil. presents expressive in-vitro antibacterial and antioxidant activity. J Pharma Pharmacol 69: 341-348.

MENDONÇA RC, FELFILI JM, WALTER BMT, SILVA JÚNIOR MC, REZENDE AV, FILGUEIRAS TS & NOGUEIRA PE. 1998. Flora Vascular do Bioma Cerrado. Cerrado, Ambiente e Flora, Embrapa, Brazil, 1 st ed, 2: 289-556.

MOREIRA DL, TEIXEIRA SS, MONTEIRO MH, DE-OLIVEIRA ACAX & PAUMGARTTEN FJR. 2014. Traditional use and safety of herbal medicines. Rev Bras Farmacogn 24(2): 248-257.

MOSMANN T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

NASCIMENTO JET ET AL. 2018. Chemical Composition and Antifungal *In Vitro* and *In Silico*, Antioxidant, and Anticholinesterase Activities of Extracts and Constituents of *Ouratea fieldingiana* (DC.) Baill. Evid Based Complement Alternat Med 2018: 1748487.

NEGRI MLS, POSSAMAI JC & NAKASHIMA T. 2009. Atividade antioxidante das folhas de espinheira-santa- *Maytenus ilicifolia*. ex Reiss., secas em diferentes temperaturas. Braz J Pharmacogn 19: 553-556.

OSAWA CC, FELÍCIO PE & GONÇALVES LAG. 2005. Teste de TBA aplicado a carnes e derivados: métodos tradicionais, modificados e alternativos. Quim Nova 28: 655-663.

PARENTE-ROCHA JA, BAILÃO AM, AMARAL AC, TABORDA CP, PACCEZ JD, BORGES CL & PEREIRA M. 2017. Antifungal resistance, metabolic routes as drug targets, and new antifungal agents: An overview about endemic dimorphic fungi. Mediators Inflamm 2017: 9870679.

PERLIN DS, RICHARDSON RR & IZQUIERDO AA. 2017. The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect Dis 17: 383-392.

PHANIENDRA A, JESTADI DB & PERIYASAMY L. 2015. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem 30: 11-26.

PICCOLO MT, MENALE C & CRISPI S. 2015. Combined anticancer therapies: an overview of the latest applications. Anti-Cancer Agents Med Chem 15: 408-422.

PRIETO P, PINEDA M & AGUILAR M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269: 337-341.

RAHMAN AHMM & PARVIN MIA. 2014. Study of Medicinal Uses on Fabaceae Family at Rajshahi, Bangladesh. Res Plant Sc 2: 6-8.

ROSELLETTI E, PERITO S, GABRIELLI E, MENCACCI A, PERICOLINI E, SABBATINI S, CASSONE A & VECCHIARELLI A. 2017. NLRP3 inflammasome is a key player in human vulvovaginal disease caused by *Candida albicans*. Sci Rep 7: 1-10.

SALMAN M, ABDEL-HAMEED ESS, BAZAID SA, AL-SHAMRANI MG & MOHAMED HF. 2014. Liquid chromatography-mass spectrometry (LC-MS) method for the determination of sugars in fresh pomegranate fruit juices. Der Pharma Chemica 6: 320-333.

SANGLARD D. 2016. Emerging Threats in Antifungal Resistant Fungal Pathogens. Front Med (Lausanne) 3: 1-10.

SANTOS JLA ET AL. 2013. Evaluation of Chemical Constituents and Antioxidant Activity of Coconut Water (*Cocus nucifera* L.) and Caffeic Acid in Cell Culture. An Acad Bras Cienc 85: 1235-1246.

SCORZONI L, SILVA ACAP, MARCOS CM, ASSATO PA, MELO WCMA, OLIVEIRA HC, COSTA-ORLANDI CB, MENDES-GIANNINI MJS & FUSCO-ALMEIDA AM. 2017. Antifungal Therapy: New Advances in the Understanding and Treatment of Mycosis. Front Microbiol 8: 1-23.

SHARMA JN, AL-OMRAN A & PARVATHY ASS. 2007. Role of nitric oxide in inflammatory diseases. Inflammopharmacology 15: 252-259.

SIMÕES M, BENNETT R & ROSA E. 2009. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. Nat Prod Rep 26: 746-757.

SOFOWORA A, OGUNBODEDE E & ONAYADE A. 2013. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med 10(5): 210-229.

SOSTRES C, GARGALLO CJ, ARROYO MT & LANAS A. 2010. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol 24: 121-132.

SOUZA ACO & AMARAL AC. 2017. Antifungal Therapy for Systemic Mycosis and the Nanobiotechnology Era: Improving Efficacy, Biodistribution and Toxicity. Front Microbiol 8: 1-13.

SPENCER ALR & SPENCER JFT. 2004. Public health microbiology: Methods and Protocols. 1st ed, New Jersey, Humana Press Inc, 568 p.

STOBIECKI M. 2000. Application of mass spectrometry for identification and structural studies of flavonoid glycosides. Phytochemistry 54(3): 237-256.

SUN J, ZHANG X, BRODERICK M & FEIN H. 2003. Measurement of nitric oxide production in biological systems by using Griess reaction assay. Sensors 3: 276-284.

TANG YL, SHI YH, ZHAO W, HAO G & LE GW. 2008. Insertion mode of a novel anionic antimicrobial peptide MDpep5 (Val-Glu-Ser-Trp-Val) from Chinese traditional edible larvae of housefly and its effect on surface potential of bacterial membrane. J Pharm Biomed Anal 48: 1187-1194.

TEODORO GR, ELLEPOLA K, SENEVIRATNE CJ & KOGA-ITO CY. 2015. Potential Use of Phenolic Acids as Anti-Candida Agents: A Review. Front Microbiol 6: 1-11.

WHALEY SG & ROGERS PD. 2016. Azole Resistance in *Candida glabrata*. Curr Infect Dis Rep 18: 1-10.

WURTZEL ET & KUTCHAN TM. 2016. Plant metabolism, the diverse chemistry set of the future. Science 353: 1232-1236.

YADAV UCS & RAMANA KV. 2013. Regulation of NF-*K*B-Induced Inflammatory Signaling by Lipid Peroxidation-Derived Aldehydes. Oxid Med Cell Longev 2013: 690545.

ZHU J & COLE RB. 2001. Ranking of gas-phase acidities and chloride affinities of monosaccharides and linkage specificity in collision-induced decompositions of negative ion electrospray generated chloride adducts of oligosaccharides. J Am Soc Mass Spectrom 12: 1193-1204.

ZOCOLER AMD, SANCHES ACC, ALBRECHT I & MELLO JCP. 2009. Antioxidant capacity of extracts and isolated compounds from *Stryphnodendron obovatum* Benth. Braz J Pharm Sci 45: 551-562.

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R.L.F. and A.P.F. provided the study design, study mentorship and critical editing of the manuscript. A.S.O.L., L.M.C. and J.T.G. contributed to the study design. A.S.O.L., L.M.C., T.F.S. and E.E.O. performed experiments and acquired and analyzed the data. A.C.M.A. and D.M.O.A. performed cultures. All authors contributed in part to writing and editing the manuscript and approved the final version.