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*Full Length Research Paper*

# Evaluation of biochemical and microbiological effects of *Cordyline dracaenoides* Kunth (Uvarana) barks

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***Cordyline dracaenoides*, known as Uvarana, is a tree that is found and spread throughout the region of "Campos Gerais" in the State of Parana, Brazil. In this work, the immunomodulatory and citotoxicity of crude extract and microbiological activity of hexanic, chloroformic, ethyl acetate and methanolic extracts were investigated. Furthermore, the phytochemical screening of the partionated extracts was analyzed and the extracts revealed the possible presence of terpenes, sterols and polyphenolic compounds (PC). It was confirmed by the measurement from Folin-Ciocalteau reagent, and it corresponds to 0.99 µg of catechin equivalent/mg of the extract and HPLC analysis. Microbiological assays showed that the extracts were active against *Staphylococcus aureus* and *Escherichia coli*, and it was determined that in chlorophormic, ethyl acetate and methanolic extracts, there are some compounds that are responsible for this activity. The immunomodulatory and citotoxicity effects were determined against the models evaluated, showing a dose-dependent effect for the crude extract.**

**Key words:** *Cordilyne dracaenoides*, immunomodulatory activity, microbiological assay, citotoxicity.

## INTRODUCTION

Considered as a divine manifestation since long ago, the use of medicinal plants for men is as antique as civilization (Rates, 2001). However the pharmacological potential of medicinal plants as a medicine source is, still today, little explored. Studies show that there are approximately 250.000 to 500.000 species of plants in the world, but only a small part of these had its phytochemical and pharmacological profile evaluated (Hamburger and Hostettmann, 1991).

In Brazil, the use of medicinal plants is spread out mainly in the rural areas for treatment of several diseases (Napolitano et al., 2005; Faria et al., 2010); it is becoming important to study these therapeutical information that have been accumulated for centuries. Technological development has made it possible for medicinal properties to be indicated by the popular knowledge that are tested and proven scientifically.

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Many of these plants used in the traditional medicine had demonstrated properties on the modulation of immunological answers (Agarwal and Singh, 1999), they had presented cytotoxic properties (Mans et al., 2000) and some type of microbiological activities (Martinez et al., 1996; Navarro et al., 1996; Mahasneh et al., 1999; Wang et al., 2011). Considering the microbiological activity, there is a great interest in the pharmaceutical area, especially in combat of some diseases caused by bacteria/yeasts and as a source of new active substances to treat microorganisms that created resistance against some drugs used routinely in clinic. Further, it is interesting to add the fact that 30% of patients interned in hospitals need some kind of medicine with microbiological action and this study which tries to discover new molecules with microbial activity becomes pertinent and opportune.

In spite of substances with immunomodulatory or antitumoral properties, although there is an extraordinary advancement in research to discover new medicines, several problems related to these diseases do not have an adequate treatment. Therefore, the search for a new

pharmacological alternative (Agarwal and Singh, 1999; Mans et al., 2000) aiming to increase the cure of those problems becomes necessary. As already affirmed, plants produced a large amount of natural substances that are responsible for protecting them against the aggressions of environment. These substances have, several times, potential antimicrobial and immunomodulatory substances.

Polyphenolic compounds present in these plants are found in great amounts in human diet. These compounds have biological activities (Ielpo et al., 2000) and for these, many scientists argue about the potentiality that plants might create new molecules. For these reasons, the study with the species *Cordyline dracaenoides* Kunt (Agavaceae) to evaluate possible pharmacological properties is desired.

*Cordilyne dracaenoides*, known as "Uvarana" is a tree frequently found and spread throughout the region of "Campos Gerais", Parana State, Brazil. It is used as an anti-inflammatory and in rheumatism treatment and associated diseases (Calixto et al., 1990; Falcão et al., 2005).

Calixto et al. (1990) studied hydroalcoholic extracts of *C. dracaenoides* obtained from roots and barks. It was observed that it had a powerful anti-inflammatory and a central depressor effects. These researchers, through chemical analysis, verified the presence of steroids**,** anthocyanins, saponins and small amounts of tannins in bark extracts.

Despite these studies, other works with this vegetal species are not found in literature. Thus, the aim of this study was to realize a phytochemical screening of Uvarana extract and to determine (1) cytotoxic effect on murine macrophages cell line (J774A.1) and human acute lymphocytic leukaemia cell line (Jurkat); (2) immunomodulatory activity on nitric oxide (NO) production on J774A.1 cells and (3) antimicrobiological proprieties against Gram-positive and Gram-negative bacteria's, and yeast.

## MATERIALS AND METHODS

### Plant and extract preparation

Whole *C. dracaenoides* Kunt (Agavaceae) plants were collected in Imbaú, Paraná, Brazil, in October 2008. A voucher specimen (register number 15393) was deposited at the herbarium of the State University of Ponta Grossa, Parana, Brazil. The barks were cleaned, dried under ambient temperature and powdered. The material was macerated with ethanol/water (70:30, v:v) for 7 days. After solvent evaporation, one part was lyophilized (crude extract), and the other part of the extract (7.45 g) was fractionated using an increasing gradient system (identified as hexan (FH), chloroform (FC), ethyl acetate (FAE) and methanol (FM), respectively), getting 0.27, 1.79, 9.48 and 40% of the extraction. After allowing the extracts, they were filtered and solvents evaporated under reduced pressure (40°C). The dried materials were lyophilized and stored in a freezer (0°C) until its use.

### Phytochemical screening of the extracts

Phytochemical screening of the extract was carried out using a conventional protocol (Wagner et al., 1984) for detecting the presence of different constituents like tannins, saponins, unsaturated sterols, triterpenoids, alkaloids and flavonoids. The extracts were monitored by thin layer chromatography (TLC) (20 × 20 cm glass plates, 0.25 mm thick, Si gel GF254, MACHEREY NAGEL®), eluted with different systems: hexane/ethyl acetate (85:15, v:v), chloroform/methanol/n-propanol/water (5:6:1:4, v:v:v:v) and chloroform/methanol (85:15, v:v). Common coloring reagents were used to screen the FH, FC, FAE and FM for phenolic compounds ($FeCl_3$, 2%), sterols (p-anisaldehyde 0.5% in $H_2SO_4$ w/v, followed by heating at 110°C), alkaloids (Dragendorff reagent) and exposed to $NH_3$ steam and UV light at 254 and 366 nm (Silva, 2002).

### Chromatographic instrumentation and chemicals

The higher performance liquid chromatographic-diode array (HPLC–DAD) system consist of two Shimadzu LC-10AD pumps (Kyoto, Japan), a SUS mixer, an auto injector model SIL 10A, a photodiode array model SPD-10AVP, and it was used with a CBM 10A interface. HPLC data acquisition was done on CLASS LC10 software. Methanol (MeOH) was HPLC-grade (J.T.Baker, Philipsburg, PA, USA). Water ($H_2O$) was purified with a Millipore Milli-Q system (Millipore, São Paulo, SP, Brazil) and it was used for all experiments. The analytical column (4.6 mm diameter and 150 mm length) used was packed with phenylhexil silica (Luna®, 10μm particle size and 100 A pore size) by the ascending slurry method, using methanol for the preparation of the slurry (50 ml) and also for the packing. These were carried out at a pressure of 7500 psi (Beltrame et al., 2006).

### *Analytical HPLC analysis*

The analytical separations were carried out using the phenyl hexil column and MeOH (B):$H_2O$ (A) as mobile phase in the following concentration: 5 to 30% in 16 min, 30 to 50% in 4 min, 50 to 100% in 30 min and then 100% of B during 5 min before returning to 5% in a period of 5 min. The flow-rate use was 1.0 ml/min. The crude extract, ethyl acetate and methanol fractions were solubilized in methanol, and the chlorophorm fraction in 2-propanol (20 mg/ml) from which 80 µl were injected in the chromatographic system. The chromatograms were recorded in the range of 190 to 400 nm.

### Measurement of total phenolic compounds (TPC)

The crude extract of Uvarana was used for the determination of TPC that was determined by Folin-Ciocalteu method as described by Sousa et al. (2007). It was performed in triplicate and the absorption of samples was measured at 720 nm against the standard solution of catechin (200 µg/ml).

### Evaluation of antioxidant activity: DPPH radical scavenging assay

Radical scavenging activity of Uvarana extracts against stable 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) (Sigma-Aldrich Co.) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced.

The changes in color (from deep-violet to light-yellow) were measured at 515 nm on a visible light spectrophotometer (Miliauskas et al., 2004). Radical scavenging activity of the extracts was measured as described subsequently. Extract solutions were prepared by dissolving crude extract lyophilized (0.625 to 20 mg) in 1 ml of methanol. In the same way the control was prepared by dissolving quercetin (3 to 96 µg/m) in 1 ml of methanol. The solution of DPPH in methanol (60 µM) was prepared at the moment of use, and 3.9 ml of this solution was mixed with 0.1 ml of each extract solution. The samples were kept in the dark at room temperature and the decrease in absorption was measured after 20 min. Absorption of blank samples containing the same amount of methanol and DPPH solution were also prepared and measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

Inhibition (%) = [(AB – AA)/AB] × 100.

where AB is the absorption of 100% of blank sample (t = 0 min) and AA is the absorption of tested extract solution (t = 20 min).

## Pharmacological properties

### *Microbiological assays*

Four microbial species taken from international collections were analyzed: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27583), *Staphylococcus aureus* (ATCC 29213) and *Candida albicans* (ATCC 10231). Microorganism suspensions were prepared in a saline solution (NaCl 0.85%, w/v) and were standardized as a 0.5 McFarland scale.

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

Different extracts of *C. dracaenoides* Kunt were dissolved in the same solvent as they are prepared (hexan, $CHCl_3$, ethyl acetate and MeOH) to a final concentration of 0.5 mg/ml. Then, they were sterilized by filtration through 0.45 µm Millipore® filters. Antimicrobial tests were carried out by the disc diffusion method (Barry and Thornsberry, 1991). Microorganism cultures were grown in tryptose liquid medium at 35°C. After 6 h of growth, each microorganism culture was inoculated on the surface of Mueller-Hinton agar plates (100 µl). Subsequently, filter paper discs (6 mm in diameter) saturated with extracts (first concentration used was 10 mg (20 µl) and later dilutions 1/2 had been prepared (5 mg), 1/4 (2.5 mg), 1/8 (1.25 mg), 1/16 (625 µg) and 1/32 (312.5 µg) of each one of the extracts) were placed on the surface of each inoculated plate. The plates were incubated at 35°C for 18 h for bacteria and for 48 h for *C. albicans*. After this, it was determined that the diameter of the inhibition zones formed around the filters and the area of inhibition was calculated. Overall, cultured microorganisms with zones equal to or greater than 7 mm were considered susceptible to the tested extract. Negative control was the own solvent used and the positive control was ciprofloxacin (5 µg/disc-NEWPROV®) for bacteria and ketoconazole (40 µg/disc) for *C. albicans*. All determinations were made in duplicate.

### *Bioautography*

Trying to find components with antimicrobial potential was carried out in the assay of bioautography (Hamburger and Cordell, 1987; Rahalison et al., 1991). Extracts with activity in the test of MIC were dissolved in its respective solvent (500 mg/ml) and applied in chromatoplates 60 × 60 mm of Si gel GF254, eluted and dried to complete solvent removal. Chromatoplates were placed in Petri's plates (10 × 100 mm, diameter) and both containing 14 ml culture, and were added 6 ml more of it (that was inoculated with *S. aureus* in concentration of 1%). After both had solidified, the Petri's plates were incubated at 35°C for 24 h. After this period, the plates were nebulizated with a solution of 2,3,5, trifenil tetrazolic chloride (TTC, 20 mg/ml) (Hamburger and Cordell, 1987) and submitted for incubation for 1 more hour at 35°C. White areas where the colored formazan did not take place indicate the presence of compounds that inhibited the growth of tested microorganisms.

### *Cytotoxicity assay*

The murine macrophage cell line (J774A.1) and human acute lymphocytic leukaemia cell line characterized as T cell line (Jurkat) were maintained in RPMI-1640 medium (CULTILAB®) containing 10% fetal calf serum (CULTILAB®). This medium was supplemented with streptomycin (l0 g/ml), penicillin (10000 UI/ml) and sodium bicarbonate (24 mM). Cells were grown in 75 $cm^2$ flasks containing 0.5 to 1 × $10^6$ cells/ml. The cells were kept in a humidified atmosphere containing 5% $CO_2$ at 37°C.

### *Cell viability*

The cytotoxic effect of Uvarana extracts expressed as cell viability, was assessed using two methods: Trypan Blue exclusion staining assay used to evaluate the cellular membrane integrity and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction assay, which is a test of metabolic competence (Mosmann, 1983). Briefly, cells were harvested and added to 24-well plates at a concentration of 2 × $10^5$ cells/m in the RPMI medium. After 24 h incubation at 37°C in a 5% $CO_2$ atmosphere, the Uvarana crude extract in various concentrations (12.5 to 800 µg/ml) were added, and the cells were treated by different periods (24, 48 and 72 h). After these periods, the viable cells (0.05 ml suspension cells) were counted by Trypan Blue exclusion staining assay (1% Trypan blue in phosphate-buffered saline (PBS)). Regression analysis was used to calculate the 50% effective concentration (EC50). To evaluate the cell viability by MTT assay, the medium in each well was replaced by a fresh medium (300 µl) containing 0.5 mg/ml of MTT. One hour later, the formazan product of MTT reduction was dissolved in DMSO, and absorbance was measured using a multi-plate reader. The effect of extracts was determined by the percentage of reduced dye in the control samples at 550 nm.

### *Morphological analysis*

Jurkat cells and J774A.1 cells at the concentration of 0.5 × $10^5$ cells/ml were incubated with RPMI 1640 (10% fetal calf serum) for 48 h in the presence of different doses of Uvarana crude extract. Cell morphology was examined by inverted microscopy (QUIMIS) for apoptotic cells.

### *NO production by murine macrophages (J774A.1)*

Macrophages cultured in 75 $cm^2$ flasks in RPMI 1640 medium were harvested and added to 96-well microtiter plates (100 µl/well) at a concentration of 1 × $10^5$ cells/well in the RPMI medium. After 6 h incubation at 37°C in a 5% $CO_2$ atmosphere, the Uvarana crude extract in various concentrations (5 to 50 µg/ml) was added. Cells

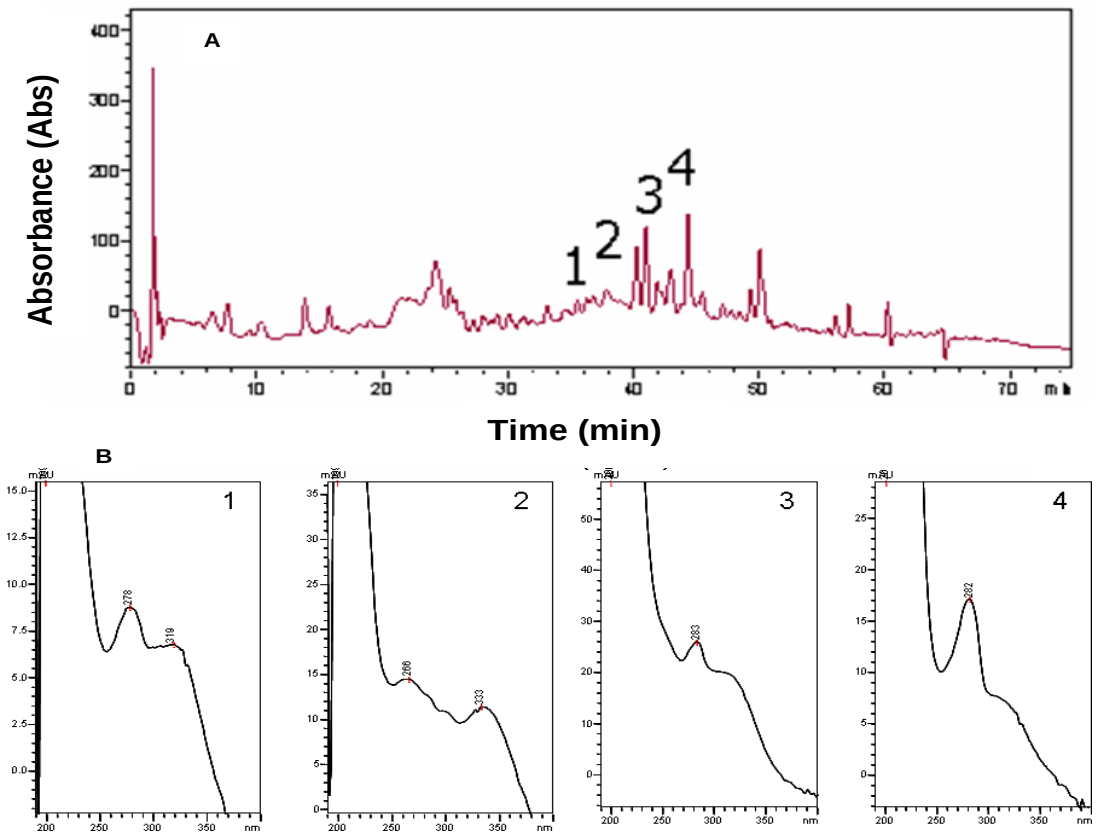


**Figure 1.** Gradient elution of the crude extraction. Mobile phase: Methanol (B) in water (A) (5 to 30% in 16 min, 30 to 50% in 4 min, 50 to 100% in 30 min and then 100% of B during 5 min. before return to 5% in a period of 5 min. The flow-rate use was 1.0 ml/min). UV spectrum of flavonoids compounds: (1) $r_t$: 35.6 min, (2) $r_t$: 36.7 min, (3) $r_t$: 41.0 min and (4) $r_t$: 44.5 min.

were stimulated to express inducible nitric oxide synthetase (iNOS) by the addition of 2.5 µg/ml of *E. coli* lipopolysaccharide (LPS) which was purchased from Sigma Chemical Laboratories. After 48 h incubation, supernatants from the treated cells were harvested and the production of NO was estimated from the accumulation of nitrite ($NO_2$). $NO_2$ was determined by Griess reaction (Ding et al., 1988) against standard curve solutions of sodium nitrite and the absorbance was evaluated at 550 nm.

#### Statistical analysis

The results are expressed as mean ± S.E.M. Comparisons between groups were performed by analysis of variance (ANOVA). Significant differences ($P < 0.05$) were found by using the Tukey-Kramer´s method (INStat software-GraphPad Software, Inc., San Diego, CA, USA) for comparison with control and are indicated in the study's figures and tables.

## RESULTS

### Phytochemical screening and HPLC analysis

Phytochemical screening of C. *dracaenoides* Kunt extracts, using common coloring reagents revealed possible presence of terpenes and sterols in the hexan fraction chloroformic, ethyl acetate and methanolic fractions showed polyphenolic compounds when revealed with anisaldehyde and confirmed by ferric chloride. None of the fractions analyzed with Dragendorff reagent indicated the alkaloids presence. The UV spectra of the chromategraphic bands of the crude extract obtained from HPLC-DAD analysis indicated the presence of polyphenolic compounds (Figure 1).

The UV spectra with maxima between 266 and 283 nm (band II/ring A) and another between 315 and 333 nm (band I/ring B) confirm the aromatic character of the compounds, correlated with the benzopirone skeleton indicating characteristics of flavonoids to the compounds. These chromatographic bands observed in the crude extraction were also present in the chloroformic, ethyl acetate and methanolic fractions. The fingerprintings of these fractions demonstrated the similarity among them, differing only in the amount of these compounds (Figure 2).

### Total phenolic compounds measurements

The content of phenolic compounds in Uvarana crude

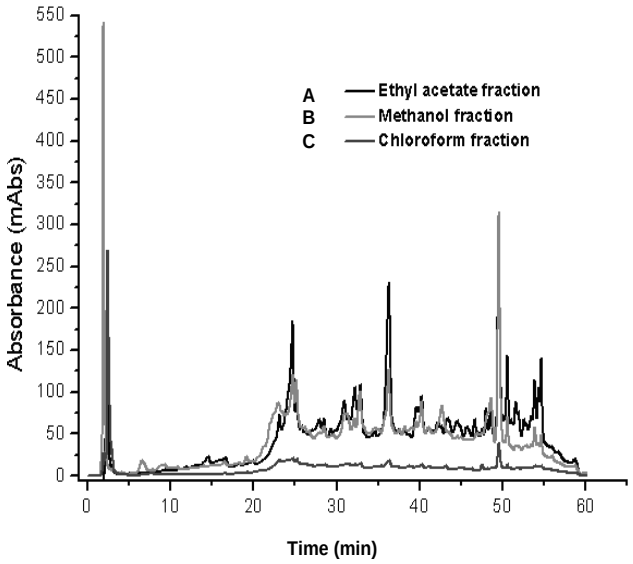


**Figure 2.** HPLC fingerprinting of the fractions: A, Ethyl acetate; B, methanol; C, chlorophorm.

extract determined from Folin-Ciocalteau reagent was 426.7 µg/ml (0.99 µg of catechin equivalent/mg of extract).

## DPPH inhibition determination

The results of DPPH inhibition (antioxidant activity) by Uvarana crude extract are summarized in Table 1. The correlation between consume DPPH (µmol/L) and concentration (µg/ml) of control or Uvarana crude extract was observed. DPPH reacts with suitable reducing agents (control and extract) and this reduction was estimated from the bleaching of radical solution (decay of absorbance at 515 nm). The control and Uvarana crude extract were effective DPPH radical scavengers. The result provided demonstrates that the activity of radical scavenger of Uvarana crude extract was in a dose-dependent manner. Thus, the EC50 values of control and crude extract of Uvarana were determined using the regression equation. As for the quercetin (control), the equation found was $y = 1.34x + 12.13$, $R^2 = 0.90$ and the EC50 corresponds to 28.5 µg/ml and to Uvarana crude extract, the equation found was: $y = 0.0056x + 17.687$, $R^2 = 0.94$ and the EC50 corresponds to 5.77 mg/ml.

## Minimum inhibitory concentration (MIC)

The hexanic, chloroform, ethyl acetate and methanol extracts were tested against four (4) different microorganisms. The tested extracts did not show any activity against *C. albicans* and *P. aeruginosa* verifying its resistance or insensibility of these microorganisms. In the assays by the agar diffusion method, the chloroformic, ethyl acetate and methanolic extracts demonstrated antimicrobial activity against the microorganisms tested, the mean zones of inhibition obtained were between 7 to 20 mm. The higher activity was observed against *S. aureus* for these three extracts tested with inhibition zones (20 mm) in the concentrations of 5 mg. For *E. coli,* zones of inhibition were obtained too (10 mm).

## Bioautography

The assay was carried out with the fractions chloroform, ethyl acetate and methanol of *C. dracaenoides* against *S. aureus*, which showed greater sensitivity to these fractions in MIC. Antibacterial action of fractions determined by bioautography presented inhibition zones. The chloroformic fraction showed inhibition zones in origin and with $R_f$ 0.14; the components of fraction ethyl acetate showed zones in origin, with $R_f$ 0.54 and 0.7 and components of methanolic fraction showed zones of inhibition in origin and with $R_f$ 0.3 and 0.6.

## Morphological changes: Optic light microscopy analysis

Under optic light microscope, both untreated cells present a typical round-shaped morphology, flat cell bodies with uniform chromatin in the nuclei (Figure 3A and D). In contrast, apoptosis was associated with cell shrinkage, nuclear condensation, fragmentation or chromatin loss (Figure 3B, C, E and F). In special cells treated with 800 µg/ml of Uvarana crude extract showed the characteristic morphological changes of apoptosis, including nuclear condensation (Figure 3C and F).

## Cytotoxicity assessments

The effect of Uvarana crude extract on Jurkat and J774A.1 viability cells were assessed by cellular membrane integrity assay (Trypan Blue exclusion), and MTT reduction assay was presented in Table 2.

## NO production in activated murine macrophage (J774A.1) determination

J774A.1 line was incubated with different doses of Uvarana crude extract (5 to 50 µM presence of LPS for 48 h) and supernatants tested for NO production as described in materials and methods. In the absence of

**Table 1.** DPPH inhibition of Quercetin (control) and Uvarana crude extract.

| | DPPH inhibition (%) | | | | | |
|---|---|---|---|---|---|---|
| **Control (µg/ml)** | **3** | **6** | **12** | **24** | **48** | **96** |
| | 10.21 ± 0.52 | 13.35 ± 1.05 | 17.06 ± 0.31 | 25.77 ± 1.20 | 38.40 ± 1.48 | 70.74 ± 2.94 |
| **Uvarana crude extract (µg/ml)** | **625** | **1250** | **2500** | **5000** | **10000** | **20000** |
| | 17.13 ± 5.63 | 22.13 ± 0.61 | 34.12 ± 1.03 | 54.39 ± 0.33 | 69.59 ± 0.54 | 82.58 ± 0.07 |

The values are presented as means ± S.E.M. of 3 determinations.

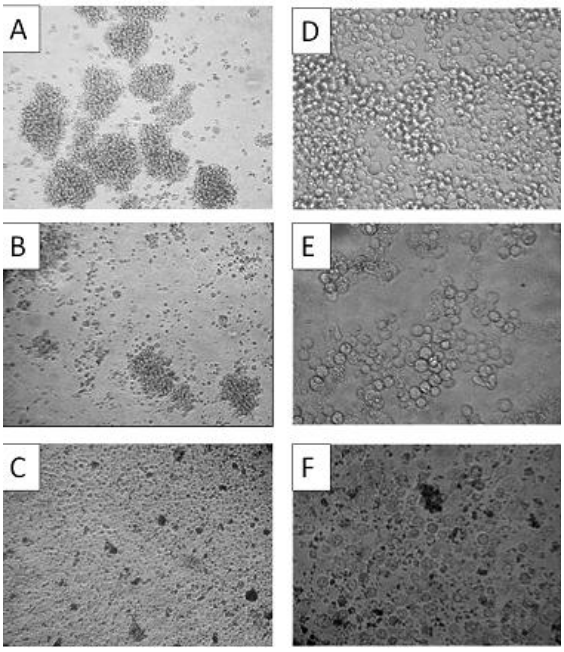


**Figure 3.** Optic light microscopic analysis of cell death. Jurkat cells (A, B and C with a 100× magnification) and J774A.1 cells (D, E and F with a 400× magnification) at the concentration $0.5 \times 10^5$ cells/ml were incubated with RPMI 1640 (10% fetal calf serum) for 48 h in the presence of different doses of Uvarana crude extract. A and D represent controls cells, B and E represent cells treated with 200 µg/ml of extract, and C and F correspond with cells treated with 800 µg/ml of extract.

LPS, there was no effect in inducing significant production of NO by J774A.1, and the most significant cell stimulation was obtained with 2.5 µg/ml of LPS (data not shown). The Uvarana crude extract significantly ($P< 0.05$) reduced NO production in a dose-dependent manner, starting from the dose of 5 µM (Figure 4). The Uvarana crude extract (5 to 50 µM) reduced the NO production by 10.7, 19.4, 35.5 and 52.6%, respectively.

## DISCUSSION

Brazil is a rich source of medicinal plants and several plant extracts are used against diseases in folk medicine, but only few of these have been scientifically investigated. Natural products derived from plants such as flavonoids, terpenes, alkaloids, etc., have received considerable attention in recent years due to their diverse pharmacological properties (Di Carlo et al., 1999). In concern, this study determined the phytochemical profile and evaluated the antimicrobiological, citotoxicity and immunomodulatory effects of the *C. dracaenoides* extracts.

The results showed that chemical characterization of the *C. dracaenoides* extracts presents terpenes and sterols in the hexan fraction and polyphenolic compounds in the others extracts (chloroformic, ethyl acetate and methanolic fractions), confirming results previously mentioned by Calixto et al. (1990). Also they were reinforced by total phenolic compounds measurement assay that determinated the amount of 0.99 µg equivalent of catechin/mg of extract.

The HPLC analysis showed that chloroformic, ethyl acetate and methanolic fractions presents qualitative equalities of these compounds. These compounds are responsible for many pharmacological activities according to Ielpo et al. (2000) and their presence in the extracts could explain some results that were obtained in this work.

Uvarana extracts presented antimicrobiological activity against *S. aureus*, and *E. coli* showed dose-dependent activity in the MIC assay. For concentrations of 1.25 mg, it was possible to see zone inhibition of 7 mm when the microorganism tested was *S. aureus* and the same zone was observed for the concentration of 2.5 mg of extracts when tested against *E. coli*. In fact, antibacterial activity can be related in the presence of polyphenolic compounds (flavonoids and tannins) in these extracts (Hamburger and Cordell, 1987; Rahalison et al., 1991; Scalbert, 1991; Cowan, 1999; Djipa et al., 2000; Harborne and Williams, 2000; Bylka et al., 2004; Veluri et al., 2004, Campos et al., 2007).

The chromatographic fingerprinting of the three organic fractions were similar regarding the chromatographic profile differing only in the UV response of the chromatographic bands with the higher response obtained from ethyl acetate fraction. These results explain the highest microbiological activity obtained by the ethyl acetate

**Table 2.** Effect of Uvarana crude extract on the viability of cells studied by MTT reduction and cellular membrane integrity assays.

| Uvarana crude extract (µg/ml) | Jurkat cells Cell viability (%) in 24 h | | J774A.1 cells Cell viability (%) in 24 h | |
|---|---|---|---|---|
| | Cellular membrane integrity | MTT reduction | Cellular membrane integrity | MTT reduction |
| 0 | 94.9 ± 0.78[a,b] | 100[b] | 95.43 ± 1.35[a] | 100[a] |
| 50 | 88.3 ± 2.81 | 96.1 ± 1.74 | 92.68 ±1.55 | 104.13 ± 2.29 |
| 100 | 87.1 ± 2.97 | 94.6 ± 1.45 | 91.86 ± 2.20 | 110.44 ± 1.23[a] |
| 200 | 57.2 ± 7.27[b] | 71.9 ± 12.79[b] | 73.46 ± 3.63[a] | 114.59 ± 2.18[a] |
| 400 | 69.1 ± 4.19[a] | 53.7 ± 1.01[b] | 56.25 ± 3.45[a] | 99.86 ± 1.93 |
| 800 | 8.9 ± 6.43[b] | 33.9 ± 1.98[b] | nd | 28.73 ± 1.65[a] |
| | **Cell viability (%) in 48 h** | | **Cell viability (%) in 48 h** | |
| 0 | 81.5 ± 2.26[a] | 100[a] | 96.93 ± 0.71[a] | 100[a] |
| 50 | 83.9 ± 6.59 | 100 ± 0.51 | 96.4 ± 0.45 | 100.28 ± 0.29 |
| 100 | 85.4 ± 3.42 | 100 ± 0.75 | 90.58 ± 0.96 | 99.12 ± 1.01 |
| 200 | 32.4 ± 7.04[a] | 98.6 ± 1.18 | 75.71 ± 2.04[a] | 99.07 ± 0.69 |
| 400 | 29.9 ± 6.98[a] | 84.6 ± 2.41[a] | 31.22 ± 5.35[a] | 93.26 ± 0.44[a] |
| 800 | 11.4 ± 4.09[a] | 43.9 ± 3.91[a] | nd | 48.63 ± 1.49[b] |
| | **Cell viability (%) in 72 h** | | **Cell viability (%) in 72 h** | |
| 0 | 98.7 ± 0.44[a] | 100[a] | 92.42 ± 1.16[a] | 100[a] |
| 50 | 95.2 ± 0.64 | 99.7 ± 0.09 | 92.99 ± 0.64 | 99.96 ± 0.10 |
| 100 | 92.6 ± 2.12 | 98.4 ± 0.60 | 84.46 ± 1.46[a] | 100.48 ± 0.24 |
| 200 | 49.3 ± 3.52[a] | 43.4 ± 3.07[a] | 25.54 ± 2.41[a] | 39.83 ± 1.08[a] |
| 400 | 22.3 ± 10.55[a] | 24.5 ± 2.03[a] | 3.41 ± 1.23[a] | 27.84 ± 0.72[a] |
| 800 | 2.08 ± 2.08[a] | 22.6 ± 1.77[a] | nd | 26.31 ± 0.67[a] |

The viability is expressed as the mean ± S.E.M. (n = 4). Similar letters correspond significantly different from untreated cells (a = P < 0.05, b = P < 0,001 and nd = not determined).

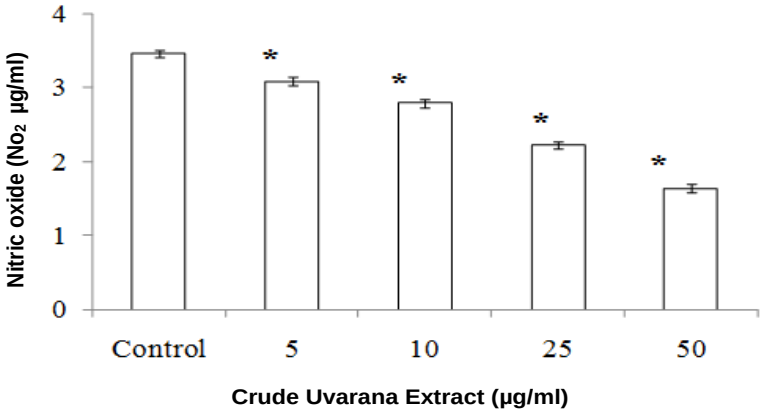


**Figure 4.** Effects of Uvarana crude extract on NO production by J774A.1 cells. The concentration (µM) of nitrite measured in the supernatant of cultured cells after treatment for 48 h with different concentration of Uvarana crude extract in the presence of 2.5 µg/ml LPS are shown. The supernatant was incubated with an equal volume of Griess reagent and the absorbance was determined at 550 nm. The values are presented as means ± S.E.M. of one experiment of the two with 8 determinations. *P < 0.05 for comparison with control.

fraction.

The bioautography test confirmed the results observed in the MIC assay when evaluated against *S. aureus* (ethyl acetate, chloroformic and methanolic extracts). For these extracts, the components showed inhibition zones for compounds with different polarities, showing that the presence of these compounds in extracts of *C. dracaenoides* can be responsible for antimicrobiological activity. The data did not establish the nature of the compounds responsible for antibacterial action; however, the preliminary phytochemical studies carried out with extracts of this plant showed that phenolic compounds could be responsible for the verified action. In fact, as observed in Figure 1, the UV spectra obtained in the chromatographic analyses by the HPLC confirm the aromatic character of the compounds, indicating the presence of flavonoids (Mabry et al., 1970).

Scalbert (1991) have found that more highly oxidized phenols have higher inhibitory action. In addition, Cowan (1999) determinated for polyphenolic compounds that the site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987).

According to Haslam (1996), others mechanisms that are responsible for the microbiological activity are the cellular metabolism modification by the metallic ions. And finally, as described by Tsuchiya et al. (1996), the activity of phenolic compounds is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic polyphenolics may also disrupt microbial membranes.

Antioxidant compounds represent an important role as a health-protecting factor. Most of the antioxidant compounds are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties (Hemalatha et al., 2010).

Haslam (1996) and Stern et al. (1996) suggest that polyphenolic compounds complex with proteins through the so-called nonspecific forces, such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation to exert its activity.

Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy, and thus inhibit the peroxidative mechanisms that lead to degenerative diseases as described by Miller et al. (2000a, b).

As mentioned earlier, Uvarana crude extract presents a high amount of phenolic compounds. It can be observed that the content of phenolics in the extract correlates with their antiradical free activity. This extract presents 9.9% of phenolic compounds and this percentage can be considered similar to the ones found in other bark extracts, for example *Myracrodruom urudeuva* and *Caesalpinia pyramidalis* (Monteiro et al., 2005). The bark extract of the *Garcinia atroviridis* Griff. ex T. Anders also showed the antioxidant activity, and the polyphenolic compounds (xanthones) are responsible for this effect (Mackeen et al., 2000).

According to Cragg and Newman (2000), over 50% of the drugs in clinical trials for antitumor activity were isolated from natural sources, such as vincristine and taxol. The rich and diverse plant resources are likely to provide effective antitumor agents. One of the best approaches in the search for antitumor agents from plant resources is the selection of plant based on ethnomedical leads, and testing the selected plants efficacy and safety through modern scientific methods.

In the present study, it was examined if the Uvarana crude extract has any effect on the death of tumoricid cells like Jurkat and J774A.1. The Uvarana crude extract showed important activity measured by endpoints of cytotoxicity MTT and cellular integrity assays indicating the presence of some cytotoxic compounds in this extract, which could be responsible for the *in vitro* activity. The cytotoxicity of the Uvarana crude extract towards Jurkat cells (cell viability: 43%) and J774A.1 cells (cell viability: 40%) at the test concentration of 200 µg/ml in culture of 72 h precluded the determination of antitumour activity. These results are confirmed by optic microscopy analysis where the morphological changes that characterized apoptosis were observed.

The phytochemical studies of other barks, such as genus *Goniothalamus* and species *Picea jezoensis* have shown results in the isolation of secondary metabolites with antitumour activities (Wiart, 2007; Wada et al., 2009).

NO production assay is simply considered to be a predictive assay for the detection of the anti-inflammatory potential of several substances and drugs. In fact, the effect of bark extracts on NO production and on the iNOS activity has been studied and may be considered promising candidates for anti-inflammatory studies (Son et al., 2008; Shrestha et al., 2008; Liang et al., 2008; Nakamura et al., 2009; Oh et al., 2009).

In concern, the inflammation that involves the immunological activity against pathogens in the hosts by one hand includes the participation of macrophages that eliminate foreign agents by phagocytosis and generate reactive nitrogen products, such as nitric oxide. Nitric oxide (NO) is a pleiotropic mediator that acts in a variety of physiological and pathophysiological processes. This small molecule is produced in these cells by inducible nitric oxide synthase (iNOS). When macrophage are activated by the endotoxin from the bacterial wall components LPS or by interferon-γ, iNOS is significantly expressed, and massive amounts of NO are produced to exert a nonspecific immune response (Marzocco et al., 2007).

In fact, an excessive production of NO by the iNOS enzyme is responsible for vasodilatation, hypotension, septic shock and inflammation (Chi at al., 2003). In addition, the NO is a potent pro-oxidant molecule that is able to induce oxidative damage which results in a number of human diseases (Cuzzocrea et al., 2001; Rao and Balachandran, 2002).

The pathophysiological importance of overproduction of NO by iNOS suggests that inhibitors of iNOS may be therapeutically useful. According to Zhou et al. (2008), phenolics compounds isolated from the bark of *Magnolia* (*M.*) *obovata* like 4-methoxyhonokiol, can inhibit LPS-mediated nuclear factor-κB (NF-κB) activation, important to iNOS expression.

## Conclusion

The research showed that Uvarana crude extract has significant anti-inflammatory properties as shown in NO production experiment and it justifies the traditional use of this plant in the treatment of various types of inflamemation (Calixto et al., 1990).

The Uvarana extract, in none toxicity concentration, caused a significant inhibition of the NO production by murine macrophages (J774A.1) in a dose-dependent manner, therefore suggests the presence of immunomodulators that could be responsible for the anti-inflammatory action.

The results contribute to a comprehensive understanding of the plant's biological properties (antimicrobial, antitumour, antioxidant and anti-inflammatory activities) and confirm the use of this plant in traditional medicine.

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