Evaluation of antitumoral and antimicrobial activity of Morinda citrifolia L. grown in Southeast Brazil[I]

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ABSTRACT

PURPOSE: To evaluate the antitumor and antimicrobial activity of ethanolic extract of *Morinda citrifolia* L. fruit cultivated in southeastern Brazil.

METHODS: Preparation ethanolic extract of the fruit of *Morinda citrifolia* L. Culture of melanoma cells B16-F10 for treatment with ethanolic extract of Morinda citrifolia L. fruit to determine cell viability by MTT and determination temporal effect of ethanolic extract fruit on the cell growth B16-F10 for 8 days. Evaluation of antimicrobial activity of ethanolic extract fruit against *Staphylococcus aureus* and *Escherichia coli* by determination of Minimum Inhibitory Concentration (MIC).

RESULTS: The ethanolic extract of *Morinda citrifolia* L. fruit (10mg/mL) decreased cellular activity and inhibited 45% the rate of cell proliferation of B16-F10 melanoma treated during period studied. The ethanolic extract of *Morinda citrifolia* L. fruit demonstrated antimicrobial activity inhibiting the growth of both microorganisms studied. *Staphylococcus aureus* was less resistant to ethanolic extract of *Morinda citrifolia* L. fruit than *Escherichia coli,* 1 mg/mL and 10 mg/mL, respectively.

CONCLUSION: What these results indicate that the ethanolic extract of the fruit of *Morinda citrifolia* L. showed antitumor activity with inhibition of viability and growth of B16-F10 cells and also showed antibacterial activity as induced inhibition of growth of *Staphylococcus aureus* and *Escherichia coli.*

Key words: *Morinda citrifolia* L.; *Morinda*; Drug Screening Assays Antitumor; Anti-Infective Agents; Antimicrobial.

Introduction

Cutaneous melanoma is considered the most serious type of skin cancer. It is a highly lethal and very invasive neoplasm, accounting for less than 5 % of all skin cancer cases. Despite its low incidence, it is considered a problem for public health due to the significant raise in the number of cases, exceeding other malignancies growth rate[1-3].

In the last few years there has been growing interest in natural products with biological activity, with relevance to anticancer activity. A large diversity of plants has been extensively investigated, these plants being secondary metabolite producers. These studies on the biotechnological potential of plants sources promising therapeutic agents are mentioned, with antibacterial, antiviral, antitumor and immunossupressive potential[3,4].

Morinda citrifolia L, known as Noni, belongs to the Rubiaceae family native to Southeast Asia and secularly used in Polynesian traditional medicine. Noni juice is widely used in complementary medicine due to its probable antioxidant, anti-inflammatory and antitumor effects against diseases such as cancer, atherosclerosis, diabetes and ulcer[5,6].

Products derived from *Morinda citrifolia* L. fruit have been commercialized in the USA since the 1990s and are distributed all over the world. A large number of beneficial effects have been claimed for Noni. However, clinical data are essentially lacking. To what e ffxtent the findings from experimental pharmacological studies are of potential clinical relevance is not clear at present[7].

Many pharmacological studies of *Morinda citrifolia* L juice and isolated compounds from the fruit has been published. These compounds including iridoids, flavonoids, lignans, coumarins and anthraquinones[7,8]. The purposes of this study were to evaluate antioxidant, antitumoral and antimicrobial activity of ethanolic extract from *Morinda citrifolia* L. fruit grown in Southeast Brazil.

Methods

Plant materials and preparation of ethanolic extract

The fruits of *Morinda citrifolia* L. were collect in the campus of the State University of Santa Cruz, Bahia, Brazil. Voucher specimens were deposited in the Herbarium of Department of Biological Sciences, State University of Santa Cruz, Bahia, Brazil. The samples were washed with running tap water and separated before being chopped into pieces. They were oven-dried at 42 °C for 5 days and ground to powder.

Plant materials used in this study were fresh fruits (seedless without core) of *Morinda citrifolia* L.. The preparation of 70% ethanolic extract of dried fruit (50 g) was obtained by grinding and exposure to organic solvent. A suspension of dried fruit (50 g) in water (150 mL) was extracted with ethanol (350 mL) for 8 days. The aqueous layer was evaporated and then followed by lyophilization to give a water-soluble fraction.

Cell Culture

The mouse melanoma B16-F10 cell line was purchased from Rio de Janeiro Cell Bank (BCRJ/UFRJ). The cells were maintained at 37 °C in an incubator with a humidified atmosphere of 5 % CO_2 and cultured in DMEM/F12 supplemented with 10 % heat-inactivated FBS, streptomycin (100 µg/mL) and penicillin (100 units/mL).

Effect of ethanolic extract of Morinda citrifolia L on B16-F10 cell growth inhibition

B16-F10 cells at 80% confluence, the cells were harvested with trypsin, and serum-free medium was used to obtained a single-cell suspension. The cells were then seeded in 06-well plates at a density of 200,000 cells/well. After 24 h, the wells were replaced with fresh medium, including FBS. Next, the wells were treated with 10mg/mL ethanolic extract of *Morinda citrifolia* L. and the cell numbers were counted following 1-8 days. A control group was prepared simultaneously and a growth curve was generated.

Cell Viability Assay

The effects of Noni extract treatment on cell viability were determined by MTT assay, which is based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase in viable cells. For all experimental groups, cells were seeded in 96-well plates at a density of 1 × 104 cells/well and treated with Noni extract at a final concentration of 1 to 80mg/mL . After 48 h, 50 µL of MTT stock solution (2 mg/mL) was added to each well to reach a total reaction volume of 250 µL, and the plates were incubated for an additional 4 h. Supernatants were aspirated, and the resulting formazan crystals were dissolved in 150 µL isopropyl alcohol. Absorbance was measured at 540 nm using a colorimetric MTT ELISA assay (VERSAmax Tunable microplate reader, Molecular Devices, CA, USA).

Antibacterial activity assay

Antibacterial activity was tested by means of a standard agar plate diffusion assay. Gram positive Staphylococcus aureus (CCBM 0324) and Gram negative Escherichia coli bacterial

strains (obtained from the Culture Collection of Microorganisms of Bahia (CCMB), Laboratory of Microbiology, University Estadual de Santa Cruz, Ilhéus) were used. Tests were repeated and was calculated at Minimum Inhibitory Concentration (MIC). MIC as recommended by the Institute of Clinical and Laboratory Standards (CLSI, 2007) and adapted[9]. Evaluation of antitumor activity was done by determining the cell growth curve in the presence of 10mg/mL ethanolic extracts of *Morinda citrifolia* L.

Statistical Analysis

Data are presented as mean ± SD of four independent experiments. Statistical analysis among groups was performed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls Multiple Range Test. GraphPad Prism v.3.0 software was used, $p < 0.05$ was considered to be statistically significant.

Results

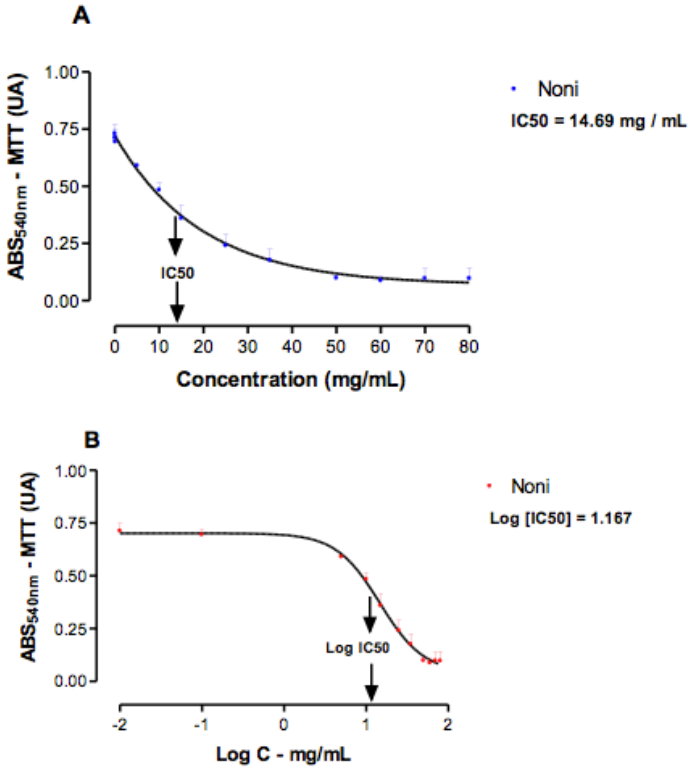

FIGURE 1 - Antiproliferative and cytotoxic effects of ethanolic extract of *Morinda citrifolia* L in B16-F10 cells. MTT viability test showing the B16-F10 cells treated for 48 h with different concentrations of extract (0 - 80 mg/mL) (A) and after treatment with extract concentration up to 5 mg/mL significantly reduced the number of cells ($p < 0.05$). (B) –Log [IC50]=1.167. The MTT data shown are performed in triplicates. Results are means ± S.E.M from four independent experiments. (*statistically significant against the control for $P < 0.05$).

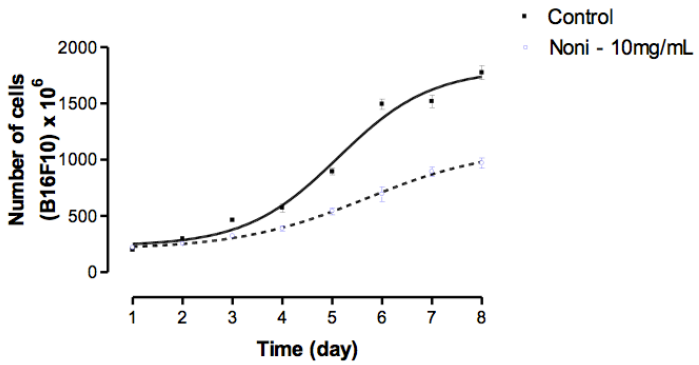

FIGURE 2 - Inhibitory activity of 10mg/mL ethanolic extract of *Morinda citrifolia* L on B16-F10 cell growth. The upper line of the graph presents the control group without ethanolic extract of *Morinda citrifolia* L treatment and the bottom line presents the group treated with ethanolic extract. At the end of each time-period, the cells were trypsinized to produce a single cell suspension and the cell number was counted. Data are presented as the mean ± standard error of the mean.

CHART 1 - Minimum Inhibitory Concentration (MIC) results; + = inhibitory concentration.

Strains	Concentration		
	10mg/mL	1mg/mL	0.1mg/mL
Staphylococcus aureus	-	+	-
Escherichia coli	+	-	-

Discussion

Malignant melanoma is a cancer with a high incidence, malignancy and poor prognosis. This cancer is highly metastatic and high mortality rate. Currently, there are no methods or effective drugs for treatment and thus new methods are necessarily expected[1,2].

Malignant melanoma cells exhibit enhanced survival and proliferation capabilities. One of the most important reasons for this is antiapoptosis capacity, which is the predominant problem for clinical tolerance of chemotherapy drugs. Therefore, the identification of an effective drug has been the focus of melanoma treatment[1,2]. Search for new chemopreventive and antitumor agents that are more effective but less toxic has kindled great interest in phytochemicals. Ethanolic extract *Morinda Citrifolia* L. fruit is one such compound which was used in this study. Morinda citrifolia L is a herbal remedy with promising anticancer properties[10].

Our results show that the rate of proliferation of B16-F10 cells is significantly inhibited by various concentrations of ethanolic extract *Morinda Citrifolia* L. fruit (0 - 80 mg/mL) (Figure 1).

Following treatment of B16-F10 cells with 10 mg/mL ethanolic extract of fruit *Morinda citrifolia* L. for 8 days, the cell proliferation rate was only 45% (Figure 2). Moreover, the time-dependent assay confirmed ethanolic extract *Morinda citrifolia* L. fruit exhibited a longlasting suppressive effect on the B16-F10 cells.

It has shown to inhibit the growth of tumor cells in experimental model systems, but little is known about its potential as an adjuvant chemotherapeutic agent[11-14].

The ethanolic extract of *Morinda citrifolia* L. fruit showed bacterial growth inhibition for *Staphylococcus aureus* and *Escherichia coli* compared to the positive control (Chart 1) Minimum Inhibitory Concentration (MIC), 1mg/mL determines the lowest concentration that is unanswered to inhibit bacterial growth. Thus, we investigated the bacterial susceptibility of gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) to the ethanolic extract. The choice of these microorganisms for the experiments is associated with the routine use of these strains for evaluation of antimicrobial activity. Moreover, these bacteria are human pathogens commonly isolated in Brazilian hospitals, representing 22.8% and 13.8% of isolates, respectively and often acquire resistance to antibiotics used (15)[14].

Thus, the search for new antibacterial agents is important for infection control. Our results show that the ethanolic extract of *Morinda citrifolia* L. has antimicrobial activity, inhibiting the growth of both gram-positive as gram-negative bacteria. The extract showed MIC ranging 1 mg /mL and 10 mg/mL, showing greater effectiveness against Staphylococcus aureus strains (Chart 1) where it exhibited a similar pattern to that caused by the antibiotic inhibition Ampicillin. For strain of Escherichia coli MIC was 10 mg/mL (Chart 1). These data corroborate others authors reported that the antimicrobial activity of the extract of *Morinda citrifolia* L, showing that its compounds may exhibit potent antibiotic activity against human pathogens such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella and Shigella*[16,17].

The inhibitory of microbial growth may be attributed to the presence of phenolic compounds in the plant. There is evidence that the Noni extract can also submit antituberculosis action, inhibiting the growth of *Mycobacterium turbeculosis*[18]. Moreover, conducted studies demonstrating satisfactory for antihelmintic activity with aqueous and ethanol extracts of *Morinda citrifolia* L. fruit. Although we found an inhibitory effect against pathogenic microorganisms, other studies should be performed to confirm and isolate the secondary metabolites that exhibit antimicrobial activity[19].

Morinda citrifolia L fruit is widely used in alternative medicine for the treatment and prevention of tumors. Currently, there are many pre-clinical trials (animal model or in vitro). These studies have opened new perspectives for the understanding and medical use of this plant. Randomized clinical trials have to be performed to conclusive determination of their effects on human disease. Especially considering the antitumoral activity, this fruit can have a major role in the anticancer therapy[10].

Conclusions

The ethanolic extract *Morinda citrifolia* L. fruit induce cell growth inhibition on *Staphylococcus aureus* and *Escherichia coli* and cell growth inhibition on B16-F10 cells. Considering the acquire resistance to antibiotics used and chemoresistance exhibited by melanoma towards conventional chemotherapy drugs, this novel compound may provide promising improvements in the therapeutic approach to infectious diseases and melanoma treatment.

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