

## Evaluation of antitumoral and antimicrobial activity of *Morinda citrifolia* L. grown in Southeast Brazil<sup>I</sup>

Thamyris Candida<sup>I</sup>, Jerônimo Pereira de França<sup>II</sup>, Alba Lucilvânia Fonseca Chaves<sup>II</sup>, Fernanda Andrade Rodrigues Lopes<sup>III</sup>, Silvana Gaiba<sup>IV</sup>, Celio Kersul do Sacramento<sup>V</sup>, Lydia Masako Ferreira<sup>VI</sup>, Lucimar Pereira de França<sup>II</sup>

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<sup>I</sup>Graduate Student, Department of Biological Sciences, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. Technical procedures.

<sup>II</sup>PhD, Associate Professor, Department of Biological Sciences, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. Scientific and intellectual content of the study, interpretation of data and critical revision.

<sup>III</sup>PhD, Nurse Graduate, Department of Biological Sciences, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. Scientific and intellectual content of the study, interpretation of data and critical revision.

<sup>IV</sup>PhD, Fellow Pos-PhD degree, Department of Biological Sciences, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. Technical procedures, acquisition and interpretation of data, manuscript writing.

<sup>V</sup>PhD, Associate Professor, Department of Agricultural and Environmental Sciences, Universidade Estadual de Santa Cruz, Ilhéus-BA, Brazil. Interpretation of data and critical revision.

<sup>VI</sup>Head and Full Professor, Plastic Surgery Division, UNIFESP, Researcher 1A-CNPq, Director Medicine III-CAPES, Sao Paulo-SP, Brazil. Interpretation of data and critical revision.

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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.

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## 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<sup>1-3</sup>.

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 immunosuppressive potential<sup>3,4</sup>.

*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<sup>5,6</sup>.

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 extent the findings from experimental pharmacological studies are of potential clinical relevance is not clear at present<sup>7</sup>.

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<sup>7,8</sup>. 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 collected 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<sub>2</sub> 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 obtain a single-cell suspension. The cells were then seeded in 96-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 × 10<sup>4</sup> 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 (VERSAmix Tunable microplate reader, Molecular Devices, CA, USA).

### *Antibacterial activity assay*

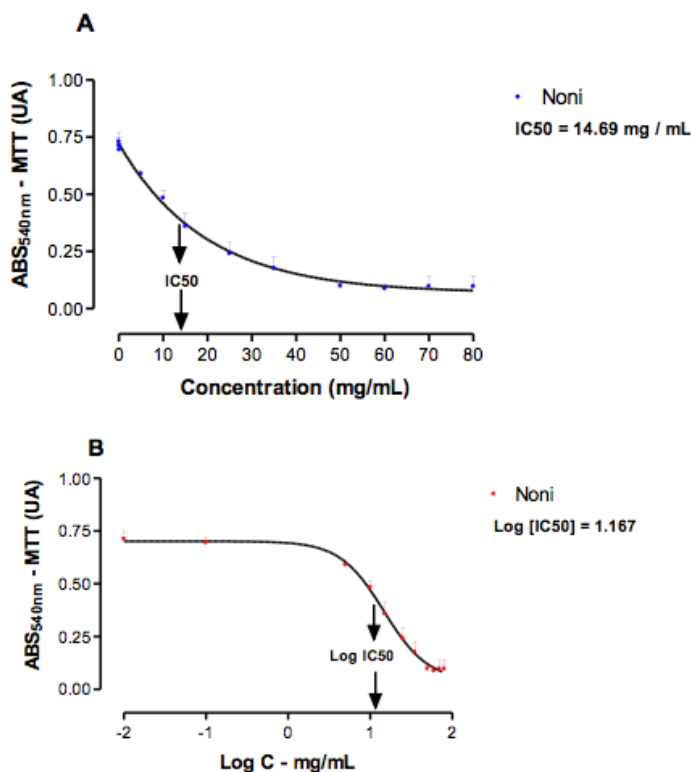
Antibacterial activity was tested by means of a standard agar plate diffusion assay. Gram positive *Staphylococcus aureus* (CCBM 0324) and Gram negative *Escherichia coli* bacterial

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<sup>9</sup>. Evaluation of antitumor activity was done by determining the cell growth curve in the presence of 10mg/mL ethanolic extracts of *Morinda citrifolia* L.

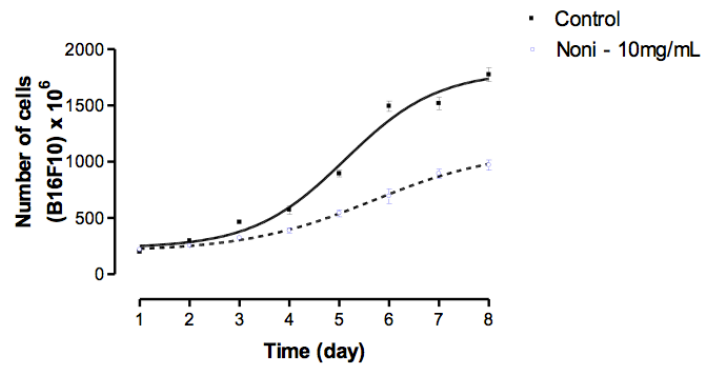
*Statistical Analysis*

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<sup>1,2</sup>.

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<sup>1,2</sup>. 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<sup>10</sup>.

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<sup>11-14</sup>.

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)<sup>14</sup>.

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*<sup>16,17</sup>.

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 tuberculosis*<sup>18</sup>. Moreover, conducted studies demonstrating satisfactory for antihelminthic 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<sup>19</sup>.

*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<sup>10</sup>.

## 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.

## References

1. Jerant AF, Johnson JT, Sheridan CD, Caffrey TJ. Early detection and treatment of skin cancer. *Am Family Physician*. 2000;62(2):357-68.
2. Lasithiotakis KG, Petrakis IE, Garbe C. Cutaneous melanoma in the elderly: epidemiology, prognosis and treatment. *Melanoma Res*. 2010 Jun;20(3):163-70.
3. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin*. 2010 Sep-Oct;60(5):277-300.
4. Rajamanickam S, Agarwal R. Natural products and colon cancer: current status and future prospects. *Drug Dev Res*. 2008 Nov 1;69(7):460-71.
5. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, Anderson G. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. *Acta Pharmacol Sin*. 2002 Dec;23(12):1127-41.
6. Chan-Blanco Y, Vaillant F, Perez AM, Reynes M, Brillouet J, Brat P. The fruit Noni (*Morinda citrifolia* L.). *J Foot Comp Anal*. 2006;19:645-54.
7. Potterat O, Hamburger M. *Morinda citrifolia* (Noni) fruit-phytochemistry, pharmacology, safety. *Planta Med*. 2007 Mar;73(3):191-9.
8. Mohamed MF, Frye RF. Effects of Herbal Supplements on Drug Glucuronidation. Review of Clinical, Animal, and In Vitro Studies. *Planta Med* 2011;77:311-21.
9. Coban AY. Rapid determination of methicillin resistance among *Staphylococcus aureus* clinical isolates by colorimetric methods. *J Clin Microbiol*. 2012;50(7):2191-3.
10. Baiju M, Mathews PP, Skariah KS, Ambika K. *Morinda Citrifolia* - Noni: A brief review of its anti cancer activity. *Health Sci*. 2012;1(3):JS004B.
11. Kamei H, Koide T, Kojima T, Hashimoto Y, Hasegawa M. Inhibition of cell growth in culture by quinones. *Cancer Biother Radiopharm*. 1998 Jun;13(3):185-8.
12. Hiramatsu T, Imoto M, Koyano T, Umezawa K. Induction of normal phenotypes in ras-transformed cells by damnacanthol from *Morinda citrifolia*. *Cancer Lett*. 1993 Sep 30;73(2-3):161-6.
13. Liu G, Bode A, Ma WY, Sang S, Ho CT, Dong Z. Two novel glycosides from the fruits of *Morinda citrifolia* (Noni) inhibit AP-1 transactivation and cell transformation in the mouse epidermal JB6 cell line. *Cancer Res*. 2001 Aug 1;61(15):5749-56.
14. Taşkın EI, Akgün-Dar K, Kapucu A, Osaç E, Doğruman H, Eraltan

- H, Ulukaya E. Apoptosis-inducing effects of *Morinda citrifolia* L. and doxorubicin on the Ehrlich ascites tumor in Balb-c mice. *Cell Biochem Funct.* 2009 Dec;27(8):542-6., 27, 542-6.
15. Sader HS, Mendes RE, Gales AC, Jones RN, Pfaller MA, Zoccoli C, Sampaio J. Perfil de sensibilidade a antimicrobianos de bactérias isoladas do trato respiratório baixo de pacientes com pneumonia internados em hospitais brasileiros - Resultados do Programa SENTRY, 1997 e 1998. *J Bras Pneumol.* 2001;27(2):59-67.
  16. Mohtar M, Shaari K, Ali NAM, Ali AM. Antimicrobial activity of selected Malaysian plants against micro-organisms related to skin infection. *J Tropical Forest Products.* 1998;4(2):199-206.
  17. Jayasinghe UL, Jayasooriya CP, Bandara BM, Ekanayake SP, Merlini L, Assante G. Antimicrobial activity of some Sri Lankan Rubiaceae and Meliaceae. *Fitoterapia.* 2002 Aug;73(5):424-7.
  18. Serafini MR, Santos RC, Guimarães AG, Dos Santos JP, da Conceição Santos AD, Alves IA, Gelain DP, de Lima Nogueira PC, Quintans-Júnior LJ, Bonjardim LR, de Souza Araújo AA. *Morinda citrifolia* Linn leaf extract possesses antioxidant activities and reduces nociceptive behavior and leukocyte migration. *J Med Food.* 2011 Oct;14(10):1159-66.
  19. Brito DR, Fernandes RM, Fernandes MZ, Ferreira MD, Rolim FR, da Silva Filho ML. Anthelmintic activity of aqueous and ethanolic extracts of *Morinda citrifolia* fruit on *Ascaridia galli*. *Rev Bras Parasitol Vet.* 2009 Oct-Dec;18(4):32-6.

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To Skin Cell Culture Laboratory, Plastic Surgery Division, Department of Surgery, UNIFESP.

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### Correspondence:

Lucimar Pereira de França  
Departamento de Ciências Biológicas  
Universidade Estadual de Santa Cruz-UESC  
Rod. Jorge Amado, Km 16  
45662-900 Ilhéus - Bahia Brasil  
Tel./Fax: (55 73)3680-5360  
lucimarfrancap@gmail.com  
jeronimopf@gmail.com

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