



## **An Analysis *In-vitro* of the Cytotoxic, Antioxidant and Antimicrobial Activity of Aqueous and Alcoholic Extracts of *Annona muricata* L. Seed and Pulp**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author RRM designed the study, coordinated and supervised the work, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Author AIS designed the study, wrote the protocol and the first draft of the manuscript. Authors FA, FS performed the statistical analysis, wrote the protocol and first draft of the manuscript, managed the analyses of the study and literature searches. Author JMM performed the statistical analysis, wrote the first draft of the manuscript and managed literature searches. Authors MICG and AZ managed the analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To evaluate the cytotoxic activity of aqueous and alcoholic extracts from *Annona muricata* L (soursop) seed and pulp on human tumor cell lines of breast, prostate and cervix; as well as the antioxidant and antimicrobial properties of those extracts.

**Study Design:** For cytotoxic activity, non-linear regressions of the values of IC<sub>50</sub> of all extracts were used. For antimicrobial and antioxidant activities, an analysis of variance with multiple range tests, using the Fisher's LSD method was applied. Each study was replicated 3 times.

**Methodology:** The methanolic extract of soursop seed was obtained by two methods: Soxhlet apparatus (SSS) and maceration (MSS). The aqueous extracts of both soursop seed (LSS) and pulp (LSP) were obtained by decoction. Human tumor cell lines from breast (MCF-7 and SKBr3), prostate (PC3) and cervix (HeLa), and fibroblasts (as control) were used to determine the cytotoxic activity by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. Antioxidant and antimicrobial activity were determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) and disc diffusion method, respectively.

**Results:** Extracts of SSS, MSS, LSP and LSS had a higher cytotoxic activity on the PC3 (0.0024 to 1.275 µg/mL) and HeLa (0.0011 to 7.194 µg/mL) cell lines with low impact on healthy cells (fibroblasts, as control), than in MCF-7 (27.09 to >100 µg/mL) and SKBr3 (20.50 to >100 µg/mL) cells. Antioxidant activity of MSS (83.23%) and LSS (84.41%) extracts were significantly ( $P < 0.05$ ) higher than those extracts of LSP (69.77%) and SSS (69.43%). Significant ( $P < 0.05$ ) antimicrobial activity against *Listeria monocytogenes*, *Salmonella enterica* ser. Enteritidis and *Staphylococcus aureus* was only observed for SSS and MSS extracts.

**Conclusion:** Results obtained in this research suggest that consumption of soursop fruit could be a good alternative to prevent illness such as cancer of prostate and cervix. However, further studies are needed to isolate and characterize the specific compounds of these extracts causing such effects.

**Keywords:** *Annona muricata* L.; cytotoxic; antioxidant; antimicrobial; tumor cells.

## 1. INTRODUCTION

Plants are one of the most important sources of medicines for treating illnesses since the beginning of human civilization [1]. In recent times, and due to historical, cultural, and other reasons, folk medicine has taken an important place, especially in developing countries, where health services are limited. The study on the medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as a source for the new drugs [2,3].

*Annona muricata* L. which is also called as the "soursop", "sir sak" or "guanabana" has been named as popular fruit tree that is cultivated throughout the tropical regions of the world. Intensive chemical investigations of the leaves, pulps and seeds of this specie have resulted in the isolation of a great number of acetogenins. The seeds and leaves of *Annona muricata* L were found to contain more than 50 mono-tetrahydro-furan acetogenins. Some of the key intermediates that are involved in the biosynthesis of these acetogenins have been

isolated recently from this specie [3,4]. The isolated compounds have displayed some biological, phytochemical or pharmacological activities, such as antitumor, cytotoxic, anti-parasitic, antimicrobial, antioxidant and pesticide properties [3,5,6,7,8].

Many of these acetogenins (epomuricenins-A and B, montecristin, cohobins-A and B, muridienins-1 and 2, muridienins-3 and 4, muricadienin and chatenaytrienins-1, 2 and 3, sabadelin and cis-panatellin) have demonstrated selective toxicity to tumor cell lines at very low dosage, as little as one part per million [5]. These acetogenins are excellent inhibitors of enzymatic processes that are found only in the membranes of cancerous tumor cells [9]. On the other hand, antioxidant compounds such as phenols (gallic and chlorogenic acid), flavonoids (myricetin, fisetin, morin, quercetin, kaempferol and isorhamnetin), anthocyanins, ascorbic acid, tocopherols, tocotrienols, carotenoids and acetogenins have been found in soursop leaf, seed and pulp [9,10,11,12]. Some extracts of phytochemical compounds of leaf, stem, root and seeds from *Annona muricata* L. such as alkaloids, flavonoids, carbohydrates, cardiac

glycosides, saponins, tannins, phytosterol, terpenoids and proteins have shown antibacterial activity against several pathogen microorganisms [9,13]. However, few studies on the cytotoxic, antimicrobial and antioxidant activity of the part of *Annona muricata* L. that is really consumed (pulp) have been carried out.

The main objectives of this research were to evaluate the *in-vitro* cytotoxic activity of aqueous and alcoholic extracts of seed and pulp of *Annona muricata* L. on human tumor cell lines of breast, prostate and cervix; in addition to the study of the antioxidant (by DPPH method) and antimicrobial (against *Salmonella enterica* ser. Enteritidis, *Staphylococcus aureus* and *Listeria monocytogenes*) activities of those isolated extracts.

## 2. MATERIALS AND METHODS

### 2.1 Fruit

*Annona muricata* L. (soursop) at commercial ripeness were selected in a local supermarket (Caracas, Venezuela) and maintained at 7°C until processing.

### 2.2 Preparation of Soursop Seeds Methanolic Extract by Soxhlet Apparatus

Dried and finely triturated seeds from soursop (300 g) were extracted with methanol (J. T. Baker, Phillipsburg, NJ) in Soxhlet apparatus during 12 hours at a ratio of 1:2 (seed:methanol) (at solvent boiling temperature, approx. 50°C) as was suggested by Leong et al. [14]. Then, the mixture was vacuum filtered using paper Whatman N°1. The solvent was removed *in vacuo* using a rotator evaporator at 45-50°C by 60 min in order to obtain the raw extract of soursop seed by Soxhlet (SSS).

### 2.3 Preparation of Soursop Seeds Methanolic Extract by Maceration

Dried and finely triturated seeds from soursop (200 g) were extracted with methanol (J. T. Baker, Phillipsburg, NJ) during 7 days at a ratio of 1:2 (seed:methanol) at room temperature (25-27°C) as suggested by Chávez et al. [15]. Then, the mixture was vacuum filtered using paper Whatman N°1. The solvent was removed *in vacuo* using a rotator evaporator at 45-50°C by

60 min to obtain the raw extract of soursop seed by maceration (MSS).

### 2.4 Preparation of Soursop Aqueous Extract by Decoction

Aqueous extracts of soursop seed (LSS) and pulp (LSP) were obtained by decoction according to methodology used by Suárez et al. [16], boiling 500 g of the sample (seed or fresh fruit without seed), in 500 mL of distilled water during 30 minutes. The decoction was vacuum filtered, and the aqueous extract stored in glass vials, and frozen for subsequent lyophilization to produce powdered forms of the extract.

### 2.5 Human Tumor Cell Lines

Human tumor cell lines from MCF-7 (breast carcinoma, without over-expression of the HER2/c-erb-2 gene), SKBr3 (breast carcinoma, in which the HER2/c-erb-2 gene is over-expressed), PC3 (prostate carcinoma) and HeLa (cervix epithelial carcinoma) were provided by Marie-France Poupon from Laboratory of Molecular Cytogenetic and Oncology of the Curie Institute (Paris, France). Human dermis fibroblasts, used as control cells, were obtained from Laboratory of Tissue Culture and Tumor Biology of the Institute of Experimental Biology (Caracas, Venezuela). All cell lines were used to determine the cytotoxic activity from soursop seed and pulp extracts. MCF-7, SKBr3 and fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS; Gibco), 2 mM glutamax (Gibco), 100 units/mL penicillin (Gibco) and 100 µg/mL streptomycin (Gibco). PC3 and HeLa were grown in Roswell Park Memorial Institute medium (RPMI 1640; Gibco) supplemented with 10% (v/v) heat inactivated FBS, 2 mM glutamax (Gibco), 100 units/mL penicillin (Gibco) and 100 µg/mL streptomycin (Gibco). For treatments, exponentially growing cells were collected, counted, re-suspended in fresh culture medium and incubated in 96 sterile well plates.

### 2.6 Cytotoxicity Assay

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay, which is based on the ability of viable cells to metabolically reduce a yellow tetrazolium salt (MTT; Sigma) to purple crystals of formazan [17]. This reaction takes

place when mitochondrial reductases are active. Cells were seeded in 96-well plates ( $5 \times 10^5$  cells/well) and incubated at 37°C for 72 hours with the extracts of the soursop seed and pulp obtained by different extraction methods at concentrations of 0; 0.001; 0.01; 0.1; 1; 5; 10; 15; 25; 100 µg/mL, respectively, in a humidified atmosphere with 5% CO<sub>2</sub>. The lyophilized extracts (LSS and LSP) were diluted in water and extracts obtained by maceration (MSS) and Soxhlet (SSS) were diluted in DMSO. The final concentration of the DMSO in culture medium was always lower than 1% (v/v), a concentration that has neither cytotoxic effect nor causes any interference with the colorimetric detection methods). After incubation, the medium was removed and the cells were treated with 100 µL MTT for 3 hours at 37°C. Subsequently, the MTT was removed and 100 µL DMSO was added. The formazan product was quantified with the help of a microplate reader TECAN-Sunrise™ at 570 nm (Tecan Group LTD, Männedorf, Switzerland). Taxol (Bristol-Myers Squibb, USA) was used as a positive control in the assay. The experiment was carried out in triplicate.

## 2.7 Selectivity Index for Cytotoxicity Assay

The selectivity index (SI) was calculated as the IC<sub>50</sub> (control cells)/IC<sub>50</sub> (tumor cell line) ratio. A selectivity index > 1 indicates that the cytotoxicity on tumor cell lines surpassed that on healthy non-tumor cells [18].

## 2.8 Determination of Antioxidant Activity

Radical scavenging ability of soursop extracts was performed by the method described by Celep et al. [19]. An aliquot of 0.2 mL of each extract (SSS, MSS, LSP, LSS) was mixed with 2.8 mL of freshly prepared 0.06 mM 1, 1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, USA) solution in methanol. The mixture was shaken with a vortex and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm (just after added the extract and after 30 min of dark incubation). Ascorbic acid (176 µg/mL) was used as reference substance (control). The absorbance was measured at 517 nm. DPPH radical-scavenging activity was calculated as follows (Eq. 1):

$$\text{DPPH radical - scavenging activity (\%)} =$$

$$\left( \frac{\text{Abscontrol} - \text{Abssample}}{\text{Abscontrol}} \right) \times 100 \quad (1)$$

Where, Abscontrol, is the absorbance value of the Ascorbic acid, and Abssample, is the absorbance of the extract solution. The experiment was made in triplicate.

## 2.9 Microbial Strains and Culture Conditions

Strains of *Salmonella enterica* ser. Enteritidis (CVCM 497), *Staphylococcus aureus* (CVCM 456) and *Listeria monocytogenes* (CVCM 449) were supplied by the "Centro Venezolano de Colecciones de Microorganismos (CVCM)" of the Institute of Experimental Biology of the Central University of Venezuela, Caracas-Venezuela for evaluating the antimicrobial activity of soursop seed and pulp extracts against these pathogens. Strains of *L. monocytogenes*, *Salmonella enterica* ser. Enteritidis, and *Staphylococcus aureus* were selected as an alternative for controlling the main emerging pathogenic microorganisms associated to ready-to-eat foods; and like a possible pharmacological alternative for controlling microorganisms resistant, which have emerged as a result of the uncontrolled use of many antibiotics.

Strains of *Salmonella enterica* ser. Enteritidis and *Staphylococcus aureus* were individually grown in 50 mL of tryptone soy broth (TSB) (Himedia, Mumbai, India), whereas the strain of *Listeria monocytogenes* was grown in 50 mL of TSB plus yeast extract (Himedia) at 0.6% (w/v). All cultures were incubated at 37°C for 24h without agitation to obtain cells in early stationary growth phase. These conditions were obtained from growth curves previously made in the Laboratory (data not shown). The maximum population reached by the microorganisms in the growth medium was approximately 10<sup>8</sup> Colony Forming Unit (CFU)/mL.

## 2.10 Antimicrobial Activity by Disc Diffusion Method

The method used in this experiment was the suggested by Kirby-Bauer [20]. Sterile filter-paper discs (Whatman N°1) measuring 9 mm in diameter were dipped in solutions containing SSS, MSS, LSP and LSS extracts individually prepared at different concentrations (0.0; 0.1; 0.3; 0.5%, v/v). Agar disc diffusion method for screening of antimicrobial activity of each extract against *Salmonella enterica* ser. Enteritidis, *Staphylococcus aureus* and *Listeria*

*monocytogenes* was used. The discs impregnated with extracts were put on Müller-Hinton agar (Himedia, Mumbai, India) plates previously inoculated with each pathogenic microorganism at  $10^8$  CFU/mL, and then incubated at 37°C for 24-48 h. The diameter of inhibition zone surrounding the filter paper disc was measured using a Vernier caliper (Hauptner, Solingen, Germany). Each extract was assayed in triplicate. Sterile distilled water was used as negative control.

## 2.11 Statistical Analysis

All experiments were performed at least three times. The values of  $IC_{50}$  (cytotoxicity) of natural products were determined by a non-linear regression of individual experiments using the program GraphPad Prism v.5.02 (GraphPad Software, San Diego, CA, USA). Analyses of variance (ANOVA) were carried out to detect differences significant statistically ( $P < 0.05$ ) in the antimicrobial and antioxidant activity of SSS, MSS, LSP and LSS extracts; and between their concentrations using statistic package Statgraphics Centurion XVI (StatPoint Technologies). Multiple range tests, using the Fisher's LSD method, were then applied to determine which extracts and concentrations of each extract were significantly ( $P < 0.05$ ) different.

## 3. RESULTS AND DISCUSSION

### 3.1 Cytotoxic Activity

Results demonstrated that SSS, MSS, LSP and LSS extracts had a significant ( $P < 0.05$ ) *in-vitro* effect on two human tumor cell lines (HeLa and PC3) with a low impact on control cells (fibroblasts) (Table 1). Whereas, for MCF-7 and SKBr3 cells, only the LSS and MSS extracts, respectively, showed a moderated cytotoxic effect (Table 1).

The MSS and SSS extracts were more effective than LSS and LSP extracts against HeLa and PC3 cells (Table 1). *In-vitro* cytotoxic activity from alcoholic extracts of soursop pericarp on cell line U 937 (Human leukemic monocyte lymphoma) has been reported [3]. Jyothi et al. [1] indicated the antitumor activity *in-vitro* of ethanolic extracts from leaf of *Annona cherimola* against bovine kidney cells (MDBK) and human larynx epidermoid carcinoma cells (Hep-2) had significant effects. Several researchers have indicated that acetogenins of *Annonaceous* are responsible of the cytotoxic activity, which suggests their potential usage as antitumor agents [1,3,21]. It is generally accepted that the mode of action of acetogenins is the inhibition of NADH-ubiquinone oxidoreductase (complex I) in the mitochondria, and inhibition suppresses ATP production, especially for cancer cells with high metabolic levels, leading to apoptosis [22,23]. In addition, Chiu et al. [24] indicated that acetogenins of *Annonaceous* such as bullatacin induced apoptosis through a reduction of intracellular cAMP and cGMP levels in human hepatoma 2.2. 15 cells.

The SSS, MSS, LSP and LSS extracts presented a highly selective cytotoxic action to HeLa and PC3 cell lines, since selective index (SI) values were higher than 1.0 (Table 2); resulting the higher SI value for the SSS extract. Meanwhile, LSS extract showed a moderate selectivity on MCF-7 cells, since the SI value was slightly higher than 1.

### 3.2 Antioxidant Activity

The free radical scavenging potential of both seed and pulp extracts from soursop was determined *in-vitro* through the DPPH method just after added the reactive and after 30 min. All extracts showed a higher antioxidant activity at time 30 min than at time 0 min (Table 3).

**Table 1. Cytotoxic activity of soursop seed and pulp extracts on various human tumor cell lines**

Extracts	Values of $IC_{50}$ ( $\mu\text{g/ml}$ )*				
	HeLa	PC3	MCF-7	SKBr3	Fibroblasts
MSS	0.0110±1.20	0.0035±1.10	70.15±1.02	20.50±1.012	17.93±1.06
LSS	0.1030±1.08	1.2750±1.08	27.09±1.03	> 100	> 100
LSP	7.1940±1.06	0.8460±1.29	>100	> 100	61.66±1.03
SSS	0.0240±1.18	0.0024±1.27	>100	> 100	56.63±1.13

\* Values are mean of three (3) determinations  $\pm$  standard deviation.  $IC_{50}$ : inhibitory concentration 50; MSS: Macerated soursop seed; LSS: Lyophilized soursop seed; SSS: Soxhlet soursop seed; LSP: Lyophilized soursop pulp. HeLa (human cervix carcinoma); PC3 (human prostate carcinoma); MCF-7 (breast carcinoma, without over-expression of the HER2/c-erb-2 gene); SKBr3 (breast carcinoma, in which the HER2/c-erb-2 gene is over-expressed); Fibroblasts (healthy cells of animal connective tissue, used as control)

**Table 2. Values of selectivity index (SI) of the seed and pulp extracts of soursop**

Extracts	Selectivity index (SI)*			
	HeLa	PC3	MCF-7	SKBr3
MSS	1.63	5123	0.25	0.81
LSS	970.1	78.43	3.69	1.0
LSP	8.57	72.88	0.62	0.62
SSS	2,359.6	23,595.8	0.56	0.56

\*Values are mean of three (3) determinations  $\pm$  standard deviation. MSS: macerated soursop seed; LSS: lyophilized soursop seed; SSS: Soxhlet soursop seed; LSP: lyophilized soursop pulp. HeLa (human cervix carcinoma); PC3 (human prostate carcinoma); MCF-7 (breast carcinoma, without over-expression of the HER2/c-erb-2 gene); SKBr3 (breast carcinoma, in which the HER2/c-erb-2 gene is over-expressed). Fibroblasts were used as reference to determine the selective effect on the tumor cells

**Table 3. Antioxidant activity of alcoholic and aqueous extracts of soursop**

Extract	Percentage (%; v/v)	
	0 min	30 min
MSS	76.45 $\pm$ 0.19 <sup>Aa</sup>	83.23 $\pm$ 1.85 <sup>Ba</sup>
LSS	70.30 $\pm$ 0.49 <sup>Ab</sup>	84.41 $\pm$ 0.01 <sup>Ba</sup>
LSP	68.53 $\pm$ 0.20 <sup>Ac</sup>	69.77 $\pm$ 0.92 <sup>Bb</sup>
SSS	64.65 $\pm$ 0.21 <sup>Ad</sup>	69.43 $\pm$ 0.11 <sup>Bb</sup>

MSS: Macerated soursop seed; LSS: Lyophilized soursop seed; SSS: Soxhlet soursop seed; LSP: Lyophilized soursop pulp. Different capital letters (A, B) in the same row indicate significant difference ( $P < 0.05$ ) between times by each extract. Different lower-case letters (a, b, c, d) in the same column indicate significant differences ( $P < 0.05$ ) between extracts by each time. Experiments were made in triplicate  $\pm$  standard deviation

In addition, MSS and LSS extracts showed an antioxidant activity significantly higher ( $P < 0.05$ ) than SSS or LSP extracts (Table 3). Moreno & Jorge [25] reported an antioxidant activity of 76.20% and a content of total phenolic compounds of 78.49 mg de gallic acid equivalents/g of dry extract to ethanolic extract of soursop seed, indicating that phenolic compounds are responsible of the antioxidant activity. Gutiérrez-Abejón et al. [10] also reported that some compounds, like flavonoids, presents in the fruit have demonstrated a strong antioxidant activity. Vijayameena et al. [9] reported that aqueous extracts of soursop seed contain high quantity of superoxide dismutase (83.4U/mg) and catalase (68 $\mu$ mol), which are enzymatic antioxidant components. The same authors indicated that acetogenins presents in

*Annona muricata* L. have an antioxidant activity, which is related to their ability to quench reactive oxygen species such as singlet molecular oxygen and peroxy radicals, thus acting, as deactivators of excited molecules or as chain breaking agents respectively [26]. The Department of Agriculture of the United States [11] reported that edible part of soursop (pulp) has a content of ascorbic acid equal to 20.60 mg/100g, which is a known compound with important antioxidant properties.

Correa-Gordillo et al. [12] performed a revision about the antioxidant activity of *Annona muricata* L. pulp and leaves but not from seed. These authors indicated that a comparison of the results between different authors was impossible due to the variability of methods and units reported by the diverse researchers. In addition, indicated that the study of the antioxidant activity of the different parts of this plant have not been carried in profundity, for this reason, it is unknown the exact mechanisms of action of antioxidants components of the plant.

Antioxidant activity showed by soursop could result in a benefice for human health, since antioxidants have the property of block the activity of other chemical compounds known as free radicals, which are highly reactive compounds that have the potential to cause damage to cells, including damage that may lead to cancer [27].

### 3.3 Antimicrobial Activity

Antimicrobial activity of soursop extracts against *L. monocytogenes*, *Salmonella enterica* ser. Enteritidis, and *Staphylococcus aureus* significantly ( $P < 0.05$ ) varied depending on kind of extract and concentration. The MSS and SSS extracts showed significant antimicrobial activity against these pathogenic microorganisms; whereas, LSS and LSP extracts did not show any antimicrobial activity. Additionally, antimicrobial activity of the extracts increased when higher concentrations were used (Table 4).

Antimicrobial activity of *Annona muricata* L has been previously reported on several microorganisms. In this sense, Pathak et al. [7] reported antimicrobial activity in methanolic and aqueous extracts of *Annona muricata* L. leaves. Those authors indicated that concentrations  $\geq$  0.8% of methanolic and aqueous extracts of soursop leaves caused antimicrobial activity on

**Table 4. Antimicrobial activity of alcoholic and aqueous soursop extracts against *L. monocytogenes*, *Salmonella enterica* ser. Enteritidis, and *Staphylococcus aureus***

Extract	Concentration (%; v/v)	Zone of inhibition (mm)		
		<i>L. monocytogenes</i>	<i>S. enteritidis</i>	<i>S. aureus</i>
MSS	0	0,25±0,07 <sup>Aa</sup>	0,20±0,00 <sup>Aa</sup>	0,25±0,07 <sup>Aa</sup>
	0,1	0,20±0,00 <sup>Aa</sup>	0,20±0,00 <sup>Aa</sup>	0,30±0,00 <sup>Aa</sup>
	0,3	0,25±0,07 <sup>Aab</sup>	0,25±0,07 <sup>Aab</sup>	0,35±0,07 <sup>Aab</sup>
	0,5	0,30±0,00 <sup>Ab</sup>	0,35±0,07 <sup>Ab</sup>	0,45±0,07 <sup>Bb</sup>
LSS	0	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
	0,1	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
	0,3	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
	0,5	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
LSP	0	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
	0,1	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
	0,3	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
	0,5	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>	0,00±0,00 <sup>Ba</sup>
SSS	0	0,20±0,00 <sup>Aa</sup>	0,15±0,07 <sup>Aa</sup>	0,25±0,00 <sup>Aa</sup>
	0,1	0,25±0,07 <sup>Aab</sup>	0,25±0,07 <sup>Aa</sup>	0,25±0,00 <sup>Aa</sup>
	0,3	0,30±0,00 <sup>Ab</sup>	0,25±0,07 <sup>Aa</sup>	0,35±0,00 <sup>Ab</sup>
	0,5	0,35±0,07 <sup>Ab</sup>	0,25±0,07 <sup>Aa</sup>	0,35±0,00 <sup>Ab</sup>

MSS: Macerated soursop seed; LSS: Lyophilized soursop seed; SSS: Soxhlet soursop seed; LSP: Lyophilized soursop pulp. Different capital letters (A, B) in the same column indicate significant difference ( $P < 0.05$ ) between extracts by each microorganism. Different lower-case letters (a, b) indicate significant differences ( $P < 0.05$ ) between concentrations by microorganism and extract. Experiments were made in triplicate  $\pm$  standard deviation

*S. aureus* and *Salmonella enterica* ser. Typhimurium, showing zones of inhibition of 18 mm and 22 mm for *S. aureus* and 16 mm and 18 mm for *S. enterica* ser. Typhimurium when methanolic and aqueous extracts were applied. Similar results were reported by Vijayameena et al. [9] and Solomon-Wisdom et al. [28], who observed that both kinds of *Annona muricata* L. leaves extracts showed antibacterial activity, but higher activity was found for alcoholic extracts than for aqueous extracts. On the other hand, the results found by Vieira et al. [6] are in contrast with those detected in the present investigation and the reported by the previous authors, since demonstrated antimicrobial activity from aqueous extract of *Annona muricata* L. peel but not from ethanolic extracts. Those differences found between authors could be due to the extract origin. It is not surprising that antimicrobial effect of natural extracts change depending on the specie, variety and part of the plant, because it is known that their antimicrobial activity is depending on the phytochemical constituents [7,28].

Significant differences ( $P < 0.05$ ) were not found between zone of inhibition produced by MSS or SSS extracts (Table 4), indicating that the extraction method have been not influenced on the efficiency of the extracts as antimicrobials.

Most phytochemicals presents in *Annona muricata* L. plant like as alkaloids, flavonoids, terpenoids and tannins have showed antimicrobial properties, suggesting that this plant or their extracts could be used for the treatment of bacterial infections [7,9]. However, like the mechanism of antimicrobial action is not well known, further studies are needed to elucidate such mechanism.

#### 4. CONCLUSION

Tumor cell lines PC3 and HeLa were more sensitive to all extracts of soursop than MCF-7 and SKBr3 cells. A higher antioxidant activity of MSS and LSS extracts were reached in comparison with extracts of LSP or SSS. Antimicrobial activity against pathogenic microorganisms was only observed for SSS and MSS extracts. Those findings provide an important new pathway that facilitates the development of chemopreventive or chemotherapeutic strategies against some cancers, and suggest that consumption of soursop could be a good alternative to prevent this illness; in addition to the scavenging and antimicrobials properties for combating free radicals and food-borne diseases, respectively. However, further studies are needed to isolate and characterize the specific compounds of these extracts that cause such effects.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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