

Original Research Article
An analysis *in-vitro* of the Cytotoxic,
antioxidant and antimicrobial activity of
aqueous and alcoholic extracts of *Annona*
***muricata* L. seed and pulp**

ABSTRACT

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

Study Design: For cytotoxic activity, a non-linear regression of the values of IC₅₀ of extracts was 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 (SS) 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 cells 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: These showed that both alcoholic and aqueous extracts of SS and pulp (SSS, MSS, LSP and LSS extracts) had a higher cytotoxic activity on the PC3 (0.0024 to 1.275 µg/mL)

and HeLa (0.0011 to 7.194 µg/mL) cells (lower IC_{50}) 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 obtained by maceration and decoction were significantly ($P < .05$) higher than those extracts of LSP (69.77%) pulp and SSS (69.43%) seed obtained by decoction and Soxhlet equipment, respectively. Significant ($P < .05$) antimicrobial activity against *Listeria monocytogenes*, *Salmonella enterica ser.* Enteritidis and *Staphylococcus aureus* was only observed for SSS and MSS alcoholic extracts of SS.

Conclusions: 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 determine isolate and characterize which 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-tetra-hydro-furan acetogenins. Some of the key

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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, cohibins-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 cells at very low dosage, as little as one part per million [5]. These acetogenins are excellent inhibitors of enzyme 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), anthocianins, ascorbic acid, tocopherols, tocotrienols, carotenoids and acetogenins have been found in soursop leaf, seed and pulp [9,10,11,12]. The leaf, stem, root and seed extract of *Annona muricata* L. have shown antibacterial activity against several pathogens associated to the phytochemical compounds such as alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterol, terpenoids and proteins [9,13]. However, few studies on the cytotoxic, antimicrobial and antioxidant activity of the [part of *Annona muricata* L.](#) ~~part~~ 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 cells 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

The 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 (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

The 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 (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

The 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 cells from MCF-7 (breast carcinoma, without overexpression of the HER2/c-erb-2 gene), SKBR3 (breast carcinoma, in which the HER2/c-erb-2 gene is overexpressed), PC3 (prostate carcinoma) and HeLa (cervix epithelial carcinoma) were provided by the PhD 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 and were used to determine the cytotoxic activity from soursoop seed and pulp extracts. Human breast cancer cell lines 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). Prostate carcinoma (PC3) and cervical epithelial carcinoma (HeLa) were grown in Roswell Park Memorial Institute medium (RPMI 1640; Gibco) supplemented with 10% (v/v) heat inactivated FBS, 2 mM glutamax, 100 units/mL penicillin and 100 µg/mL streptomycin. Human dermis fibroblast, used as control cells, from Laboratory of Tissue Culture and Tumor Biology of the Institute of Experimental Biology (Caracas, Venezuela) were obtained from primary culture and grown in DMEM medium supplemented with 10% (v/v) heat inactivated FBS, 2 mM glutamax, 100 units/mL penicillin and 100 µg/mL streptomycin. 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 grown in 96-well plates (5×10^5 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% CO₂. The lyophilized extracts ([LSS and LSP](#)) were diluted in [waterDMSO](#) and extracts obtained by maceration ([MSS](#)) and Soxhlet ([SSS](#)) were diluted in [DMSOwater](#). 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 ~~to the mixture~~. The ~~solubilized~~ 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 IC₅₀ (control cells)/IC₅₀ (tumor cell line) ratio. A selectivity index > 1 indicates that the cytotoxicity on tumor cells surpassed that on healthy non-tumor cells [18].

2.8. Determination of antioxidant activity

The 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):

$$DPPH\ radical - scavenging\ activity\ (\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100 \quad \text{Eq.1}$$

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](#) (CVCN 497), *Staphylococcus aureus* (CVCN 456) and *Listeria monocytogenes* (CVCN 449) were supplied by the "Centro Venezolano de Colecciones de Microorganismos (CVCN)" 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 *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 cell 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 10⁸ 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 aqueous or alcoholic extracts from soursop seed and pulp](#) individually prepared at different concentrations (0.0; 0.1; 0.3; 0.5 %, v/v). Agar disc diffusion method was used for screening of antimicrobial activity of each extract against

Salmonella enterica ser. Enteritidis, *Staphylococcus aureus* and *Listeria monocytogenes*. 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 them incubated at 37 °C for 24-48 h; and size of inhibition zone diameters surrounding 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). Analysis of variance (ANOVA) were carried out to detected differences statistically significant ($P < 0.05$) in the antimicrobial and antioxidant activity of SSS, MSS, LSP and LSS extracts ~~aqueous and alcoholic extracts of soursop seed and pulp~~; 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 ($P < .05$) different.

3. RESULTS AND DISCUSSION

3.1. Cytotoxic activity

Results demonstrated that ~~both SSS, MSS, LSP and LSS alcoholic and aqueous extracts of soursop seed and pulp~~ had a significant ($P < .05$) ~~in-vitro~~ effect on two human tumor cells lines (HeLa and PC3) with a low impact on control ~~healthy~~ cells (fibroblasts) (Table 1; Fig. 1). Whereas for MCF-7 and SKBr3 cells, only the ~~aqueous extract of soursop seed obtained by decoction~~ (LSS) extract and ~~the alcoholic extract of soursop seed obtained by~~

maceration (MSS) extracts, respectively, showed a moderated cytotoxic effect (Table 1; Fig. 1).

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

Extracts	Values of IC ₅₀ (µg/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 ± standard deviation. IC₅₀: lethal/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 overexpression of the HER2/c-erb-2 gene); SKBr3 (breast carcinoma, in which the HER2/c-erb-2 gene is overexpressed); Fibroblasts (healthy cells of animal connective tissue, used as control). Experiments were made in triplicate and determinations by duplicated ± SD

Figure 1. Cytotoxic effect of different extracts of pulp and seeds of soursop on viability of tumor cells and control cells. A) Maceration soursop seed soursop, B) Lyophilized soursop seed, C) Lyophilized soursop pulp, D) Soxhlet soursop seed. (♦) Control cells; (■) HeLa; (●) PC3; (▲) MCF 7; (▼) SKBr3.

The Alcoholic extracts obtained by maceration (MSS) and extraction with Soxhlet equipment (SSS) extracts were more effective than aqueous extracts obtained by decoction (LSS and LSP) extracts against HeLa and PC3 cells (Table 1). In-vitro cytotoxic activity from alcoholic extracts of soursop pericarp has been reported to on cell line U 937 (Human leukemic monocyte lymphoma cell line) 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. Different/Several researchers have indicated that acetogenins of *Annonaceae* are

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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 [22]. ~~h~~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 ~~Both alcoholic and aqueous extracts of soursop pulp and seed~~ 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 ~~alcoholic extract obtained by Soxhlet equipment (SSS) extract~~. Meanwhile, ~~seed aqueous extract of soursop (LSS) extract~~ showed a moderate selectivity on MCF-7 cells, since the SI value was slightly higher than 1.

Tabla 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 overexpression of the HER2/c-erb-2 gene); **SKBr3** (breast carcinoma, in which the HER2/c-erb-2 gene is overexpressed). ~~Fibroblasts were used as reference to determine the selective effect on the tumor cells.~~

3.2. Antioxidant Activity

The free radical scavenging potential of both seed and pulp extracts from soursop was determined in-vitro/in-vitro through the DPPH method just after added the reactive and after

30 min. ~~All Both alcoholic and aqueous extracts of soursop seed and pulp~~ showed a higher antioxidant activity at time 30 min than at time 0 min (Table 3).

Table 3. Antioxidant Activity of alcoholic and aqueous extracts of soursop

Extract	Percentage (%; v/v)	
	0 min	30 min
MSS	76.45 ± 0.19 ^{Aa}	83.23 ± 1.85 ^{Ba}
LSS	70.30 ± 0.49 ^{Ab}	84.41 ± 0.01 ^{Ba}
LSP	68.53 ± 0.20 ^{Ac}	69.77 ± 0.92 ^{Bb}
SSS	64.65 ± 0.21 ^{Ad}	69.43 ± 0.11 ^{Bb}

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 ($P < .05$) between times by each extract. Different lower-case letters (a, b, c, d) in the same column indicate significant difference ($P < .05$) between extracts by each time. Experiments were made in triplicate ~~and determinations by duplicated~~ ± standard deviationSD.

In addition, MSS (~~83.23 ± 1.85%~~) and LSS (~~84.41 ± 0.01%~~) extracts showed an antioxidant activity significantly higher ($P < .05$) than SSS (~~69.43 ± 0.11%~~) or LSP (~~69.77 ± 0.92%~~) 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 (68μmol), 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 significantly ($P < .05$) varied depending on kind of extract and concentration. ~~The Soursop seed alcoholic extracts (MSS and SSS) extracts~~ showed significant antimicrobial activity against *L. monocytogenes*, *Salmonella enterica ser.* Enteritidis, and *Staphylococcus aureus*; whereas, ~~aqueous extracts of soursop seed and pulp (LSS and LSP) extracts~~ did not show any antimicrobial activity. Additionally, antimicrobial activity of the extracts increased when higher concentrations were used (Table 4).

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

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

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 ($P < .05$) between extracts by each microorganism. Different lower-case letters (a, b) indicate significant difference ($P < .05$) between concentrations by microorganism and extract. Experiments were made in triplicate ~~and determinations by duplicated~~ ± standard deviationSD.

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 *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 is known that their antimicrobial activity is depending on the phytochemical constituents [7,28].

Differences significant ($P < .05$) were not found between zone of inhibition produced by MSS or SSS alcoholic extracts obtained from soursop seed by maceration (MSS) or Soxhlet equipment (SSS) (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. CONCLUSIONS

The soursop seed and pulp extracts obtained by several extraction methods showed high cytotoxic activity on tumor cell lines PC3 and HeLa were more sensible to all extracts of soursop than in comparison to control cells (fibroblasts); whereas, moderate cytotoxic activity was observed on tumor line MCF-7 and SKBr3 cells. A higher In addition, antioxidant activity of MSS and LSS soursop seed extracts obtained by maceration and decoction extracts were reached in comparison with significantly ($P < .05$) higher than those extracts of LSP pulp obtained by decoction or SSS extracts of seed obtained by Soxhlet equipment. Antimicrobial activity against pathogenic microorganisms *Listeria monocytogenes*, *Salmonella enterica* ser. Enteritidis and *Staphylococcus aureus* was only observed for SSS and MSS alcoholic extracts of soursop seed. Those findings provide an important new pathway that facilitates

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the development of chemopreventive or chemotherapeutic strategies against some cancers, and suggest that consumption of *ing-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~~determine which~~ specific compounds of these extracts that cause such effects.

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