

Nutritional composition, antioxidant activity and anticancer potential of *Syzygium cumini* (L.) and *Syzygium malaccense* (L.) fruits

Composição nutricional, atividade antioxidante e potencial anticâncer das frutas de *Syzygium cumini* (L.) e *Syzygium malaccense* (L.)

Composición nutricional, actividad antioxidante y potencial anticancerígeno de frutos de *Syzygium cumini* (L.) y *Syzygium malaccense* (L.)

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Abstract

Intending to highlight new fruits with nutraceutical potential, the present work reports the nutritional and antioxidant content of *Syzygium cumini* (L.) Skeels (*S. cumini*) and *Syzygium malaccense* (L.) Merr. & LM Perry (*S. malaccense*),

and evaluates the anticancer potential against CP-H460 (lung carcinoma line) and its functionality over HEK-293 (healthy embryonic kidney line), two human cells. For this, the physical-chemical characterization of the lyophilized fruits was carried out, and the content of total phenolic compounds (Folin-Ciocalteu) and the antioxidant potential (DPPH, FRAP, and ORAC) was determined. For anticancer activity, aqueous extracts were prepared and evaluated using the MTT (3-[4,5-dimethyl-triazol-2-yl]-2,5-diphenyltetrazolium) assay for periods of 20 hours. These two species are rich in dietary fibers, mainly insoluble fibers, and are sources of natural compounds and antioxidants, which possibly explain the protective potential against cancer cells. Thus, it is expected, as two source fruits, results obtained ($p < 0.01$), mainly *S. cumini* in contact with CP-H460, which reduces the growth of a cell with lung carcinoma. This finding revealed that these fruits have antiproliferative activity against a lung carcinoma cell, where the highest concentration tested (2 mg/mL) was able to inhibit almost 80% of cell proliferation. Besides, when *S. cumini* was evaluated in HEK-293, all concentrations evaluated showed cell viability superior to the positive control ($p < 0.01$). In conclusion, both *S. cumini* and *S. malaccense* can be used as nutraceuticals in complementary therapies given their nutritional properties.

Keywords: *Syzygium*; Natural antioxidant; Human embryonic kidney; Lung cancer; Nutraceuticals; Complementary therapy.

Resumo

Com a intenção de destacar novas frutas com potencial nutracêutico, o presente trabalho relata o conteúdo nutricional e antioxidante de *Syzygium cumini* (L.) Skeels (*S. cumini*) e *Syzygium malaccense* (L.) Merr. & LM Perry (*S. malaccense*), e avalia o potencial anticâncer contra CP-H460 (linhagem de carcinoma de pulmão) e sua funcionalidade sob HEK-293 (linhagem de rim embrionário saudável), duas células humanas. Para isso, foi realizada a caracterização físico-química das frutas liofilizadas, determinado o conteúdo de compostos fenólicos totais (Folin-Ciocalteu) e o potencial antioxidante (DPPH, FRAP e ORAC). Para a atividade anticâncer, extratos aquosos foram preparados e avaliados empregando-se o ensaio colorimétrico de MTT (3-[4,5-dimetil-triazol-2-il]-2,5-difeniltetrazólio) por um período de 20 horas. Essas duas espécies são ricas em fibras alimentares, principalmente fibras insolúveis, e são fontes de compostos e antioxidantes naturais, o que possivelmente explica o potencial protetor contra as células cancerígenas. Assim, como esperado, as duas frutas apresentaram resultados significativos ($p < 0,01$), principalmente *S. cumini* em contato com CP-H460, que impediu o crescimento de uma célula com carcinoma de pulmão. Esse achado revela que essa fruta apresenta atividade antiproliferativa contra uma célula de carcinoma de pulmão, onde a maior concentração testada (2 mg/mL) foi capaz de inibir quase 80% da proliferação celular. Além disso, quando *S. cumini* foi avaliado em HEK-293, todas as concentrações testadas apresentaram viabilidade celular superior ao controle positivo ($p < 0,01$). Em conclusão, tanto *S. cumini* quanto *S. malaccense* podem ser utilizadas como nutracêuticas em terapias complementares em vista de suas propriedades nutricionais.

Palavras-chave: *Syzygium*; Antioxidante natural; Rim embrionário humano; Câncer de pulmão; Nutracêuticos; Terapia complementar.

Resumen

Con la intención de destacar nuevas frutas con potencial nutracéutico, el presente trabajo reporta el contenido nutricional y antioxidante de *Syzygium cumini* (L.) Skeels (*S. cumini*) y *Syzygium malaccense* (L.) Merr. & LM Perry (*S. malaccense*), y evalúa el potencial anticanceroso contra CP-H460 (linaje de carcinoma de pulmón) y su funcionalidad sobre HEK-293 (linaje de riñón embrionario sano), dos células humanas. Para ello, se realizó la caracterización físico-química de los frutos liofilizados, determinando el contenido de compuestos fenólicos totales (Folin-Ciocalteu) y el potencial antioxidante (DPPH, FRAP y ORAC). Para la actividad anticancerígena, se prepararon y evaluaron extractos acuosos usando el ensayo MTT (3-[4,5-dimetil-triazol-2-il]-2,5-difeniltetrazolio) durante un período de 20 horas. Estas dos especies son ricas en fibras dietéticas, principalmente fibras insolubles, y son fuentes de compuestos naturales y antioxidantes, lo que posiblemente explique el potencial protector contra las células cancerosas. Así, como se esperaba, los dos frutos mostraron resultados significativos ($p < 0.01$), principalmente *S. cumini* en contacto con CP-H460, lo que impidió el crecimiento de una célula con carcinoma de pulmón. Este hallazgo revela que esta fruta tiene actividad antiproliferativa contra una célula de carcinoma de pulmón, donde la concentración más alta probada (2 mg/mL) fue capaz de inhibir casi el 80% de la proliferación celular. Además, cuando se evaluó *S. cumini* en HEK-293, todas las concentraciones probadas mostraron una viabilidad celular superior al control positivo ($p < 0.01$). En conclusión, tanto *S. cumini* como *S. malaccense* pueden utilizarse como nutracéuticos en terapias complementarias por sus propiedades nutricionales.

Palabras clave: *Syzygium*; Antioxidante natural; Riñón embrionario humano; Cancer de pulmon; Nutracéuticos; Terapia complementaria.

1. Introduction

Syzygium corresponds to one of the broadest genera of the Myrtaceae family, covering 1.200 to 1.800 species found in Africa, South Asia, Latin America, and the Pacific regions (Kishore, et al., 2019; Rochetti, et al., 2020). *Syzygium cumini* (L.)

Skeels (*S. cumini*), popularly known as jambolan, jamun, black olive, or purple plum, and *Syzygium malaccense* (L.) Merr. and LM Perry (*S. malaccense*), popularly known as red jambo or red apple, are exotic fruits that had their origin in India. Actually, they can be found in various regions of the world, including in Brazil, mainly in the Northeast and South Coast regions (Ayyanar, Subash-Babu & Ignacimuthu, 2013; Tavares, et al., 2016; Farias, et al., 2020). While *S. cumini* has a dark purple color when ripe and an astringent flavor, *S. malaccense* resembles a red apple and has a sweet taste. Both fruits can be consumed both *in nature* and in the preparation of drinks, jams, and even wine (Tavares, et al., 2016; Seraglio, 2018).

Currently, the preparation of extracts of these fruits has also been arousing interest, since stand out for their high bioactive potential and have high antioxidant properties against pro-oxidative damage that is induced by several factors, and that makes them able to act directly in the protection against cancer cells. Cancer starts when a normal cell begins to undergo various transformations and becomes an abnormal cell. So, the bioactive compounds present in natural products can interfere in different phases of the transformation of this cell, being able to block or reverse the initial stage of carcinogenesis in several periods. They can also halt or slow the development and progression of precancerous cells into malignant cells (Kee, et al., 2019; Farias, et al., 2020; Sunny, et al., 2020).

Until then, the extracts of *S. cumini* and *S. malaccense* have been more studied concerning their nutritional and chemical composition (Faria; Marques & Mercadante, 2011; Nunes, et al., 2016; Peixoto, et al., 2016; Tavares, et al., 2016; Batista, et al., 2017), and some therapeutic properties (similar for both fruits), such as type 2 diabetes mellitus (Farias, et al., 2020), and potential antiobesity (Xu, et al., 2019; Batista, et al., 2020). Regarding the anticancer potential, few studies address this issue and among them, the anticancer potential of the extract of the *S. malaccense* bark in a human hepatoma cell line (Vuolo, et al. 2018), presenting an inhibitory effect on the growth and proliferation of this type of cells. For *S. cumini*, the effect of the extract against breast carcinoma cells has been reported (Nazif, 2007), the effect on a lung carcinoma strain (Aqil et al., 2012), and the hepatoprotective effect of fruits in rats (Das & Sharma, 2009).

In summary, further studies on this subject are needed since the consumption of the fruits of *S. cumini* and *S. malaccense* can bring complementary nutritional benefits to the patient undergoing cancer treatment. According to the latest World Cancer Report by the International Cancer Research Agency (IARC), low consumption of fruits and vegetables is related to the increased incidence of various types of cancer (Wild, Weiderpass & Stewart, 2020). The incentive of alternatives derived from natural products has been gaining prominence in the scope of complementary therapies. However, despite its medicinal and nutritional importance of fruits, this species has not been so far analyzed for these parameters. Therefore, in this study, we aimed to evaluate the nutritional and antioxidant contents of *S. cumini* and *S. malaccense* fruits and evaluate the anticancer potential against CP-H460 and its functionality over HEK-293, two human cells.

2. Methodology

2.1 Sample preparation

S. cumini and *S. malaccense* were collected in Southern Brazil, and a voucher was deposited at the Municipal Botanical Museum of Curitiba, Parana, Brazil (n° MBM-095008 and n° MBM-379581, respectively). The research is registered under n° A340881 (SisGen – Brazil). Mature fruits were frozen (-18°C), lyophilized, and stored under vacuum.

2.2 Physico-chemical composition of the fruits

Moisture, fixed mineral residue, pH, titratable acidity, protein, fat and dietary fibers, were determined by official AOAC methods (AOAC 2000; AOAC 2005). Carbohydrates were calculated by difference considering the sum of moisture, protein, fixed mineral residue, fat and dietary fibers. Total soluble solids were read in a digital refractometer (AIQ, RTD-95, São Paulo, BR). The Total Energy Value was calculated according to Atwater's conversion (Osborne & Voogt, 1978).

2.3 Preparation of extracts

Two different extracts were made, one methanolic, used to identify total phenolic compounds and antioxidant activity (since there is a better extraction of components from this solvent), and an aqueous extract, used to assess the anticancer potential (since this is the closest to fresh fruit consumption). For the methanolic extract, the lyophilized fruits were dissolved in methanol: water (50:50) (v/v), according to Alves et al. (2007). The extracts were centrifuged at 2000 rpm for 15 minutes (Fanen 280R, São Paulo, Brazil®) and a new extraction was performed with ketone: water (70:30) (v/v). The extracts were filtered and concentrated in a rotary evaporator (USC-1400 Unique, São Paulo, Brasil®) under reduced pressure at 40 °C. For the aqueous extract, lyophilized samples were mixed with distilled water for 15 minutes in ultrasound (T 22-25 °C) and allowed for one day in magnetic stirring at room temperature (Bursal & Gulçin, 2011). The extracts were filtered, frozen, and lyophilized.


2.4 Total Phenolic contents (TPC)

TPCs were evaluated by spectrophotometry using the Folin-Ciocalteu reagent (Zielinski & Kozłowska, 2000; Pires, et al., 2017). Concentrations were prepared from the homogenization of the methanolic extract of the samples in methanol. Afterward, the aliquots of the test solutions were homogenized and the solutions remained at room temperature (25 °C) in the absence of light for 30 minutes. After that, we measured the absorbance in a spectrophotometer (Agilent Technologies, Cary 60 UV-VIS, Santa Clara, CA) at 700 nm. TPC was calculated using a calibration curve built with the gallic acid standard at different concentrations (2, 7, 10, 25, 50, 75, and 90 µg.mL⁻¹). The results were expressed as milligrams of gallic acid equivalent (mg GAE g⁻¹).

2.5 Profile of phenolic compounds

Over the years, several compounds have been identified for *S. cumini* and *S. malaccense*, and therefore, we compiled the compounds already mentioned in the literature for both species (Table 1).

Table 1 – Profile of bioactive compounds cited in the literature of *S. cumini* and *S. malaccense*.

Specie	Class	Compounds	Reference
 (The authors, 2020)	Anthocyanins	Cyanidin 3,5-diglucoside	Faria, Marques and Mercadante (2011)
		Delphinidin 3,5-diglucoside	Farias et al. (2020)
		Delphinidin acetyl-diglucoside	
		Delphinidin-3-O-glucoside	
		Malvidin 3-glucoside	
		Malvidin 3,5-diglucoside	
		Petunidin 3-glucoside	
		Peonidin 3,5-diglucoside	
		Petunidin 3,5-diglucoside	
		Phenolic acids	Ellagic acid
Chlorogenic acid	Branco et al. (2016)		
<i>p</i> - coumaric acid	Azima et al. (2017)		
Vanillic acid	Seraglio et al. (2018)		
Ferulic acid	Farias et al. (2020)		
Gallic acid	Tavares et al. (2016)		
Syringic acid	Chang et al. (2018)		
3,4- dihydroxybenzoic acid			

	Catechin	
	Epicatechin	
	Epigallocatechin	
	Gallacatechin	
	Laricitrin-3- <i>O</i> -galactoside	
	Laricitrin-3- <i>O</i> -glucoside	
	Myricetin	
	Myricetin-3- <i>O</i> -galactoside	
	Myricetin-3- <i>O</i> -glucoside	
	Myricetin-3- <i>O</i> -glucoside	
	Myricetin-3- <i>O</i> -pentoside	
	Myricetin-3- <i>O</i> -rhamnoside	
	Quercetin	
	Rutin	
	Syringetin-3- <i>O</i> -galactoside	
	Syringetin-3- <i>O</i> -glucoside	
	<hr/>	
Tannins	Gallotannins	Chang et al. (2018)
	Ellagitannins	
	<hr/>	
Carotenoids	<i>All-trans</i> -lutein	Faria, Marques and Mercadante (2011)
	<i>All-trans</i> - β –carotene	
	Phytoene	
	<hr/>	
Anthocyanins	Cyanidin 3,5-diglucoside	Nunes et al. (2016)
	Cyanidin 3-glucoside	Batista et al. (2017)
	Peonidin 3-glucoside	Farias et al. (2020)
	<hr/>	
Phenolic acids	Benzoic acid	Reynertson et al. (2008)
	Chlorogenic acid	Batista et al. (2017)
	Ellagic acid	Vuolo et al. (2018)
	Gallic acid	Farias et al. (2020)
	<i>p</i> - coumaric acid	
	<hr/>	
Flavanols and Procyanidins	Catechin	Reynertson et al. (2008)
	Epicatechin	Nunes et al. (2016)
	Epicatechin gallate	Farias et al. (2020)
	Isorhamnetin	
	Isoquercitrin	
	Kaempferol	
	Procyanidin	
	Quercetin	
	Quercitrin	
	Rutin	



(The authors, 2020)

These data were reported in the literature by the authors mentioned in this table. Source: Authors.

2.6 Antioxidant properties

2.6.1 Antioxidant properties by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate)

A methanol solution containing 0.06 mmol.L^{-1} DPPH was prepared and stored at 20°C for later use. We prepared the working solution by diluting the stock solution of DPPH (0.06 mmol.L^{-1}) in methanol until we obtained a solution with an absorbance of approximately 0.980 ± 0.02 at 515 nm (Brand-Willians, Cuvelier & Berset, 1995; Rufino, et al., 2011). Results were expressed in μmol equivalent of Trolox 100 g^{-1} .

2.6.2 Ferric reducing antioxidant power (FRAP) assay

A solution of the methanolic extract with FeCl_3 (3 mmol.L^{-1} in 5 mmol.L^{-1} citric acid) was mixed and incubated for 30 minutes in a water bath at 37°C . Then, 2,4,6-tripyridyl-s-triazine (TPTZ) solution (3.6 mL) was added, vortexed and after 10 minutes, reading at 620 nm (HP 8452A spectrophotometer, Cheadle Heath, Stockport Cheshire, United Kingdom). Results were expressed in μmol equivalent of Trolox 100 g^{-1} (Benzie & Strain, 1996).

2.6.3 Oxygen Radical Absorbance Capacity (ORAC) assay

Aliquots of $20 \mu\text{L}$ of the samples were added to $120 \mu\text{L}$ fluorescein solution (61.2 nM) and incubated for 10 minutes at 37°C . After, $60 \mu\text{L}$ of 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) solution (19 mM) was added to the microplates starting the reaction. Fluorescence intensity was measured kinetically every 1 minute (excitation: $485/20 \text{ nm}$ and emission: $528/20 \text{ nm}$) until the fluorescence value is less than or equal to 0.5% of the initial fluorescence. The antioxidant activity was expressed as μmol equivalent of Trolox 100 g^{-1} (Zulueta, Esteve & Frigola, 2009).

2.7 Cell proliferation assay

We tested two cell lines, CP-H460, considered a lung carcinoma line, and HEK-293, considered a lineage of a human embryonic kidney, according (Schiller, et al., 1992; Aguilar, 2011), with some modifications. For this, the colorimetric analysis was used with 3-[4,5-dimethyl-triazol-2-yl]-2,5-diphenyltetrazolium (MTT).

Cells were trypsinized and subcultured into 96-well plates, seeded at the density of 3×10^4 cells/well, and allowed to adhere. After, $15 \mu\text{L}$ of MTT (5 mg.mL^{-1}) was added and incubated for 4 hours. Subsequently, $70 \mu\text{L}$ of 10% Sodium Dodecyl Sulfate (SDS) was added to the wells and the plates were maintained for another 16 hours at 37°C . Absorbance at 595 nm was measured at a spectrophotometer. Data were expressed in cell viability (%) of the fruits in comparison with the positive control (RPMI medium and MTT).

2.8 Statistical analysis

All measurements were carried out in triplicate, and the results were expressed as mean values \pm standard deviation. Statistical significance analysis was performed by T student test ($p < 0.01$) for nutritional composition and antioxidant capacity, and by ANOVA followed by Tukey's test ($p < 0.01$) for cell viability. For this, statistical software GraphPad Prism 6 (Graphpad Software, Inc, San Diego, CA) was used.

3. Results and Discussion

3.1 Physico-chemical composition of the fruits

S. cumini and *S. malaccense* are fruits with high amounts of carbohydrates, mainly of dietary fibers, when *S. malaccense* stands out for the content of dietary fibers ($19.50 \pm 0.34 \text{ } 100 \text{ g}^{-1}$), almost double the content found in *S. cumini* ($11.38 \pm 0.50 \text{ } 100 \text{ g}^{-1}$). Also, the two fruits in our study showed higher values of dietary fiber when compared to two other fruits

of the Myrtaceae family, *Eugenia brasiliensis* Lamarck (4.65–5.94 100 g⁻¹), popularly known as Brazilian cherry, and *Myrciaria jaboticaba* (Vell.) O. Berg (3.47-3.88 100 g⁻¹), popularly known as jaboticaba (Schulz, et al., 2020). The high values of dietary fiber make the two studied fruits of the *Syzygium* genus of great interest for consumption and even food enrichment.

Table 2 - Physico-chemical composition of lyophilized *S. cumini* and *S. malaccense* fruits.

Parameter	<i>S. cumini</i>	<i>S. malaccense</i>	p-value
Moisture (100 g ⁻¹)	8.63 ± 0.59	11.9 ± 0.25*	0.0003
Fixed mineral residue (100 g ⁻¹)	1.99 ± 0.24	4.79 ± 0.17*	0.0005
Protein (100 g ⁻¹)	0.33 ± 0.01	2.90 ± 0.03*	<0.0001
Fats (100 g ⁻¹)	0.36 ± 0.06	1.85 ± 0.10*	<0.0001
Carbohydrates (100 g ⁻¹)	77,31 ± 0.34*	59.06 ± 0.21	<0.0001
Total dietary fiber (100 g ⁻¹)	11,38 ± 0,50	19.50 ± 0.34*	<0.0001
Soluble fiber (100 g ⁻¹)	1,98 ± 0,78	0.56 ± 0.07	0.0347
Insoluble fiber (100 g ⁻¹)	9.4 ± 0,24	18.94 ± 0.06*	<0.0001
pH	3.94 ± 0.09	3.77 ± 0.01	0.0263
Total soluble solids % (°Brix)	11.73 ± 0.05*	8.37 ± 0.01	<0.0001
Titrateable acidity (g citric acid)	1.19 ± 0.07*	0.58 ± 0.002	0.0001
Total Energy Value (kcal)	313.08	263.95	

Mean values + or - standard deviations of samples in triplicate. Asterisks in the same line denote significant differences (T student test p <0.01).Source: Authors.

Moreover, *S. cumini* has a protein content nine times higher when compared to *S. malaccense*. On the other hand, *S. malaccense* has more total soluble solids and a higher pH (Table 2). In general, eating foods with various nutritional components, such as dietary fiber, have many benefits to the individual's body and health since it is associated with a lower occurrence of chronic intestinal disorders and diseases, like obesity, diabetes, cardiovascular diseases, and cancer (Al-Sheraji, et al., 2011). Some studies have linked an inverse association between fruit consumption with high fiber concentrations and cancer risk, especially prostate cancer (Deschasaux, et al., 2014), upper digestive tract, and lung cancer (Bradbury, Appleby & Key, 2014).

Until then the relationship mechanism between the protection of food with dietary fiber against cancer cells is due to the anti-inflammatory properties of the fibers (King, et al., 2007). In foods, fibers are used to slow curing, control moisture, and the formation of ice crystals and increase stability (Al-Sheraji, et al., 2011). All of these properties increase the interest in using fruits in the medicinal and nutraceutical area.

3.2 Total Phenolic Contents (TPC) and antioxidant properties

The methanolic extract of the two fruits evaluated presented great sources TPC (Table3), and *S. cumini* stood out, exhibit values almost three times higher (596.06 ± 44.06 mg GAE 100g⁻¹) than *S. malaccense* (202.30 ± 4.20 GAE 100g⁻¹) (p<0.01). With these values, *S. cumini* is considered a fruit with a high content of TPC (> 500 mg GAE 100 g⁻¹) and average content of TPC for *S. Malaccense* (100–500 mg GAE 100 g⁻¹) (Souza, et al., 2012).

Table 3 - Total phenolic contents and antioxidant activity of the fruits.

Parameter		<i>S. cumini</i>	<i>S. malaccense</i>
Total phenolic compounds (mg GAE 100g ⁻¹)		596.06 ± 44.06*	202.30 ± 4.20
Antioxidant Activity (µmol TEAC 100g ⁻¹)	DPPH	3426.59 ± 10.78*	160.73 ± 34.31
	FRAP	2942.26 ± 168.47*	249.52 ± 16.37
	ORAC	3176.54 ± 60.24*	372.42 ± 9.50

Mean values + or - standard deviations of samples in triplicate. Asterisks in the same line denote significant differences (T student test p <0.01).Source: Authors.

Both fruits have different bioactive compounds already described in the literature, mainly *S. cumini*, which has around 23 different phenolic acids and almost 10 types of anthocyanin classes (Table 1), widely used as a source of natural additive. This mixture of natural bioactive compounds together with the dietary fibers present in the two fruits evaluated is of great relevance for complementary therapies since it is already described in the literature that natural foods that have complex mixtures of nutrients offer different health benefits, due to their synergistic effect (Singh, et al., 2019).

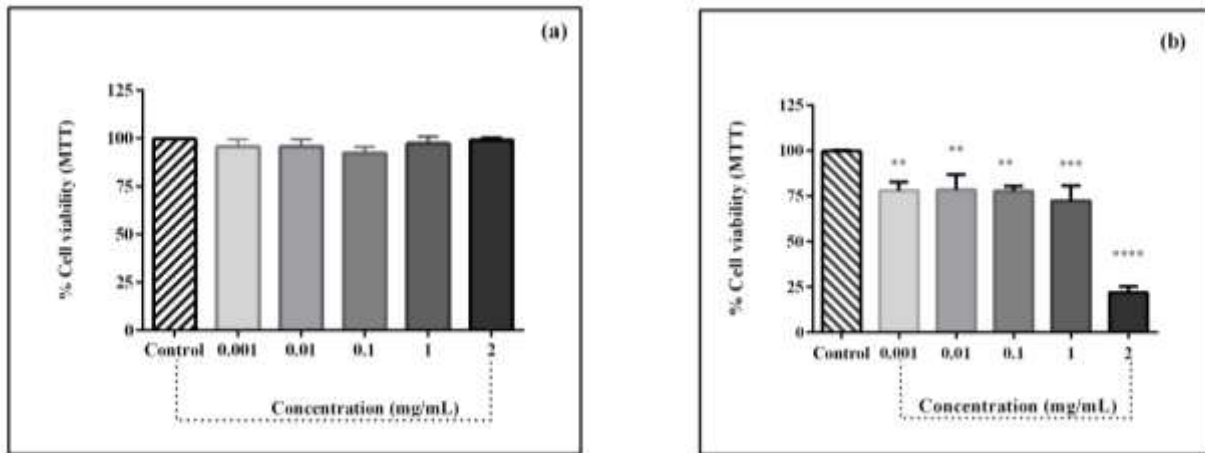
Also, we highlight that the large amounts of phenolic compounds are often used as a quick screening for the antioxidant potential of food (Singh, et al., 2019). In this way, according to the values found in this study, the two fruits are good sources of natural antioxidants, mainly by the DPPH method for *S. cumini* (3426.59 ± 10.78 mg GAE 100g⁻¹) and ORAC for *S. malaccense* (372.42 ± 9.50 mg GAE 100g⁻¹).

In summary, increasing the consumption of natural foods with bioactive compounds and, consequently, antioxidant potential, has in recent years become a good alternative to reduce oxidative damage of the organism that is associated with stress and can generate several pathologies, such as Diabetes mellitus, Arterial hypertension, Mal Alzheimer's disease, Parkinson's disease and cancer (Ali, et al., 2008).

3.3 Cell proliferation

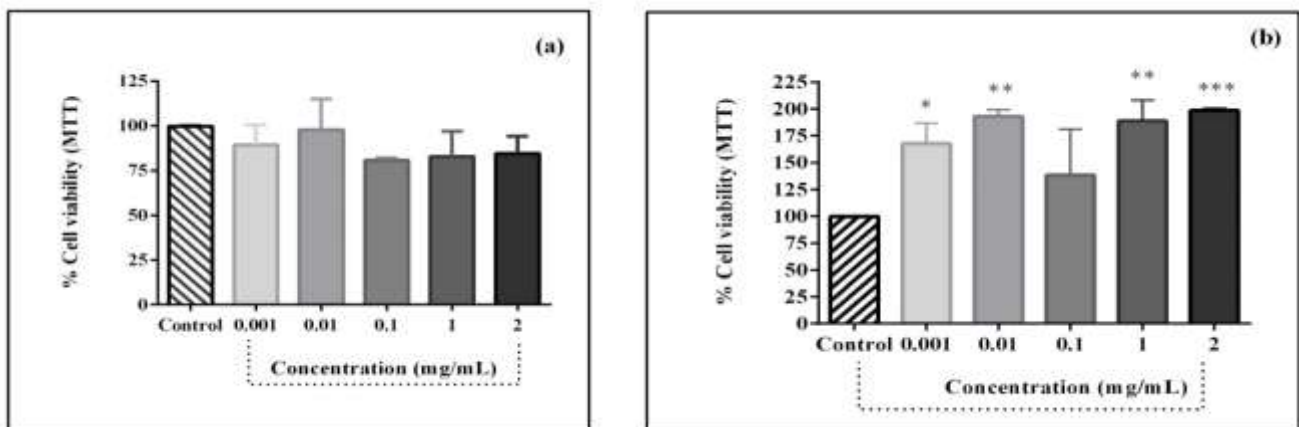
Figure 1 and Figure 2 show the percentage of cell viability of the two fruits tested against CP-H460 and HEK-293. *S. cumini* shows a very potent ant proliferative effect against the CP-H460 cancer cell line. As we increased the concentration of *S. cumini* extract in contact with this cell, it decreased the proliferation (Figure 1b), revealing a concentration-dependent protective effect. On the other hand, this same extract has no ant proliferative effect on a healthy cell (HEK-293). Thus, we can emphasize that the aqueous extract of *S. cumini* may arouse interest as a new agent capable of inhibiting the growth of cancer cells and that in addition, it does not interfere in human cells considered healthy.

Figure 1 - Cell viability of *S. malaccense* (a) and *S. cumini* (b) aqueous extract after 20 hours of the induced oxidative stress in cells CP-H460.



Mean values + or - standard deviations. Asterisks represent the statistical difference found using ANOVA followed by Tukey's test ($p < 0.01$). Source: Authors.

Figure 2 - Cell viability of *S. malaccense* (a) and *S. cumini* (b) aqueous extract after 20 hours of the induced oxidative stress in cells HEK-293.



Mean values + or - standard deviations. Asterisks in the same line denote significant differences (ANOVA followed by Tukey's test ($p < 0.01$)). Source: Authors.

The greatest finding in our study, *S. cumini*, where the highest concentration of the extract (2 mg/mL), was able to inhibit approximately 80% of the viability of the lung carcinoma cell. This type of cancer is currently considered the most prevalent type, both for its incidence (millions of new cases registered annually) and for its low survival rate (Wild, Weiderpass & Stewart, 2020). Furthermore, in addition to inhibiting the growth of a cancer cell, the fruit is even more valued when not present toxicity in a cell considered healthy, as is the case of *S. cumini*. We emphasize that the higher concentration of the fruit extract increased almost 100 times more the proliferation of that cell line ($p < 0.01$). However, for this line, the extract showed a synergistic effect, where the concentration of 0.01 mg / mL showed the second-highest percentage of cell viability, behind only the concentration of 2 mg/mL. This effect was already highlighted in our study and it happens due to the mixture of compounds that the fruit presents (Singh, et al., 2019).

Fruits are known for their high levels of antioxidants and phenolic compounds that they present, and we found in this study a high antioxidant potential mainly in *S. cumini*. This content of antioxidants present in the fruit can decrease the levels

of reactive oxygen species in the body, and in this way, they can prevent damage to DNA and possible mutations that will prevent the progression of the cancer cell (Zhang, et al., 2008; Hogan, et al., 2010). There are already assumptions about the action that natural products can present in the proliferation of cancer cells (Tsai, et al., 2018), and Aqil et al. (2012) reported a similar effect of the ethanolic extract of the fruit against lung carcinoma, however of an A549 strain, different from that used in this study (CP-H460). Also, an ethanolic extract of *S. cumini* fruit has been reported on its protective effect against breast carcinoma cells. In this study, the amount of anthocyanins presents in the fruit was associated with the protective effect (Nazif, 2007), but in our study the extraction was different and there may be interference from other compounds, like dietary fibers.

Besides, other fruits of the Myrtaceae family, have been related to chemopreventive effects, such as *Eugenia brasiliensis* (for breast cancer), *Myrciaria cauliflora* (for colon cancer), *Myrciaria jaboticaba* (for leukemia, prostate, lung, breast, hepatic and cervical cancer) (Schulz, et al., 2020). Thus, *S. cumini* appears as a new alternative for chemoprevention. For *S. malaccense*, it is important to emphasize that the effects found were not statistically significant in the doses tested for the two cell lines. But, on the other hand, it also does not inhibit the growth of a normal embryonic cell, expected effect when it comes to fruits, since it has antioxidant properties that act without control of acute kidney injury and chronic kidney disease (Reyes-Fermin, et al., 2020).

4. Conclusion

Under preliminary laboratory conditions, *S. cumini* demonstrated the ability to decrease the viability of a lung carcinoma cell (almost 80%), and thus, could be considered a promising anticancer agent in complementary therapies. Furthermore, these both *S. cumini* and *S. malaccense* fruits can be used as a nutraceutical due to their nutritional properties because they are rich in dietary fiber, mainly insoluble fiber, and the two species have a good content of phenolic compounds and antioxidant properties.

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