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Original Article

Bioactive properties of *Persea lingue* Ness (Lauraceae) fruit and leaf extracts

Propriedades bioativas de extratos de folhas e frutos de *Persea lingue* Ness (Lauraceae)

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Abstract

Persea lingue Ness is a tree species that lives mainly in temperate forests of south-central Chile. Its leaves are used in ethnomedicine, the fruit is a drupe similar to that of the avocado and has not been studied. The aim of this study was to determine the cytotoxicity in leukemia cell and antibacterial activity, along with some chemical content characteristics of *P. lingue* fruit and leaf extracts. The antibacterial activity was determined by the inhibition of bacterial growth in liquid medium assay against Gram-positive and Gram-negative bacteria. The leukemia cell lines Kasumi-1 and Jurkat were used to evaluate the cytotoxic activity by using propidium iodide and AlamarBlue assays. Total phenolic, flavonoid, condensed tannin, alkaloid and lipid contents were evaluated in the fruit and in the leaf extracts. The antioxidant activity of both extracts were also elavaluated. Leaf extract presented the highest content of total phenols, condensed tannins and flavonoids, and also the highest antioxidant activity. While the fruit extract has a higher amount of lipids and alkaloids and the high antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus megaterium* and *Micrococcus luteus*. The leaf extract only showed activity against *M. luteus*. Concerning the cytotoxic activity, only the fruit extract showed cytotoxicity against the cell lines Jurkat and Kasumi-1. *P. lingue* fruit extract is a potential source of biologically active molecules for the development of new drugs to be used in some types of leukemia, as well as antibacterial agent.

Keywords: *Persea lingue*, antibacterial activity, cytotoxic activity, secondary metabolite.

Resumo

Persea lingue Ness é uma árvore que vive principalmente na floresta temperada do centro-sul do Chile. As folhas são usadas na etnomedicina. O fruto é uma drupa similar ao abacate e que nunca foi pesquisada anteriormente. O objetivo deste estudo foi o de avaliar a citotoxicidade em células leucêmicas e as atividades antibacterianas, assim como algumas características químicas do extrato de fruto e da folha do *P. lingue*. As atividades antibacterianas foram determinadas pelo método da inibição do crescimento bacteriano em meio líquido empregando-se bactérias Gram-positivas e Gram-negativas. As linhagens celulares leucêmicas, Kasumi-1 e Jurkat foram usadas para avaliar a atividade citotóxica em ensaios empregando-se iodeto de propídio e AlamarBlue. Foram avaliados os teores totais de fenóis, flavonóides, taninos condensados, alcalóides e lipídeos presentes nos extratos das folhas e dos frutos. As atividades antioxidantes de ambos os extratos também foram avaliadas. O extrato das folhas foi o que apresentou o maior conteúdo de fenóis, taninos condensados e flavonóides totais e a maior atividade antioxidante. Já o extrato de fruto apresentou a maior quantidade de lipídios e alcaloides e a melhor atividade antibacteriana contra *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus megaterium* e *Micrococcus luteus*. Já o extrato das folhas apresentou apenas atividade contra *M. luteus*. Em relação à atividade citotóxica, apenas o extrato do fruto apresentou citotoxicidade contra as linhagens celulares Jurkat e Kasumi-1. Em resumo, o extrato do fruto de *P. lingue* é uma potencial fonte de moléculas com atividade biológica para o desenvolvimento de novos fármacos a serem utilizados em alguns tipos de leucemia, bem como agente antibacteriano.

Palavras clave: *Persea lingue*, atividade antibacteriana, atividade citotóxica, metabolito secundário.

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1. Introduction

The interest in searching for new chemical compounds biologically active has raised research on flora with a history of traditional folk medicine (Fulda and Efferth, 2015). The main goal is to develop new drugs against diseases such as cancer, diabetes, Alzheimer, among others; and also find new molecules with antibacterial activity due to antibiotic resistance and the lack of new antimicrobials (Elisha et al., 2017).

Vascular plants produce a wide variety of secondary metabolites, such as polyphenols, alkaloids, and terpenes (Delgoda and Murray, 2017). These compounds are used as protection mechanisms against phytopathogens, such as viruses, bacteria and fungi, also from herbivores and insect attack; as well as being antioxidants, free radical scavengers, UV light absorbers and antiproliferative agents (Fulda and Efferth, 2015; Wink, 2003; Kennedy and Wightman, 2011; Freiesleben and Jäger, 2014).

The species of the Lauraceae family have a wide variety of uses, ranging from spices to drugs (Palazzo et al., 2009). One of its main genera is *Persea*, with about 190 species distributed in the tropical and subtropical zones of America and Asia (van der Werff, 2002); highlighting the species *Persea americana* Mill. (avocado), reporting an important biological activity as antioxidant, antimicrobial, antitumor, antidiabetic and antimalarial (Lima et al., 2012; Guzmán-Rodríguez et al., 2013; Jesus et al., 2015), especially the fruit of this species, where they found different beneficial properties for human health (Yasir et al., 2010), including phytochemical compounds with potential in use for some types of cancer (Dreher and Davenport, 2013).

Persea lingue Ness, is a tree species up to 30 meters high and lives mainly in the temperate forests of south-central Chile, and its conservation status is of least concern (LC) in most of the Chilean territory according to the latest report of the Ministry of Environment (Chile, 2019). This plant, has been highly exploited in the past for the excellent quality of its wood used in the manufacture of fine furniture, and by the use of their husk, used in leather tanning due to its high tannin content (Donoso and Escobar, 2006; Cob et al., 2010); also has use in ethnomedicine by using its leaves to treat some diseases (Holler et al., 2012a, b). The study of compounds with chemical or medicinal properties of fruit, it is practically nil. The fruit of *P. lingue* is a drupe that resembles the fruit of the avocado, but of smaller size, with 14 to 17 mm in length and with 11 to 14 mm of diameter. It presents a violet color to bright black when ripe, with bitter taste, and serves as food mainly for avifauna, and local rodents (Vergara et al., 2010; Gho-Illanes et al., 2015). As for human consumption, there are few reports, the Mapuche's, a people originally from Chile, formerly prepared an alcoholic beverage, "chicha", based on fermentation of their fruit (Pardo and Pizarro, 2016).

In order to find new extracts of plants with a pharmacological potential, the objective of this study was to determine the cytotoxicity against leukemia cells linkage and antibacterial activity, along with some chemical content characteristics of *Persea lingue* fruit and leaf extracts.

2. Material and Methods

2.1. Plant material

The fruit and leaf samples of *P. lingue* were collected in the Province of Cautín, Region of La Araucanía, Chile; identified by Dr. Alicia Marticorena and specimens were deposited at the Herbarium CONC (Universidad de Concepción) with register N° 189838. They were washed with distilled water and dried at 30 ± 2 °C for 72 h. The samples were ground for further maceration. The maceration was performed with 10 g of dry material in 50 mL of methanol, the resulting solution was sonicated for 15 min (× 3). Subsequently, the blend was filtered on Whatman No. 1 paper and the solvent eliminated on a rotary evaporator (BÜCHI R-210) at reduced pressure and at a temperature of 40 °C, finally the resulting sample was lyophilized.

2.2. Chemical analysis

2.2.1. Liquid chromatography/electrospray mass spectrometry (LC/ESI-MS)

LC/ESI-MS data were obtained on a mass spectrometer model 3100 coupled to a liquid chromatograph model Alliance 2695 both from Waters (Milford, MA, USA) using a Phenomenex (Torrance, CA, USA) Gemini C_{18} column (2.0 mm × 150 mm, 3.0 µm particle size, and 110 Å pore size). Solvent A was 0.1% TFA in water, and solvent B was 90% methanol in solvent A. The gradient was 5-95% B for 30 min, and molecules were detected at 220 and 280 nm. Mass measurements were performed in a positive mode with the mass range between 100 and 2000 m/z. Tentatively identified compounds were found based on the comparison of spectra with the ones reported in the literature.

2.3. Antioxidant assays

2.3.1. DPPH radical scavenging activity assay

The antioxidant activity was determined by free radical DPPH (2,2′-diphenyl-1-picrylhydrazyl) method proposed by Brand-Williams et al. (1995) with some modifications. To 0.1 mL of extract was added 3.9 mL of DPPH methanolic solution (100 µM), a blank was prepared with methanol instead of sample, finally the mixture was stirred left in the dark for 30 min. The absorbance of blend was measured at wavelength at 515 nm. The result was expressed as percent inhibition of DPPH• by the following Equation 1,

$$\%inhibition = \left[1 - \frac{AB}{AE}\right] \times 100 \quad (1)$$

where, AB is the absorbance of sample and AE is the absorbance of the blank.

For the determination of IC_{50} with DPPH• radical, a curve between 5 to 100 µg/mL was used for the fruit extract and 1 to 20 µg/mL for leaf extracts and Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, chemical control). The IC_{50} was determined from the percent inhibition plot versus the sample concentration. The IC_{50} evaluations of the DPPH• radical were performed in triplicate.

2.3.2. ABTS radical scavenging activity assay

The antioxidant assay was determined according to method proposed by Ozgen et al. (2006). The ABTS reagent (2,2-azino-bis-3- ethylbenzothiazoline-6-sulfonic acid) was used, 2.45 mM potassium persulfate solution was mixed with that of 7 mM ABTS in water, the mixture was allowed to stand for 16 h. The absorbance of $ABTS^{\bullet+}$ radical solution was adjusted to 0.700 ± 0.01 at 734 nm by the addition of sodium acetate-acetic acid buffer (20 mM at pH 4.5). Finally, 0.1 mL of methanolic extract was added 2.9 mL of the solution containing the free radical $ABTS^{\bullet+}$, in addition to a blank without sample. The solutions were left in dark for 30 min. The absorbance of the mixture was measured at 734 nm. The result was expressed as percentage of inhibition of $ABTS^{\bullet+}$ using Equation 1. For the IC_{50} of $ABTS^{\bullet+}$ a curve between 20 to 80 µg/mL was used for fruit extract and 10 to 30 µg/mL for leaf extract and Trolox. The IC_{50} was determined from the percent inhibition plot versus the sample concentration. The IC_{50} determinations of $ABTS^{\bullet+}$ were performed in triplicate.

2.3.3. Total phenolic content

The analysis of total phenols was performed by spectrophotometry based on a colorimetric oxidation-reduction reaction described by Ashraf et al. (2016) with some modifications. 0.1 mL of the methanolic extract (2.4 mg/mL) was added 1.6 ml of distilled water, 0.4 mL of Folin-Ciocalteu reagent, 0.80 mL of Na_2CO_3 (10% w/v) and 1.1 mL of water, the mixture was then left for 30 min at room temperature and in the dark, absorbances were measured at 765 nm. The calibration curve was done with gallic acid (Sigma-Aldrich), between 0.25 to 20 mg/L. The content of total phenols was expressed in mg EAG/g (mg equivalents of gallic acid per gram of dry extract).

2.3.4. Total flavonoid content (flavone/flavonol)

The total flavonoids content were determined by spectrophotometry based on the formation of a colored complex between Al (III) ions and carbonyl, and hydroxyl groups of flavonoid as described by Lillo et al. (2016). 0.1 mL of the methanolic extract (2.4 mg/mL) was added 0.2 mL of $AlCl_3$ (2% w/v) in ethanol, 0.2 mL of 1 M sodium acetate and 3.5 mL methanol, the mixture was then left for 30 min at room temperature and in the dark, absorbances were measured at 425 nm. The standard curve was made with quercetin (Sigma-Aldrich), between 0.0625 to 25 mg/L. The content of total flavonoids was expressed in mg EQ/g (mg equivalent of quercetin per gram of dry extract).

2.3.5. Total condensed tannin content (proanthocyanidins)

The contents of condensed tannins were measured according to the conditions of Price et al. (1978). An aliquot of 20 µL (2.4 mg/mL) of extract was added 180 µL of methanol, mixed with 1.2 mL of 4% w/v vanillin in methanol, 600 µL of HCl and reacted for 30 min; the absorbance was determined at 500 nm. A standard curve was established with (+)-catechin (Sigma-Aldrich), between 1 to 20 mg/L. The content of total condensed tannins was expressed as mg EC/g (mg equivalent of catechin per gram of dry extract).

2.3.6. Total alkaloid content

The alkaloid content was evaluated by colorimetric method described by Nuñez et al. (2018). 5 mL of chloroform extract was taken and 5 mL of phosphate buffer pH 4.7 and 5 mL of bromocresol green solution (34.9 mg bromocresol green with 15 mL of NaOH 1N) were added, the mixture was transferred to a separating funnel, after three time liquid-liquid extractions with 2 mL of chloroform. The absorbance of the resulting organic phase was read at 470 nm. The calibration curve was done with berberine (Sigma-Aldrich) from 2 to 10 mg/L. The content of total alkaloids was expressed in mg EB/g (mg equivalents of berberine per gram of dry extract).

2.3.7. Total lipid content

The determination of the total lipids was made by the methodology proposed by Knight et al. (1972) with some modifications. Lipid quantification is performed by the Sulfo-Phosfo-Vanillin reagent (SFV), generating a stable pink colorimetric reaction between the carbonium ion derived from the unsaturated hydrocarbon chain of lipids and the aromatic phosphate ester (vanillin). The lyophilized sample was previously solubilized in methanol: chloroform: water (2:1:0.8 v/v/v) to extract lipids. To 30 µL of sample is added 270 µL of concentrated sulfuric acid, the mixture is heated at 100 °C for 10 min., cooled to room temperature and add 750 µl of vanillin reagent (600 mg vanillin in 500 mL phosphoric acid), heat at 37 °C for 15 min. and cool to room temperature in darkness for 45 min. The absorbance was determined at 530 nm. The calibration curve was done with oleic acid from 1 to 50 mg/L. The content of total lipids was expressed in mg OA/g (mg equivalents of oleic acid per gram of dry extract).

2.4. Antibacterial activity

2.4.1. Minimal inhibitory concentration (MIC)

Antibacterial activity was evaluated by a liquid medium inhibitory growth of bacterial assay as described by Sutti et al. (2015). In a 96-well micro plate, 20 µL of plant extract (4 mg/mL in DMSO 8% w/v), were applied to wells of 80 µL of the culture medium Poor Broth (PB) used for bacteria in the logarithmic phase of growth. The absorbance of the cultures was measured after 24 h incubation at 30 °C on a microplate reader Victor3 (1420 Multilabel Counter/ Victor3 – Perkin Elmer) at 595 nm.

The fruit and leaf extracts of *P. lingue* was tested against six bacterial strains, Gram-positive: *Staphylococcus aureus* ATCC 29213, *Bacillus megaterium* ATCC 10778 and *Micrococcus luteus* A270 and Gram-negative: *Escherichia coli* D31, *Enterobacter cloacae* β12 and *Pseudomonas aeruginosa* ATCC 27853. The concentration range was 800 to 1.56 µg/mL. Streptomycin was used as a positive control, and 8% DMSO was also used as solvent control. MICs were expressed as the interval of concentrations [a]–[b], where [a] is the highest concentration tested at which the microorganisms were growing and [b] is the lowest concentration that causes 100% growth inhibition. All analyzes were performed in triplicate.

2.5. Cytotoxic activity

2.5.1. Cell lines and culture conditions

The acute myeloblastic leukemia cell line Kasumi-1 and human acute T lymphocytic leukemia cell line Jurkat was cultured in suspension in RPMI 1640 medium (Cultilab, Brazil) supplemented with 10% fetal calf serum (FBS, Cultilab, Brazil), 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere at 37 °C under 5% CO_2.

2.5.2. Propidium iodide (PI)/Calcein assay

The cytotoxic activity was evaluated using the method propidium iodide/calcein described by Vermes et al. (1995) with some modifications. Cells of Kasumi-1 and Jurkat were plated onto 96-well microplates (10^5 cell/mL) in RPMI 1640 supplemented with 10% FBS in the absence or presence of the extracts *P. lingue* (100 µg/mL), for 48 h in a humidified atmosphere at 37 °C and 5% CO_2. After this period, the cells were washed with PBS and resuspended, to which 5 µg/mL of PI and 1 µM of calcein were added. The cells were incubated for 20 min at room temperature and protected from light. After incubation, the cells were washed with PBS and immediately analyzed on an Accuri C6 flow cytometer and Acurri C6 software. A total of 10,000 events were collected per sample. For the determination of the EC_{50} a curve was realized from 0.5 to 100 µg/mL for Jurkat, the EC_{50} is the concentration of extract that gives half-maximal response cell viability (%), the EC_{50} value was calculated using a nonlinear regression curve.

2.5.3. AlamarBlue assay

This cytotoxic activity was evaluated using the method AlamarBlue described by Nakayama et al. (1997). The adherent cells of Jurkat and Kasumi-1, were plated onto 96-well microplates (2×10^4 cells/mL), and after 24 h were washed with PBS and subsequently incubated with the extracts of *P. lingue* (100 µg/mL) for 48 h. After incubation, the medium was aspirated and 200 mL of sodium resazurin (Sigma-Aldrich, USA) solution prepared in culture medium without serum was added (1 mg/mL), the solution was in contact with the cells for 4 h. Then, 100 µL of the medium was transferred to 96-well black plates and the reduction of resazurin was measured by fluorescence analysis ($\lambda_{Ex.}$ = 560 nm, $\lambda_{Em.}$ = 590 nm) performed on a microplate reader FlexStation 3. Cell viability was normalized assuming control as 100%.

2.6. Statistic analysis

All data collected for the chemical compounds were presented as the mean ± standard deviation of at least three independent experiments and three replicates, for cytotoxic and antibacterial analysis with two independent experiments and three replicates. To determine if there were significant differences between the fruit and leaf extracts in each analyzes, a test t-student or test W of Mann-Whitney with a significance level of 95% were employed. A non-parametric Kruskal-Wallis test was performed, with a significance level of 95% to observe differences in the IC_{50} of $DPPH^+$ and $ABTS^{\bullet+}$. A Spearman correlation was used between the antibacterial and cytotoxic variables with the chemical variables and between Antioxidant $DPPH^+$ and $ABTS^{\bullet+}$ with the chemical variables. All analyses were performed with Statgraphic Centurion software.

3. Results

3.1. Chemical analysis

Analysis of the LC/ESI-MS results shows in the leaf extract (see Table 1), the presence of some types of polyphenolic compounds such as flavonoids derivative quercetin and kaempferol, as well as several condensed tannins; differing from the fruit extract, which contains few polyphenolic compounds and other no-identified compounds.

The antioxidant activities determined by DPPH and ABTS of *P. lingue* fruit and leaf extracts are presented in Figure 1. The amount of leaf extract to obtain an IC_{50} in the DPPH assay is statistically lower ($P < 0.05$) in relation to fruit extract (7.8 and 71.5 µg/mL, respectively); in addition, the leaf extract has an approximate value for the chemical control Trolox. The same trend is observed for IC_{50} by the ABTS test, with a statistically significant difference ($P < 0.05$) between both extracts.

Table 1. ESI-MS product ions obtained from the $[M-H]^+$ ions of extracts *Persea lingue*.

Compound tentative	RT (min)	$[M-H]^+$	main fragment
Leaf			
Procyanidin trimer type A	14.86	865	713, 291
Procyanidin tetramer	14.17	1154	865, 579,291
Unknown	15.08	305	
Rutin	23.57	611	303
Quercetina 3-*O*-glucoside	23.67	465	303
Kaempferol 3-*O*-glucoside	25.15	449	327, 287
Ellagitannin (elaeocarpusin)	26.50	1111	303
Isorhamnetin	27.37	317	287
Fruit			
Unknown	3.29	365	
Ellagic acid	12.08	303	
Procyanidin trimer	16.44	865	579
Unknown	18.16	327	
Petunidin	19.35	317	
(Epi)cathequingallate	20.60	307	195
Unknown	26.08	610	331

RT: retention time.

In the content of the different compounds analyzed, leaf extract of *P. lingue* showed a higher concentration of phenolics, flavonoids and condensed tannins (see Table 2), being statistically superior to extract of the fruit, which has a very low amount of total flavonoids and total condensed tannins. In the case of total alkaloids, the fruit extract has almost double the concentration of the leaf extract ($P < 0.05$). There is a high level of total lipids in the leaf and fruit, being in the latter almost 50% higher ($P < 0.05$).

3.2. Antibacterial activity

MICs of leaf and fruit extracts of *P. lingue* is shown in Table 3. The differences in MIC values between the extracts of fruits and leaf are notable in most of bacteria tested, especially with *E. coli*, *S. aureus* and *P. aureginosa*, where the fruit extract has a high efficacy to inhibit the development of these microorganisms, with very lows MICs, while the leaf extract had no inhibitory effect in any concentration employed. Against *B. megaterium* and *M. luteus*, both extracts have inhibitory activity, but fruit extract is much more effective. The only bacterium whose growth was not inhibited by the two extracts was *E. cloacae*. Solvent control of 8% DMSO showed no inhibition of bacterial growth.

3.3. Cytotoxic activity

The cytotoxic activity evaluated by PI/calcein assay, this methodology evaluate the cell membrane integrity, *P. lingue* extracts against Jurkat and Kasumi-1 cell (see Table 4) evidenced a better performance with the fruit extract which showed an excellent effect on cellular viability levels. A statistical difference was observed with the leaf extracts that cause a small reduction in the cellular viability (62% against Jurkat). For the Kasumi-1 cell, the fruit extract was again highlighted by the high cytotoxic activity, which had an effect on the cellular viability levels of approximately 2%, whereas for the leaf extract the effect on the viability levels observed was 90%, and therefore statistically inferior to the one observe with the fruit.

With AlamarBlue assay (see Table 4), which measure cell metabolism, the extracts present a similar behavior to the previous assay, although with lower levels of cytotoxic for the same cell lines. Fruit extract has a high cytotoxic activity causing a reduction in the cellular viability against Jurkat, and against Kasumi-1 cell. The fruit extract presented statistical difference ($P < 0.05$) with the leaf extract.

The EC_{50} value for the fruit extract obtained from the concentration-response curve (see Figure 2) against the Jurkat cell is 5.5 µg/mL to obtain a 50% cell viability after 48 h of incubation, with the adjusted model equation: *cell viability (%) = 85.9579 – 17.8673×ln(concentration)*; $r^2 = 0.9837$.

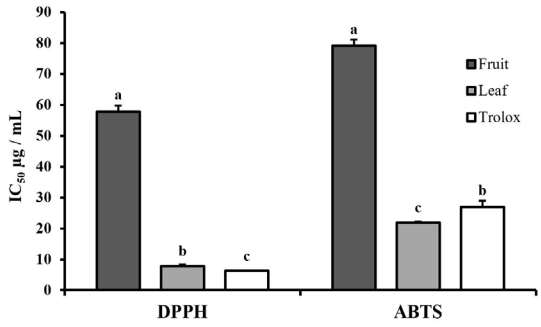

Figure 1. Antioxidant activity evaluated by DPPH and ABTS methods from fruit and leaf of *Persea lingue* extracts. Bars indicate mean value IC_{50} and respective standard deviation. Different letters indicate statistical difference, Kruskal-Wallis ($P < 0.05$, n=3). Trolox chemical control.

4. Discussion

4.1. Chemical characterization

Among the extracts of fruit and leaf of *P. lingue*, there is a clear differentiation in the antioxidant power that each one has and in the content of secondary metabolites. In general, phenolic compounds, have a high antioxidant power due to its chemical structure, which consists of at least one aromatic ring with a variable number of hydroxyl groups, which determine its antioxidant capacity (Balasundram et al., 2006), as well as the flavonoids (flavones, flavonols and proanthocyanidins) with various hydroxyl groups in their three rings, allow them to capture free radicals through the donation of hydrogen atoms (Procházková et al., 2011). Therefore, the high concentration of proanthocyanidins and flavones in leaf extract is what induce the high *in vitro* antioxidant power of this sample. Some of most common flavonoids in plants are the flavones quercetin and kaempferol. When they are methylated or glycosylated they form larger molecules as quercetin-3-*O*-glucoside, these flavones are possibly found in high concentrations in the leaf extract and also contains different types of proanthocyanidins. These observation corroborates with the highest index of the total phenols and flavonoids and the high antioxidant power,

Table 2. The content of total phenolics, flavonoids, condensed tannin, alkaloids and lipids from fruit and leaf *Persea lingue* extract.

	Total Phenolics	Total Flavonoids*	Total Condensed Tannins*	Total Alkaloids	Total Lipids
	(mg EAG/g)	(mg EQ/g)	(mg EC/g)	(mg EB/g)	(mg OA/g)
Fruit	2.032 ± 0.051b	0.070 ± 0.007b	0.177 ± 0.036b	0.184 ± 0.014a	26.69 ± 3.09a
Leaf	4.863 ± 0.115a	0.762 ± 0.036a	2.221 ± 0.139a	0.088 ± 0.021b	16,41 ± 1.14b

Values indicate average and respective standard deviation. Different letters indicate statistical difference, t-student or W Man-Whitney* ($P < 0.05$).

notoriously differentiating from the fruit extract. The former who concentrates another type of phytochemical like alkaloids and unsaturated lipids, the latter are abundant in fruits of the genus *Persea* (Dreher and Davenport 2013; Rodriguez-Saona et al., 2000). The concentrations of the phytochemicals, flavones and flavonols, present in the extracts had greater influence on the antioxidant power as observed in our study, for both DPPH and ABTS assays (Table 5). Possibly the other phenolic type compounds (condensed tannins, phenolic acids, hydroxycinnamic acids) also influence the antioxidant power, but in a lower extent.

Table 3. The minimum inhibitory concentration (MIC) of *Persea lingue* fruit and leaf extract against bacteria tested in microdilution assays.

Bacteria	MIC (µg/mL)	
Gram-Negative	**Fruit**	**Leaf**
Escherichia coli D31	12.5 - 25	ND
Pseudomonas aeruginosa ATCC 27853	12.5 - 25	ND
Enterobacter cloacae β12	ND	ND
Gram-Positive		
Staphylococcus aureus ATCC 29213	6.25 - 12.5	ND
Bacillus megaterium ATCC 10778	6.25 - 12.5	200 - 400
Micrococcus luteus A270	3.12 - 6.25	25 - 50

ND: not detected.

Table 4. Cytotoxic activity in cell lines Jurkat and Kasumi-1 with PI/Calcein and AlamarBlue assay, from fruit and leaf *Persea lingue* extract (100 µg/mL).

	Cell Viability (%)		
	Control	**Fruit**	**Leaf**
PI/Calcein			
Jurkat	96.7 ± 1.2	1.7 ± 0.6 a	62.0 ± 2.0 b
Kasumi-1	97.3 ± 0.6	2.0 ± 1.0 a	90.0 ± 2.6 b
AlamarBlue			
Jurkat	100	7.1 ± 0.8 a	83.8 ± 3.5 b
Kasumi-1	100	46.7 ± 1.8 a	89.3 ± 3.3 b

Values indicate percent of viability cell and respective standard deviation. Different letters indicate statistical difference, t-student ($P < 0.05$).

4.2. Antibacterial activity

One of the properties of the secondary metabolites of plants is to provide protection against pathogens such as fungi, bacteria, and viruses. From our results of antibacterial activities, the extract that had the greatest efficacy against *E. coli*, *S. aureus*, *M. luteus*, *B. megaterium* and *P. aeruginosa* was the extract of the fruit, with MICs equal to or less than 12.5 - 25 µg/mL. In contrast, leaf extract has activity only against *M. luteus* and *B. megaterium*, which is in agreement with that observed by Mølgaard et al. (2011), who did not find antibacterial activity against *E. coli*, *S. aureus* and *P. aeruginosa* in *P. lingue* leaf extract. However, Avello Lorca et al. (2012) found that *P. lingue* leaf oil has a mild antibacterial activity against *E. coli*, *Bacillus subtillis*, *P. aeruginosa* and *S. aureus*. Some investigations have found antimicrobial activity in the *Persea americana* extract against *P. aeruginosa*, *E. coli* and *S. aureus* (Raymond Chia and Dykes 2010; Rodríguez-Carpena et al., 2011). There are no studies so far regarding the antimicrobial activity of *P. lingue* fruit. The correlation analysis (see Table 5)

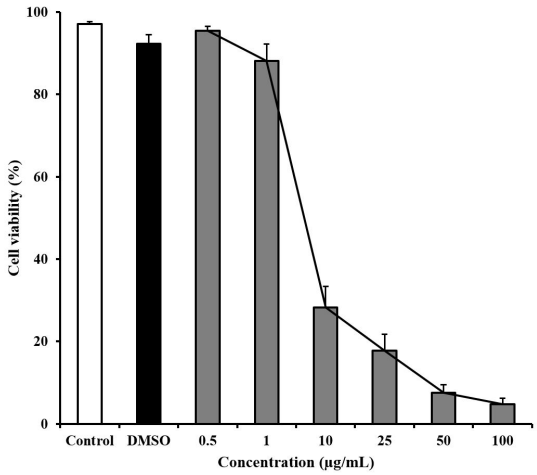

Figure 2. Concentration-response curve of *Persea lingue* fruit extract treatment of Jurkat cell line. EC_{50} obtained by curve fitting of viable cells after 48 h treatment for Jurkat cell line, *cell viability (%) = 85.9579 – 17.8673×ln(concentration)*; $r^2 = 0.9837$. Bars indicate mean value and respective standard deviation (n=3).

Table 5. Spearman correlations: (a) between biological and chemical variables, (b) between antioxidant and chemical variables.

		Total Phenols	Total Flavonoids	Total Tannin condensed	Total Alkaloid	Total Lipids
(a)	Cytotoxic	-0.79**	-0.76*	-0.73*	0.85**	0.78*
	MIC	-0.79**	-0.88**	-0.87**	0.87**	0.87**
(b)	DPPH	ns	0.88*	ns	-0.94*	-0.94*
	ABTS	0.89*	0.88*	ns	ns	-0.94

*: $P < 0.05$; **: $P < 0.01$; ns: not significant.

suggests that antibacterial activity of the fruit is possibly mediated by the content of lipids or alkaloids and not by polyphenolic compounds.

The most important phytochemical compounds found in the *P. lingue* fruit have the potential to inhibit the development of Gram negative and Gram positive bacteria, possibly for presence of alkaloids. Thus some alkaloids as isoquinolines has been able to inhibit the cell function of bacteria through various mechanisms such as damaging the cell structure, causing Z-ring disturbance and inhibiting cell division. They also act as protein and DNA synthesis inhibitors that result in bacterial death. Isoquinoline can cause a remarkable increase in the DNA cleavage by targeting and inhibiting the bacterial topoisomerase IA. The quinolone alkaloid can inhibit type II topoisomerase enzymes and consequently, inhibit the DNA replication as well (Cushnie et al., 2014; Khameneh et al., 2019).

The lipids or fatty acids present in the fruit also have a high antibacterial potential, fatty acids are known to naturally insert into cell membranes and physically disturb their functionality, and cis-double bonds present in unsaturated fatty acids can form fixed bends in their carbon chain that occupy a greater cross-section and introduce disorder into neighbor phospholipid acyl chains, which leads to a greater fluidity of the membrane, generating cell disintegration (Salinas-Salazar et al., 2017).

4.3. Cytotoxic activity

From the first cytotoxicity analyzes by PI/calcein, observed against the Jurkat and Katsumi-1 cell, the fruit extract is notable for causing a high cell death, demonstrating its efficacy against these leukemia cell lines studied. Additionally, AlamarBlue assay, also showed a high cytotoxic activity against the Jurkat cell. There is evidence that leafs and seeds of the *P. americana* species have cytotoxic properties against the Jurkat cell (Bonilla-Porras et al., 2013), which is in agreement with the data obtained with *P. lingue* fruit in this study. A crude extract is generally considered to have *in vitro* cytotoxic activity, if the EC_{50} value following incubation between 48 and 72 h is less than 20 µg/mL (Lee and Houghton 2005). Therefore, the fruit extract has a high potential for cytotoxic activity against the Jurkat cell due to the low EC_{50} value obtained (5.5 µg/mL), that can be considered very active (Nordin et al., 2018).

Some researchers have also found a positive correlation between antioxidant power and anticancer activity of plant extracts (Li et al., 2007; Wang et al., 2007). However with *P. lingue* species, the opposite was observed, may be due to the low antioxidant activity found in the fruit, indicating that anticancer potential of this species is not due to phenolic compounds or another compounds with high antioxidant power (as show in Table 5). It is important to consider that many species belonging to the genus *Persea* contain lipidic compounds like avocadofurans and acetogenins, which are present in the bark, leaves and fruits of these species. These compounds provide a high biological activity such as antimicrobial, insecticidal, anticancer, antithrombotic, and others (Yasir et al., 2010; Ma et al., 1989, 1990; Reis et al., 2019; Fraga and Terrero 1996; Rodriguez-Saona et al., 2000; Rodriguez-Saona and Trumble, 2000; Domergue et al., 2000; Scora and Scora 2001; Pino et al., 2004; Butt et al., 2006; Rodriguez-Sanchez et al., 2015). Therefore, it is possible that the high antibacterial and especially cytotoxic activity detected in the *P. lingue* fruit may be due to the presence of some of these phytochemicals, which is found in high quantity of lipids in the fruit and with an important positive correlation (see Table 5).

It is important to evaluate the level of cytotoxicity of the extracts to observe if the phytochemicals present in *P. lingue* fruit do not significantly damage normal cells, which is essential for their potential therapeutic use.

5. Conclusions

The *Persea lingue* fruit is a potential source of biologically active molecules for development of new drugs to be used in some types of leukemia, as well as antibacterial agent. This study is one of the first contributions and possibly the only one to investigate the anticancer and antibacterial properties, and the chemical characterization of this plant.

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