Persea americana: Phenolic profile, Antioxidant potential, Antimicrobial Activity and *in silico* Prediction of Pharmacokinetic and Toxicological Properties

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Deuschle *et al.*: Phenolic Profile from *Persea americana* Leaves

The objective of this study was to carry out a phytochemical evaluation of the hydroethanol extract and chloroform, ethyl acetate and butanol fractions of the leaves of *Persea americana* and correlate with its antioxidant and antimicrobial activities, in order to verify which phytoconstituent is responsible for these activities. In addition, an *in silico* study of the active constituents identified was carried out to evaluate pharmacokinetic and toxicological properties. The butanol fraction yielded high content of phenolic compounds, total flavonoids, vitamin C and a very high content of rutin (20.6±0.5 mg/g) and quercetin (6.1±0.1 mg/g). The hydroethanol extract demonstrated IC_{50} of 6.7±1.7 in the 2,2-diphenyl 1-picrylhydrazyl method, ethyl acetate fraction demonstrated greater ability to scavenge H_2O_2 (28.5±0.1) and butanol fraction to reduce Fe^{3+} to Fe^{2+} (55.1±0.1). Significant antifungal activity was found in the hydroethanol extract and the ethyl acetate and butanol fractions (MIC of 32 µg/ml against *Candida glabrata* and *Candida glabrata* fluconazole resistant). The ethyl acetate fraction showed antimycobacterial activity with an MIC of 156.25 µg/ml for all species tested. The phytoconstituents identified have no carcinogenic potential but rutin and quercetin might be mutagenic and inhibit cytochrome P450 isozymes, CYP1A2 and CYP3A4. The results provided a good understanding of the constituents present in the leaves of the plant and their potential for pharmaceutical, food and cosmetics application.

Key words: Phenolic compounds, flavonoids, tannins, avocado, free radicals

Persea americana Mill. (Lauracea), popularly known as the avocado, is ill-suited to tropical regions; however, it grows well in temperate regions, especially Central America. Their leaves have been ascribed antitussive, antidiabetic effects, in addition vessel relaxing action, reducing blood pressure and used in seizures and epilepsy in childhood[1-3]. Among the activities that have already been described for the leaves of the species, antifungal, antiinflammatory, antioxidant and analgesic activities are the main ones[4,5,1]. *P. americana* contains a variety of compounds with biological activities important for human health. Lima *et al.*[3] reported the presence of triterpenoids glycosides, coumarins, alkaloids, saponins, tannins, flavonoids, and reducing sugars as constituents in avocado leaves.

The antioxidant potential of the plants has been associated mainly with the presence of phenolic compounds[6]. Phenolic compounds that were reported in the leaves of *P. americana*, included, gallic, caffeic, coumaric, ferulic, chlorogenic, resorcylic, sinapic, syringic, vanillic, hydroxybenzoic, protocatechuic and pyrocatechuic acids. Flavonoids found in the leaves were, rutin, quercetin, quercetrin, quercetin-3-*O*-arapyranoside, quercetin-3-*O*-β-glucopyranoside, quercetin-3-*O*-β-D-glucoside, apigenin, luteolin, luteolin-7-*O*-glucoside, isohamnetin, catechin, epicatechin, kaempferol-3-*O*-arabinopyranoside and kaempferol-3-*O*- rhamnopyranoside[6-9].

Many compounds present in plants, which are responsible for the inhibition of bacteria and fungi, can

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constitute an important niche in order to be exploited for the development of new drugs and medications[10]. The phenolics, flavonoids, alkaloids, and terpenoids are responsible for the inhibition of microbial growth are among the chemical constituents responsible for the inhibition of microbial growth[11,12]. *P. americana* contains phenolics which could be exploited in relation to these therapeutic benefits[5].

Odo *et al*.[13] evaluated the acute toxicity and lethality (LD$_{50}$) of methanol and chloroform fractions of leaves of *P. americana* in Wistar rats. The result of this study demonstrated that there was no lethality or any sign of toxicity in animals at 10, 100, and 1000 mg/kg doses at the end of the first phase of the study. At the end of the second phase of the study, no death or sign of toxicity occurred in groups of animals receiving 1900 and 2600 mg/kg of both fractions. However, there were deaths and obvious signs of toxicity (slowness, edema in the face and eyes) in the group of rats that received 5000 mg/kg of either the methanol or the chloroform fraction within 24 h after administration, which is probably due to the cytotoxic nature of the solvents used. Thus, that study indicated that leaves of *P. americana* were considered safe with a distant risk of acute toxicity.

Thus, the objective of this study was to evaluate phytochemical constitution and determine the antioxidant and antimicrobial activity of the hydroethanol extract and fractions of *P. americana* leaves, in order to identify beneficial effects and the constituents responsible for these effects. In addition, to perform an *in silico* study of the active constituents identified to understand their pharmacokinetic and toxicological properties.

MATERIALS AND METHODS

All reagents used in the work were of analytical grade. Standards of rutin, quercetin, catechin, gallic, and chlorogenic acid were acquired from Sigma Chemical Co. (St. Louis, USA). Methanol was of HPLC grade and acquired from Sigma Chemical Co. (St. Louis, USA). Solvents for the extractions, chloroform, ethyl acetate, ethanol, methanol, and *n*-butanol were purchased from Merck (Darmstadt, Germany). Mueller-Hinton broth was obtained from Merck and the oleic acid-albumin-dextrose-catalase (OADC) from Difco Laboratories (Detroit, Michigan). Aqueous solutions were prepared with ultra-pure water from a Milli-Q Synergy UV system (Merck Millipore, Darmstadt, Germany). High performance liquid chromatography (HPLC-DAD)

was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto-Sampler (SIL-20A), equipped with Shimadzu LC-20 AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV/Vis detector DAD SPD-M20A and Software LC solution 1.22 SP and the spectrophotometric analyses was performed on a Shimadzu spectrophotometer (UV-1201).

Plant collection and extraction:

Leaves of the species were collected in February of 2014 in the city of Cruz Alta, Rio Grande do Sul, Brazil, with the following geographical coordinates, longitude- -53° 36′ 23″ W; latitude- -28° 38′ 19″ S; altitude- 452 m[14]. Botanical identification was performed at the Department of Biology, University of Cruz Alta (UNICRUZ), where a voucher specimen has been deposited in the Herbarium of Poisonous and Medicinal plants under the registration code 1109. The species was identified as *Persea americana*, var. americana.

Fresh leaves were coarsely divided and dried in an oven with air circulation at 45° for 7 d. The dried plant material was extracted with aqueous ethanol (ethanol:water 70:30, v/v) by maceration as described previously (Deuschle *et al*.)[15]. The hydroethanol extract obtained was further fractionated in separation funnels by sequential extraction using increasingly polar solvents such as chloroform, ethyl acetate, and *n*-butanol (approximately 3×200 ml of each solvent). The organic fractions were concentrated and dried in a rotary evaporator. The hydroethanol extract was lyophilized to obtain dry extract. The extract and fractions were designated as, *P. americana* hydroethanol extract (PAHE), *P. americana* chloroform fraction (PACL), *P. americana* ethyl acetate fraction (PAEA) and *P. americana* butanol fraction (PABT).

TLC analysis:

Phytochemical screening of the extract and fractions was carried out in order to detect essential oil components, terpenoids, saponins, and alkaloids using thin layer chromatograph (TLC)[16]. The solid phase consisted of aluminum TLC plates coated with silica-gel 60 with the F$_{254}$ fluorescent indicator. The mobile phases consisted of specific mixtures of solvents for each class of substances; terpenoids- ethyl acetate:methanol:water (77:15:8); saponins- chloroform:glacial acetic acid:methanol:water (64:32:12:8); alkaloids-toluene:ethyl acetate:diethylamine (70:20:10);

derived anthracenics- ethyl acetate:methanol:water (100:13:5:10); cardiac glycosides- ethyl acetate:methanol:water (100:13:5:10). Standards were applied for comparison. After elution, results were observed under UV/Vis light (254 and 365 nm), and afterwards sprayed with specific reagents (essential oils- sulfuric acid anisaldehyde; terpenoids and saponins- vanillin sulfuric acid; alkaloids- Dragendorff reagent; derived anthracenics- potassium hydroxide and cardiac glycosides- Kedde reagent).

Spectrophotometric analysis:

Phenolic compounds content was determined using the Folin-Ciocalteu method in alkaline medium, as described by Swaint and Hillis[17]. For this, the sample was diluted to a concentration of 1 mg/ml and the absorbance was measured in triplicate at a wavelength of 750 nm. The total phenol content was expressed as milligrams of gallic acid equivalents per gram of dry plant (mg GAE/g), based on the calibration curve of gallic acid.

Total flavonoid content was established according to the method described by Zhishen et al.[18], using aluminum chloride. The sample was diluted to a concentration of 1 mg/ml in methanol. The absorbance was then read at 510 nm. Tests were performed in triplicate and the content of flavonoids was determined in milligrams of quercetin equivalents per gram of dry plant (mg QE/g), based on the calibration curve of quercetin.

The condensed tannin determination was carried out using the method described by Morrison et al.[19]. The sample was diluted to a concentration of 25 mg/ml in methanol. The solution was heated at 60° for 10 min and the absorbance was determined at 500 nm. Analyses were performed in triplicate. The condensed tannin content was expressed as milligrams of catechin equivalents per gram of dry plant (mg CE/g), based on the calibration curve of catechin.

Ascorbic acid content was determined in extracts (1 mg/ml) using the method of Benderitter et al.[20], based on the reaction with 4-dinitrophenylhydrazine, calculated using a vitamin C standard curve and expressed in milligrams of ascorbic acid per gram of dry plant (mg AAE/g).

HPLC-DAD analysis:

Reversed-phase chromatograph analyses were carried out in gradient conditions using C-18 column (4.6× 250 mm) packed with 5 µm particles. The solvents in the mobile phase were 0.33 M acetic acid in water (A) and methanol (B), and the composition gradient was: 0.0-5 min 8 % B; 5-20 min 20 % B; 20-30 min 30 % B; 30-40 min 50 % B; 40-50 min 60 % B; 50-60 min 70 % B; 60-70 min 20 % B; 70-80 min 10 % B as the column washing step[21]. The mobile phase and all solutions and samples were filtered through a 0.45 µm Millipore membrane filter and then degassed by an ultrasonic bath before use. Quantification was carried out by integration of the peak using external standard method. The flow rate was 0.8 ml/min, the injection volume was 40 µl. Detected compounds were quantified of 327 nm at 356 nm. The chromatographic peaks were confirmed by comparing their retention time and DAD-UV spectra with those of the reference standards. All chromatographic operations were carried out at room temperature (25°±1°) and in triplicate.

2,2-Diphenyl 1-picrylhydrazyl (DPPH) radical method:

The antioxidant capacity was evaluated according to the method previously described by Choi et al.[22]. Spectrophotometric analysis was used to determine the inhibition concentration (IC_{50}; concentration that offers 50 % inhibition) of the extracts. The extracts were diluted in ethanol in the following concentrations: 250, 125, 62.5, 31.25, 62 and 7.81 µg/ml and 2.5 ml mixed with 1.0 ml of a 0.3 mM DPPH solution in ethanol. After 30 min, the readings were determined at 518 nm. A solution of DPPH (1 ml, 0.3 mM) dissolved in ethanol (2.5 ml) was used as the negative control. Ascorbic acid was used as positive control, at the same concentrations as the samples. The assay was performed in triplicate and the antioxidant capacity was calculated according to the Eqn. 1, % inhibition = 100-[(Abs sample–Abs blank)×100]/Abs control, where, Abs sample is the test absorbance; Abs blank is the blank absorbance, and Abs control is the DPPH solution in ethanol absorbance.

Ferric reducing antioxidant power (FRAP assay):

To 30 µl of extraction solution (25, 50, 100, 250, 500, and 1000 µg/ml), 90 µl distilled water and 900 µl of FRAP reagent (acetate buffer 300 mM/l, 2,4,6-tripyridyl-S-triazine 10 mM/l, and ferric chloride 20 mM/l in a ratio of 10:1:1) were added. The solutions were mixed and kept at 37° for 30 min. A standard curve was prepared with ferrous sulphate solution at concentrations of 0.1, 0.5, 1, 1.5, and 2 mM. The absorbance was determined at 593 nm. Gallic acid was used as the standard (positive control), at the same concentrations as the samples.

The reducing power of the extract and the fractions (at the concentrations of 25-1000 µg/ml) was presented as mM Fe^{2+}/ml extract[23].

Hydrogen peroxide scavenging capacity:

The ability of the extracts (50 µg/ml) to scavenge hydrogen peroxide was determined according to the method of Ruch et al.[24]. Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. Gallic acid was used as the standard (positive control), in the same concentrations as the samples. The percentage of hydrogen peroxide scavenging of the extracts and standard compounds were calculated according to the Eqn. 2, % scavenged $[H_2O_2] = [(AC–AS)/AC]\times100$, where AC is the absorbance of the control and AS is the absorbance in the presence of the sample (extract) or standard.

Nitric oxide scavenging capacity:

The scavenging effect of the extracts on nitric oxide radicals was measured according to the method of Sreejayan and Rao[25]. Gallic acid was used as the positive control. For the assay, sodium nitroprusside (10 mM) was mixed with different concentrations of gallic acid or samples (1, 10, 25, and 50 µg/ml), incubated for 150 min and then mixed with 0.5 ml of Griess reagent and measured at 546 nm. In the negative control, the sample was substituted with phosphate-buffered saline. The NO scavenging ability was calculated using the following Eqn. 3, % scavenging (nitric oxide) = $[1−(A\ sample/A\ control)]\times100$.

Antibacterial and antifungal activity assays:

Susceptibility tests were performed according to the Clinical and Laboratory Standards Institute (CLSI) microdilution technique, M07-A9[26] for bacteria, M27-A3[27] for yeasts and M38-A2[28] for filamentous fungi, with concentrations ranging from 1000 mg/ml a 0.25 mg/ml. The results of the tests were expressed as the minimal inhibitory concentration (MIC).

Fast-growing Mycobacteria (except *M. smegmatis*):

The susceptibility tests were evaluated by broth microdilution method according to the standard protocol CLSI M24-A2[29]. The extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml and diluted in Mueller-Hinton broth to the desired concentrations. To establish the MIC of each extract, different concentrations of the same antimicrobial agent were used in a 1:2 serial dilution

series. The inoculum was standardized to MacFarland's 0.5 scale. For the preparation of the final inoculum (5×10^5 CFU/ml), 50 µl of the bacterial suspension was transferred to a test tube containing 10 ml of Mueller-Hinton broth. In a sterile microplate titration, 100 µl of the inoculum and antibiotic dilutions were added in the same volume. After incubation for 72 h at 30°, readings were taken from the plates to determine the MIC.

Other microorganisms:

The inocula were standardized to the MacFarland 0.5 scale in Middlebrook 7H9 medium (MD7H9), supplemented with 10 % OADC and 0.2 % glycerol. For *M. smegmatis*, the suspension was diluted in MD7H9 to a concentration of 10^5 CFU/ml. The extracts were dissolved in DMSO at a concentration of 10 mg/ml and diluted in MD7H9 to the desired concentrations. Susceptibility tests were performed using the broth microdilution method. After incubation at 30° for 48 h (*M. smegmatis*) for 5 d (*M. avium*), readings were taken from the plates and the MIC was determined. To verify the presence of microorganism growth, the dye 2,3,5-triphenyltetrazolium chloride (TTC-Sigma) was used. The MIC for use in microbiological assays is considered the lowest concentration of sample that can produce visible inhibition of microorganism growth[29,30]. Negative controls of all assays consisted of dilutions of extract/fractions in the culture medium at the same assay concentrations, but without inoculum.

***In silico* toxicology and pharmacological properties:**

The LAZAR[31] and AdmetSAR[32] open source softwares were used to predict the toxicity and pharmacokinetic properties of chlorogenic acid, rutin, and quercetin, which were the constituents identified.

Statistical analysis:

The results of the determinations were subjected to analysis of variance (ANOVA) followed by post hoc multiple comparison using the Tukey test ($p\leq0.05$). The analyses were performed using GraphPad Prism for Windows, version 5.03 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

With the results obtained in this study, capacities of different solvents used to extract the phenolic compounds, which directly influenced the antioxidant and antimicrobial activities was tested. In general, more polar solvents have a greater potential in extracting

such compounds. The TLC analysis demonstrated the presence of saponins and anthracenic derivatives for PAHE and PABT and only anthracenic derivatives for PAEA. The other extracts tested negative for the tested compounds. The total phenolic content, total flavonoids, condensed tannins, vitamin C content are shown in Table 1. The highest content of flavonoids and polyphenols was observed in PABT. For the determination of condensed tannins, the highest value was found in PACL. The presence of tannins, flavonoids, and saponins may be associated with antidiarrheal and antispastic effects already described for *P. americana* leaves in traditional medicine[13]. Moreover, phenolic compounds such as flavonoids and tannins are secondary metabolites that are related to the antioxidant activities of plants[33].

Ascorbic acid (vitamin C) is a potent inhibitor of singlet oxygen, superoxide anion, hydroxyl radical, hydrogen peroxide and reactive nitrogen species[34,35]. All samples showed different levels of vitamin C, but the butanol fraction presented the highest amount. The presence of vitamin C in the *P. americana* leaf extract also contributed to the antioxidant activity of this sample, determined in this study using DPPH, FRAP, H_2O_2 and nitric oxide scavenging methods. Ethyl acetate is an intermediate polarity solvent; this fraction contained lower levels of polyphenols. In the chloroform fraction, none of the compounds were identified since as this was the most non-polar solvent[36].

The extracts and fractions were analysed using HPLC-DAD for the determination of phenolic compounds. The results indicated the presence of the following compounds with retention times (Rt). PAHE-chlorogenic acid (20.208; peak 1), rutin (32.548; peak 2) and quercetin (40.10; peak 3); PAEA- chlorogenic acid (20.194; peak 1), rutin (32.548; peak 2) and quercetin (41.02; peak 3); PABT- chlorogenic acid (20.185; peak 1), rutin (Rt= 32.487; peak 2) and quercetin (40.975; peak 3, fig. 1), and in PACL, no compounds were found. The quantification of chlorogenic acid, quercetin, and rutin by HPLC-DAD based on the reference standards is shown in fig. 1. The presence of chlorogenic acid, rutin and quercetin in the leaves of *P. americana* was reported by other authors[6-9].

The IC_{50} values of DPPH and H_2O_2 scavenging activity are shown in Table 1. Reducing power of the extracts was assessed based on their ability to reduce Fe^{3+} to Fe^{2+}, as presented in fig. 2. NO scavenging activity is presented in Table 2. In relation to antioxidant capacity determined by the DPPH assay (Table 1), it was observed that all samples showed the ability to reduce DPPH radicals. The lowest IC_{50} was found for PAHE (6.73 µg/ml), and was very close to the IC_{50} of vitamin C, which was used as the standard (5.08 µg/ml).

The reducing power of the extract and the fractions was determined by the FRAP method, which demonstrates the capacity to reduce Fe^{3+} to Fe^{2+}, thereby directly measuring the concentration of antioxidant electron donors[34,37]. The butanol fraction exhibited greater capability than gallic acid, the positive standard. The results for the hydroethanol extract and ethyl acetate fraction were similar to that of the standard, while the chloroform fraction showed the least reducing power (fig. 2). Table 2 shows that the extracts were
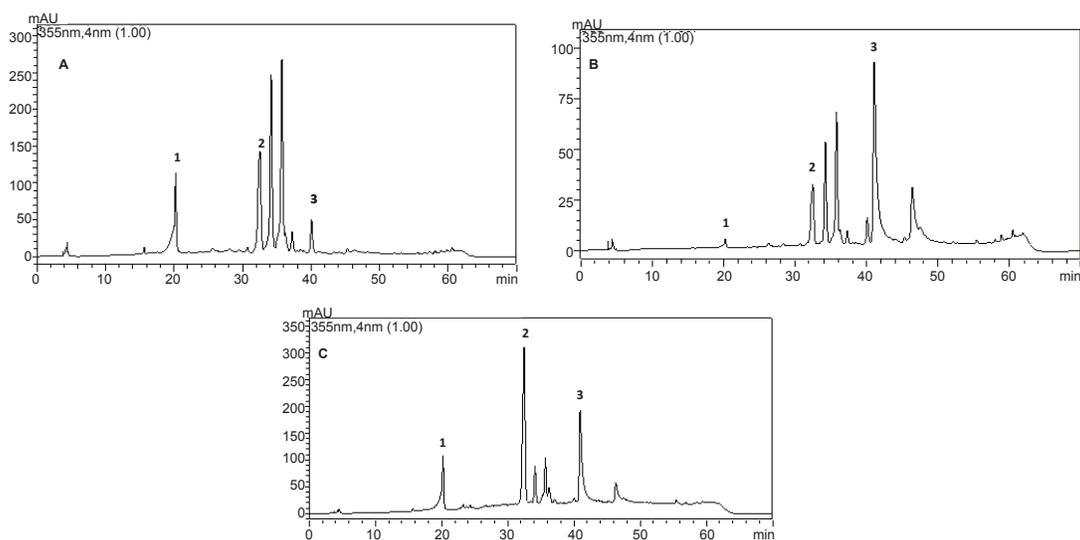
Fig. 1: High performance liquid chromatograms from the leaves of *Persea americana*
A. hydroethanolic extract (PAHE), B. ethyl acetate extract (PAEA), C. butanol extract (PABT). 1. chlorogenic acid, 2. rutin and 3. quercetin

TABLE 1: TOTAL PHENOLS, FLAVONOIDS, CONDENSED TANNINS, VITAMIN C AND ANTIOXIDANT ACTIVITY OF *PERSEA AMERICANA*

Extract	Total phenols (mg GAE/g) ±SE*	Total flavonoids (mg QE/g) ±SE*	Condensed tannins (mg CAE/g) ±SE*	Ascorbic acid (mg AAE/g) ±SE*	IC$_{50}$ DPPH (μg/ml) ±SE*	Scavenging H$_2$O$_2$ (%) ±SE*
PAHE	99.4±1.2[a]	42.5±1.9[a]	69.7±1.5[a]	5.9±0.1[a]	6.7±1.7[a]	5.7±0.1[a]
PACL	19.7±1.3[b]	9.3±1.2[b]	86.7±1.3[b]	2.1±0.1[b]	19.0±1.5[b]	3.4±0.2[b]
PAEA	85.2±0.1[c]	54.9±1.8[c]	22.9±0.8[c]	5.4±0.1[a]	19.7±0.8[c]	28.5±0.1[c]
PABT	127.0±0.1[d]	93.6±0.7[d]	22.3±0.4[c]	11.3±0.7[c]	14.2±3.2[d]	8.0±0.3[d]
AAE	ND	ND	ND	ND	5.0±0.4[e]	ND
GAE	ND	ND	ND	ND	ND	4.5±0.1[e]

*Results are expressed as mean±standard error (SE) of three determinations. GAE- gallic acid equivalents, QE- quercetin equivalents, CAE- catechin equivalents, AAE- ascorbic acid equivalents, ND- not determined. Means marked with different letters are significantly different (p<0.05) in the same column. PAHE- *P. americana* hydroethanol extract, PACL- *P. americana* chloroform fraction, PAEA- *P. americana* ethyl acetate fraction, and PABT- *P. americana* butanol fraction

TABLE 2: NO SCAVENGING CAPACITY OF *PERSEA AMERICANA*

Extract/concentration (μg/ml)	GA (%) ±SE*	PAHE (%) ±SE*	PACL(%) ±SE*	PAEA (%) ±SE*	PABT (%) ±SE*
1	94.5±0.1[a]	28.3±0.4[a]	45.4±0.4[a]	45.4±0.4[a]	51.6±0.3[a]
10	79.3±4.0[b]	35.6±0.1[b]	48.4±3.6[b]	46.6±0.2[b]	55.0±0.1[b]
25	77.5±1.1[c]	40.5±0.5[c]	49.3±1.6[c]	47.5±1.7[a]	55.1±0.2[c]
50	78.2±4.1[d]	38.5±0.6[d]	49.9±0.3[c]	44.7±1.8[c]	55.1±0.3[d]

*Results are expressed as mean±standard error (SE) of three determinations. Means marked with different letters are significantly different (p<0.05) in the same column. GA is gallic acid
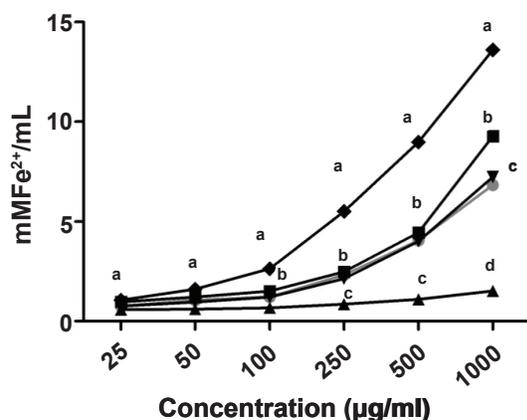
Fig. 2: FRAP activity of *Persea americana* extract and fractions and gallic acid
Ferric Reducing antioxidant power (FRAP) activity of PABT (—♦—), PAAE (—▼—), PACL (—▲—), PAHE (—■—) and GAA (—●—), each value is expressed as means±standard error (n=3). Different letters represent significant differences (p<0.05)

significantly less active than the gallic acid standard in the nitric oxide scavenging capacity test, but the butanol fraction at the higher concentration tested (50 μg/ml) showed a nitric oxide scavenging capacity of 58 %. The ability of the extracts to scavenge H$_2$O$_2$ radicals was also tested (Table 1). This test results showed that PAHE was similar to the standard, PABT was slightly higher and PAEA exhibited activity

significantly greater than that of the standard. The demonstrated antioxidant capacity could be due to the presence of phenolic compounds identified and quantified (especially rutin and quercetin). Phenolic compounds are considered to be strong natural antioxidants, due the redox capacity of their hydroxyl groups, thereby being able to remove free radicals[38,39].

Table 3 showed the results of antifungal screening. Good antifungal activity was observed for PAHE, with MIC 32 μg/ml for *Candida glabrata* fluconazole-resistant, *C. glabrata* and *C. albicans*; PAEA and PABT also gave MIC of 32 μg/ml for *C. glabrata* and *C. glabrata* fluconazole-resistant. In addition, the results indicated that PAEA presented a MIC of 128 μg/ml for *C. parapsiloisis* and *Cryptococcus neoforms* and PABT, a MIC of 128 μg/ml for *C. neoforms* and *Sacharomyces cerevisiae*. The remaining antifungal MICs were very high and not relevant. According to Cushinie and Lamb[12] MIC values lower than or equal to 100 μg/ml for antimicrobial activity related to the presence of flavonoids are considered remarkable. The results obtained for *P. americana* are within this range. Flavonoids have been known to have antimicrobial activity, and the presence of rutin and quercetin in the extracts likely contributed to this activity[40]. In a study by Araruna *et al.*[40], the antifungal activity of rutin presented an MIC of 32 μg/ml against three *Candida* species

(*C. albicans*, *C. krusei*, and *C. tropicalis*). In our study, the presence of this compound also contributed to such inhibition. The mechanism of antifungal activity of flavonoids is probably due to the ability to complex with extracellular and soluble proteins of the fungal cell wall[41].

These extracts had low MICs, which is extremely important, since many fungi are resistant to most antifungals. Infections caused by *Candida* species have increased significantly in the last 30 y, especially in immunocompromised patients[42]. *C. albicans* is the most frequently isolated pathogen in patients in this condition[42,43], with high morbidity and mortality[44]. Although *C. albicans* is the most frequently isolated species, there has been a marked increase in the frequency of other species such as *C. glabrata*, which stands out due to its lower sensitivity to antifungal agents, mainly to the azoles[45].

Bacteria used in antibacterial screening activity were *Enterococcus faecalis* ATCC 91299, *Escherichia coli* ATCC 5922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella pullorum* ATCC 9140 and *Staphylococcus aureus* ATCC 29213. However, none of the compounds showed activity until the highest concentration tested, which was 1280 µg/ml. Although there have been studies that correlated antibacterial activity with the polyphenol and total flavonoid contents, this is also dependent on factors such as the amounts of these compounds present in the extracts and the target microorganism[46,47]. Moreover, Pereira *et al.*[48] demonstrated that rutin isolated from the plant *Solanum palinacanthum* Dunal (Solanaceae) does not inhibit the growth of *Staphylococcus aureus*,

confirming the negative results, obtained in present study.

The results of the antimycobacterial activity assessment are summarized in Table 4. Non-tubercular mycobacteria are generally ubiquitous microorganisms in the environment and, from a clinical point of view, are important opportunistic pathogens for which the therapeutic arsenal is restricted[49]. The best activity exhibited against all species tested was by the ethyl acetate fraction (MIC 156.25 µg/ml). According to Tosun *et al.*[50], a plant extract is considered active against mycobacteria, which can prevent growth at a concentration below 200 µg/ml. As Santhosh and Suriyanarayanan[51] have stated, plant extracts with high concentrations of flavonoids, particularly those belonging to the class of flavones and flavonols (quercetin), have demonstrated antimycobacterial activity. The results obtained in our study confirm some previous data regarding *P. americana* phenolic compounds. However, literature does not show data comparing the extracts obtained with the solvents used in our study.

Using the LAZAR tool (Table 5), it was observed that these substances are not likely to have carcinogenic potential, however, rutin and quercetin could be mutagenic. In addition, quercetin penetrates blood brain barrier, which could be related to the effects on the central nervous system. According to Table 6, the data related to the search through the AdmetSAR tool showed that all the substances studied showed intestinal absorption and do not inhibit P-glycoprotein. P-glycoprotein acts as a pump in the efflux of drugs and xenobiotics and participates in the processes of the

TABLE 3: MIC VALUES FOR *PERSEA AMERICANA* FRONT AGAINST YEAST AND FILAMENTOUS FUNGI

Strains	ATCC	PAHE	PACL	PAEA	PABT
Candida tropicalis	CI*	≥1280	≥1280	≥1280	≥1280
Candida parapsilosis	22018	256	1280	128	256
Candida glabrata	2001	32	512	32	32
Candida glabrata - FR	CI*	32	512	32	32
Candida dubliniensis	CBS 7987	256	≥1280	256	512
Candida dubliniensis - FR	CI*	256	≥1280	256	512
Candida albicans	14053	32	≥1280	256	512
Cryptococcus neoformans	90012	256	1280	128	128
Sacharomyces cerevisae	2601	512	≥1280	≥1280	128
Candida guilliermondii	CI*	512	1280	256	256
Aspergillus fumigatus	CI*	≥1280	≥1280	≥1280	≥1280
Aspergillus flavus	CI*	≥1280	≥1280	≥1280	≥1280

*CI- clinical isolate; FR- fluconazole-resistant, MIC- minimum inhibitory concentration

TABLE 4: MIC VALUES FOR *PERSEA AMERICANA* FRONT AGAINST MYCOBACTERIAL NON TUBERCULOSUS (MNT)

Strains	ATCC	PAHE	PACL	PAEA	PABT
M. abscessos	19977	>2500	625	156.25	312.5
M. fortuitum	6841	>2500	12500	156.25	625
M. Massiliense	48898	>2500	12500	156.25	312.5
M. smegmatis	700084	2500	2500	156.25	12500
M. avium	LR541CDC	2500	12500	156.25	625

MIC- minimum inhibitory concentration

TABLE 5: PREDICTION OF TOXITY USING LAZAR

Evaluated parameters	Compounds		
	Chlorogenic acid	Ruin	Quercetin
Blood brain barrier penetration (Human)	Non-penetrating	Non-penetrating	Penetrating
Carcinogenicity (rat)	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic
Carcinogenicity (rodents)	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic
Mutagenicity (*Salmonella typhimurium*)	Non-mutagenic	Mutagenic	Mutagenic

TABLE 6: PREDICTION OF TOXITY AND PHARMACOKINETIC PROPERTIES USING ADMETSAR

Evaluated parameters	Compounds		
	Chlorogenic acid	Ruin	Quercetin
Human intestinal absorption	Positive	Positive	Positive
P-glycoprotein	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C9 Substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 Substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 Substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 1A2 Inhibitor	Non-inhibitor	Inhibitor	Inhibitor
CYP450 2C9 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4 Inhibitor	Non-inhibitor	Inhibitor	Inhibitor

absorption, distribution, metabolism and excretion of drugs[52].

It has also been found that rutin and quercetin could be inhibitors of CYP1A2 and CYP3A4 isozymes of cytochrome P450. Cytochrome P450 corresponds to the main family of enzymes related to the metabolism of drugs and xenobiotics. Regulation of these enzymes occurs in various forms, for example, genetic variation and xenobiotic or endogenous induction and repression (as for example, by cytokines and hormones)[53,54].

The results demonstrated that the secondary metabolites presented in *P. americana* leaves have antioxidant and antimicrobial activities. For this reason, the present study provides a basis for the use of this extract and its fractions in mycobacterial and fungal infections, mainly against *Candida* species and also as free radical scavengers, important as preventive of various disorders related to oxidative damage.

Conflict of interest:

Authors declare no conflicts of interest.

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