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Biochemical assessment of *Persea americana* leaves extracts: Antioxidant, Antimicrobial and Cytotoxic effects

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Abstract

This investigation aimed to assess the antioxidant, antimicrobial, and anticancer activity of *Persea americana* leaves extracts (ethanol, ethyl acetate, and petroleum ether). The total phenolic and flavonoid contents were measured, while the different phenolic compounds were recognized using the HPLC instrument. Antioxidant activity was measured using not only DPPH but also ABTS. In addition, antimicrobial activity was estimated *via* the agar well diffusion method against microbial strains *(Bacillus Subtilis, Aspergillus fumigatus, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa,* and *Escherichia coli).* Anticancer activity was determined against the human lung normal fibroblast (Wi38) and human lung carcinoma (A549) in addition to human liver carcinoma (HepG2) cell lines by the MTT protocol. The results showed that the highest total phenolic and flavonoid content was found in the ethanolic extract, with 217.58 ± 1.52 mg GAE/g and 71.55 ± 1.57 mg QE/g, respectively, compared with other extracts. The ethanolic extract contains very high concentrations of chlorogenic acid, naringenin, rutin, ferulic acid, syringic acid, gallic acid, coumaric acid, caffeic acid, methyl gallate, vanillin, and ellagic acid. The ethanolic extract gave the highest antioxidant activity using DPPH and ABTS (IC_{50} = 14.33 and 16.03 µg/ml, respectively). It also has the highest antimicrobial activity against the different microorganisms examined. The ethyl acetate extract has the highest anticancer activity against A549, HepG2 and Wi38 cell lines (IC_{50} = 44.28, 88.91 and 90.52 µg/ml, respectively). *P. americana* leaves extracts have strong antioxidant ability, strong antimicrobial activity, and very good cytotoxic activity.

Key words: Avocado leaves, phenolic compounds, scavenging activity, antibacterial, cytotoxicity.

1. Introduction

Medicinal plants have been used as traditional therapies for a range of human illnesses for thousands of years in many parts of the globe, being more popular in underdeveloped nations due to cultural acceptance and the idea that they are safe and non-toxic because they are natural [1]. The benefits of using plant products to combat human illnesses are their low cost, biodegradability, and widespread availability [2].

Polyphenols have gained popularity as a result of their medicinal usefulness, which is attributed to their antioxidant capabilities as well as anti-mutagenic, anti-carcinogenic, and antibacterial characteristics. The pharmaceutical and food sectors are particularly interested in the antioxidant properties of plant polyphenols [3]. Antioxidants are compounds that play a role in preventing or postponing the cell damage caused by unstable molecules known as free radicals that are created by the body in reaction to environmental and other stimuli. Natural antioxidants boost the plasma's antioxidant capacity and lower the risk of illnesses including cancer, atherosclerosis, heart disease, and stroke [4].

Antimicrobial health problems impose constraints on the treatment of moderate-to-severe microbial illnesses [5]. Antimicrobial resistance is formed when bacteria generate enzymes such as beta-lactamases

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that break down the antibiotic, decreasing its plasma concentration and therefore allowing the bacteria to survive and evolve molecular mechanisms that protect them from that specific type of antibiotic [6, 7].

Cancer is a non-contagious illness and has one of the highest fatality rates in the world [8]. Each year, 12.7 million new cancer diagnoses are recorded, with 7.6 million people dying as a result [9]. Certain medicinal plants contain novel biologically active compounds that can be used to treat cancer [10]. Avocado, or *Persea americana*, is a plant that has cancer-fighting properties [11].

Avocado is a tropical fruit native to America that belongs to the *Lauraceae* family and is scientifically known as *P. americana* Mill. Avocado pear is a fruit that is not common but possesses meaningful physico-chemical properties and nutritional content. Common varieties of avocado include Fuerte, Hass, Bacon, Gwen, Pinkerton, Reed, and Zutano. Additionally, *P. americana* has been shown to have analgesic, anti-inflammatory, antiviral, vasodilator, hypotensive, antiulcer, wound healing, antioxidant, hypoglycemic, antihepatotoxic, and anticancer properties [12].

Recently, there has been an increasing interest in the utilization and recycling of waste, as its accumulation leads to negative effects on the environment. Avocado leaves are considered a by-product and underutilized although being rich in naturally occurring bioactive compounds [13]. Avocado leaves, according to reports, are utilized as a traditional medication. The leaves have been used to treat diuresis, inflammation, hypertension, hypoglycemia, diarrhea, throat inflammation, and bleeding [14]. Avocado leaves include a variety of chemical components that are beneficial for human health such as flavonoids, phenols, tannins, alkaloids, and saponins [15]. The leaves may be used to brew tea and as a flavor in meat and bean meals [12].

The present study aimed to evaluate total phenolic and flavonoid contents by spectrophotometer, phenolic compounds by HPLC instrument, the antioxidant activity by DPPH and ABTS, the antimicrobial activity against gram-positive and gram-negative bacteria and fungi, and *in-vitro* cytotoxic activity against normal cell line (human lung fibroblast; WI 38) and carcinoma cell lines (human lung; A 549 and human liver; HepG2) by the MTT protocol of *P. americana* leaves extracts.

2. Materials and Methods

2.1. Chemicals and materials

Fuerte *P. americana* leaves were collected from the Horticultural Research Station in Al-Qanater Al-Khairia, Agricultural Research Center. Analytically pure solvents were used for analysis; ethanol, ethyl acetate, petroleum ether (40-60°C), and methanol. All chemicals and reagents (gallic acid, Folin-Ciocalteau reagent, quercetin, ascorbic acid, DPPH, and ABTS) were obtained from Sigma Chemicals Company, USA.

2.2. Microorganisms and cancer cell lines

All pathogenic microorganisms, gram-positive bacteria (*B. Subtilis* ATCC 6633 and *E. faecalis* ATCC 10541), gram-negative bacteria (*E. coli* ATCC 8739 and *P. aeruginosa* ATCC 90274), and fungi (*C. albicans* ATCC 10221 *and A. fumigatus*) were obtained from the Science Faculty, Al-Azhar University, Egypt. Cell lines, normal cell lines (human lung fibroblast; Wi38) and carcinoma cell lines (human lung; A549) and human liver; HepG2) were also obtained from the Science Faculty, Al-Azhar University, Egypt.

2.3. Preparation of plant extracts

Fresh leaves were rinsed in distilled water to eliminate any dirt, and then air-dried for two weeks at room temperature. Using an electronic blender, the dried leaves were crushed to a fine powder and kept in an airtight container in the refrigerator until analysis. The dried powder leaves of *P. americana* (200 g) were extracted successively into 2000 ml of each solvent: ethanol, ethyl acetate, and petroleum ether (40-60°C) by maceration for 48 h. Then, the ethanol extract (EE), ethyl acetate extract (EAE), and petroleum ether extract (PEE) were filtered through Whatman No. 1 filter paper. Using a rotary vacuum evaporator set to 45°C, the filtrates were evaporated. The crude extracts were weighed and dried in a freeze-drier before being stored in brown bottles in the refrigerator until further examination according to Womeni *et al.* [16].

2.4. Determination of total phenolic content (TPC)

According to Gao *et al.* [17], the folin-ciocalteau colorimetric technique is used to determine the TPC of *P. americana* leaves extracts. The results were determined and represented as mg gallic acid equivalents per gram of dry extract (mg GAE/g dry extract).

2.5. Determination of total flavonoid content (TFC)

The aluminium chloride technique reported by Quettier-Deleu *et al.* [18] was used to determine the TFC of *P. americana* leaves extracts. The results were determined and represented in mg of quercetin equivalents per gram of dry extract (mg QE/g dry extract).

2.6. Identification of phenolic compounds using HPLC

For the identification of phenolic compounds of *P. americana* leaves crude extracts, HPLC instrument was used according to Loon *et al.* [19]. An Agilent 1260 series HPLC analyzer was used for the analysis. The samples were separated using the Eclipse C18 column (4.6 mm x 250 mm i.d., 5 m). At a flow rate of 1 ml/min, both water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were used as the mobile phase. In the following order : (82 percent A) at 0 min; (80 percent A) at 0–5 min; (60 percent A) at 5-8 min; (60 percent A) at 8-12 min; (85 percent A) at 12-15 min and (82 percent A) at 15-16 min, the mobile phase in a linear gradient was coded. A wavelength of 280 nm (for phenolic compounds) and 330 nm (for flavonoid compounds) was used to monitor the multi-wavelength detector. About 10 µl injection volume was injected into each of the sample solutions. At 35°C, the column temperature was maintained.

2.7. Antioxidant activity

2.7.1. DPPH free radical scavenging assay

P. americana leaves extracts at different concentrations (25, 50, 100, 200, 400, 800, and 1000 µg/ml) were tested for their ability to inhibit free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Brand-Williams *et al.* [20]. Ascorbic acid was used as a standard. The IC_{50} of all extracts was calculated. The DPPH scavenging activity percentage was computed as inhibition percent according to the following formula:

$$\text{Inhibition percentage} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100$$

Where:

- A control represents the absorbance of the control at zero time.
- A sample represents the absorbance of the samples at 30 minutes.

2.7.2. ABTS scavenging assay

The ABTS [2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] assay compares antioxidants to ascorbic acid as a standard in terms of their ability to scavenge ABTS formed in the aqueous phase. The ABTS is made by the reaction of the ABTS salt with a strong oxidizing agent (potassium persulfate). The amount of hydrogen-donating antioxidants that reduce the blue-green ABTS radical is assessed [20]. *P. americana* leaves extracts at different concentrations (25, 50, 100, 200, 400, 800 and 1000 µg/ml) were evaluated [21]. The ABTS scavenging activity percentage was determined as inhibition percent by the following equation:

$$\text{Inhibition percentage} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100$$

Where:

- A control represents the absorbance of the control at zero time.
- A sample represents the absorbance of the samples at one min.

2.8. Antimicrobial activity

2.8.1. Agar Well-diffusion method

The antimicrobial activity of *P. americana* leaves extracts was determined according to Magaldi *et al* [22]. Bacterial strains were inoculated into peptone water to get a dilution of 1.0×10^6 CFU/ml. The nutrient agar media was used for bacteria, and potato dextrose agar media was used for fungi. After pouring media into plates, it was inoculated by bacterial strain by surface spreading. Wells (6 mm diameter) were poked aseptically using a sterile cork borer. About 100µl of *P. americana* leaves extracts (EE and EAE) (10 mg/ml) and the antibiotic Gentamycin (10 mcg/disc) as a standard were introduced into the wells. Plates were incubated after 15 minutes for 24 hours at 35°C for bacteria and 48-72 hours at 27°C for fungi. The inhibition zones were measured in millimeter. For each sample, the experiment was repeated three times, and the average data was recorded.

2.9. Cytotoxic activity

Cytotoxicity of *P. americana* leaves extracts (EE and EAE) was determined by viability assay (MTT protocol) using 3 cell lines; normal (human lung fibroblast; Wi38) and carcinoma (human lung; A549) and human liver carcinoma; HepG2), Science Faculty, Al-Azhar University, Egypt [23, 24]. The 96 well tissue culture plate was injected with 1 X 105 cells/ml (100ul/well), which were treated with different concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/ml) of *P. americana* leaves extracts (EE and EAE) and the drug doxorubicin (as a standard) at different concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/ml) separately and incubated in 5% CO_2 at 37°C for 24 hours. After adding 0.5 mg/ml MTT, the plate was incubated for 4 hours at 37°C with 5% CO_2. Formazan (MTT metabolic product) should be resuspended in DMSO. At 570 nm, the absorbance was measured to evaluate the number of viable cells and the concentrations required to kill 50% of cancer

cells (IC_{50}) were calculated. By using the following formula, the percentage of cell viability was calculated:

Cell viability (%) = [(Control OD – Sample OD)/Control OD] X 100.

2.10. Statistical analysis

The data is presented as a mean ± standard error (n = 3). The data was analysed using SPSS (version 20) as described in Steel and Torrie, [25], with a p-value < 0.05.

3. Result and Discussion

3.1. Total phenolic content of P. americana leaves extracts

Phenolic compounds are the most abundant secondary metabolites present in the plants that help in their defence mechanisms [26]. The EE of *P. americana* leaves has the highest TPC, which registered 217.58 ± 1.52 mg GAE/g, followed by EAE (55.5 ± 0.61 mg GAE/g), while the PEE has the lowest TPC at 39.12 ± 0.47 mg GAE/g (Fig. 1). The TPC of EE was higher than that of Abd Elkader *et al*. [27], who found that the TPC of EE was 178.95 mg GAE/g. *P. americana* leaves contain high phenolic compounds, which are regarded as good natural strong antioxidants [16].

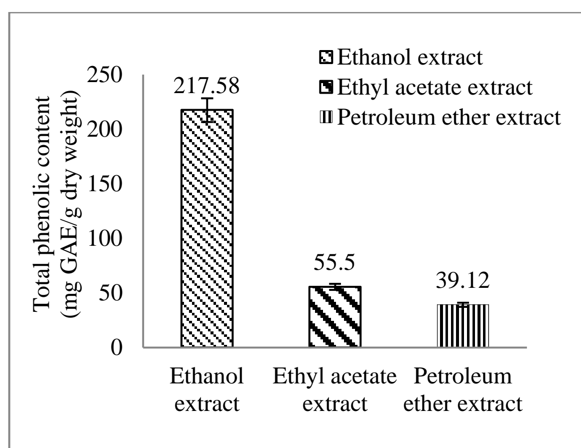

Fig.1. Total phenolic content of *P. americana* leaves extracts

3.2. Total flavonoid content of P. americana leaves extracts

The highest TFC was recorded at 71.55 ± 1.57 mg QE/g for the EE followed by EAE at 19.73 ± 0.53 mg QE/g, while the PEE has the lowest TFC at 10.41 ± 0.13 mg QE/g (Fig. 2). The TFC of EE was consistent with Abd Elkader *et al*. [27], who stated that the TFC of EE was 70.08 mg QE/g. *P. americana* leaves contain flavonoids, which have potent antioxidant activity through a variety of action mechanisms [28]. This was due to their structures being more complicated than phenolic acids [29].

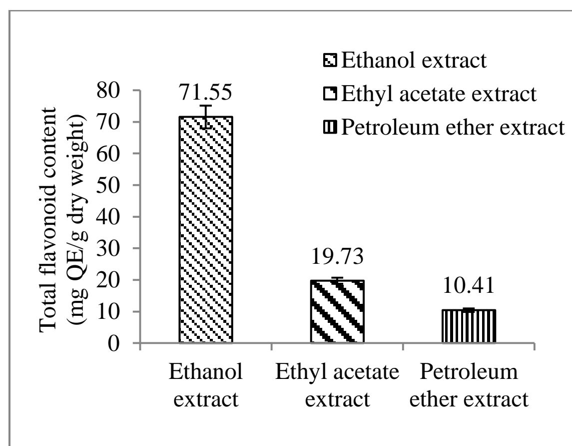

Fig.2. Total flavonoid content of *P. americana* leaves extracts

3.3. Identification of phenolic compounds using HPLC

Phenolic compounds of *P. americana* leaves extracts were quantified by using the HPLC technique. Among 16 available standard phenolic compounds, 13 compounds were detected in the EE. The EAE contained 14 compounds, while the PEE contained only 8 compounds. The results in Table (1) showed that the EE contained very high concentrations of chlorogenic acid (22250.72 µg/g), naringenin (6470.32 µg/g), rutin (3216.15 µg/g), ferulic acid (2763.13 µg/g), syringic acid (2545.46 µg/g), coumaric acid (1327.95 µg/g), gallic acid (1326.13 µg/g), caffeic acid (1209.95 µg/g), methyl gallate (655.33 µg/g), vanillin (571.88 µg/g) and ellagic acid (229.44 µg/g) compared with EAE and PEE. EAE contained a higher concentration of cinnamic acid (107.99 µg/g). PEE contained high concentrations of kaempferol (648.23 µg/g) and taxifolin (172.21 µg/g) compared with EE and EAE.

3.4. Antioxidant activity

3.4.1. DPPH free radical scavenging assay

The results of the antioxidant activity of *P. americana* leaves extracts showed that they exhibit a considerable scavenging effect; nevertheless, they were less effective than ascorbic acid as standard. The EE gave the highest inhibition percentage of 95.80% ± 0.42, 94.78% ± 0.22, and 92.94% ± 0.36 at

concentrations of 100, 200, 400 µg/ml, respectively, compared with EAE and PEE, while it was less than the inhibition percentage of ascorbic acid at the same concentrations (Table 2). The IC_{50} values of DPPH radicals of EE, EAE, and PEE were 14.33, 22.19, and 49.85 µg/ml, respectively. These results are higher than Abd Elkader *et al*. [27], who found that the EE gave the highest inhibition percentage of 91.03% ± 0.19 at a concentration of 1000 µg/ml, and the IC_{50} value was 421.6 µg/ml. EE has the highest antioxidant activity. This is due to the high TPC and the presence of phenolic and flavonoid compounds, which were determined in this study. Plants with high phenolic content had strong DPPH radical scavenging activity [16, 30].

Table 1. Phenolic compounds of *P. americana* leaves extracts by HPLC

Phenolic compounds	Retention time (min)	Concentration (µg /g)		
		ethanol extract	ethyl acetate extract	petroleum ether extract
Gallic acid	3.116	1326.13	145.80	81.27
Chlorogenic acid	3.911	22250.72	2311.26	62.95
Methyl gallate	5.053	655.33	8.61	0.00
Caffeic acid	5.419	1209.95	62.60	0.00
Syringic acid	5.894	2545.46	93.78	7.64
Ellagic acid	7.795	229.44	32.75	0.00
Coumaric acid	8.136	1327.95	132.68	3.70
Vanillin	8.749	571.88	43.37	21.54
Ferulic acid	9.626	2763.13	172.49	0.00
Cinnamic acid	13.295	13.29	107.99	61.37
Rutin	7.103	3216.15	16.75	0.00
Naringenin	10.025	6470.32	770.66	0.00
Taxifolin	12.056	0.00	151.24	172.21
Kaempferol	14.169	10.15	37.09	648.23

Table 2. Antioxidant activity (%) of *P. americana* leaves extracts by DPPH free radical scavenging

Concentration (ppm)	Inhibition (%)			
	Ascorbic acid	Ethanol extract	Ethyl acetate extract	Petroleum ether extract
25	96.49 ± 0.01^{g}	87.22 ± 0.22^{f}	56.32 ± 0.06^{f}	49.27 ± 0.42^{e}
50	96.76 ± 0.01^{f}	92.95 ± 0.15^{c}	59.37 ± 0.43^{e}	50.15 ± 0.04^{d}
100	97.04 ± 0.01^{e}	95.80 ± 0.42^{a}	63.38 ± 0.26^{d}	56.04 ± 0.47^{c}
200	97.14 ± 0.01^{d}	94.78 ± 0.22^{b}	67.72 ± 1.10^{c}	66.07 ± 0.08^{b}
400	97.65 ± 0.01^{c}	92.94 ± 0.36^{c}	78.94 ± 1.20^{b}	66.48 ± 0.12^{b}
800	98.37 ± 0.01^{b}	91.60 ± 0.50^{d}	90.42 ± 0.56^{a}	70.34 ± 0.16^{a}
1000	99.14 ± 0.01^{a}	88.45 ± 0.21^{e}	92.2 ± 0.21^{a}	70.63 ± 0.05^{a}
LSD 5%	0.02	0.97	2.07	0.77
IC_{50} (ppm)	12.95	14.33	22.19	49.85

Values are mean ± SE (n=3). The mean values within a column indicate significant differences ($p<0.05$). LSD is the least significant difference. The IC_{50} values were determined from dose – effect curves by linear regression.

3.4.2. ABTS scavenging assay

The antioxidant activity of *P. americana* leaves extracts revealed that the leaves have a considerable scavenging effect, but it was less than that of ascorbic acid. As shown in Table (3), the EE gave the highest inhibition percentage of 95.92% ± 0.19 and 96.47% ± 0.20 at concentrations of 50 and 100 µg/ml, respectively, compared with other extracts (EAE and PEE), while it was less than the inhibition percentage of ascorbic acid at the same concentrations. The PEE gave the lowest inhibition percentage. The IC_{50} values of the ABTS radicals of EE, EAE and PEE were 16.03, 307.22 and 1279.75 µg/ml, respectively. The IC_{50} values proved that the EE has the highest antioxidant activity. By the two methods used (DPPH and ABTS), the EE has the highest antioxidant activity, while the PEE has the lowest antioxidant activity.

Table 3. Antioxidant activity of *P. americana* leaves extracts by ABTS scavenging in different concentrations

Concentration (ppm)	Inhibition (%)			
	Ascorbic acid	Ethanol extract	Ethyl acetate extract	Petroleum ether extract
25	96.97±0.01^{g}	77.97±0.21^{f}	30.27±1.65^{f}	23.94±0.50^{g}
50	97.36±0.01^{f}	95.92±0.19^{a}	32.19±1.44^{f}	29.21±0.25^{f}
100	97.62±0.01^{e}	96.47±0.20^{a}	38.26±0.68^{e}	30.91±0.14^{e}
200	98.11±0.01^{d}	92.87±0.33^{b}	47.68±1.75^{d}	32.14±0.16^{d}
400	98.48±0.01^{c}	91.28±0.31^{c}	65.10±0.46^{c}	33.66±0.29^{c}
800	99.29±0.01^{b}	89.78±0.50^{d}	71.50±0.07^{b}	35.77±0.13^{b}
1000	99.60±0.01^{a}	83.62±0.25^{e}	87.72±1.29^{a}	39.07±0.48^{a}
LSD 5%	0.03	0.91	3.66	0.95
IC_{50} (ppm)	12.89	16.03	307.22	1279.75

Values are mean ± SE (n=3). The mean values within a column indicate significant differences ($p<0.05$). LSD is the least significant difference. The IC_{50} values were determined from dose – effect curves by linear regression.

3.5. Antimicrobial activity

3.5.1. Agar Well-diffusion method

The data in Table (4) showed inhibition zones of EE and EAE of *P. americana* leaves by the agar well diffusion method against *B. Subtilis, E. faecalis, E. coli, P. aeruginosa, C. albicans, and A. fumigatus* when compared with control (gentamycin). The efficacy of the extract against microbial growth is responsible for the size of the resulting zones. The results showed that the antimicrobial activity of *P. americana* leaves extracts was higher than that of the gentamycin. EE has the highest inhibition zone on *B. Subtilis* (30±0.35 mm), *C. albicans* (25±0.17 mm), *P. aeruginosa* (24±0.29 mm), *E. coli* (21 ± 0.23 mm) and *A. fumigatus* (18±0.29 mm) compared with gentamycin and EAE. EAE has the highest inhibition zone on *E. faecalis* (25±0.12 mm) compared with EE and control. EE has the highest inhibition zone on *B. Subtilis* (30±0.35 mm) and the least inhibition zone on *A. fumigatus* (18±0.29 mm), while the EAE has the highest inhibition zone on *E. faecalis* (25±0.12 mm) and the least inhibition zone on *A. fumigatus* (16±0.40 mm). The zone of inhibition indicated that the EE has the highest antibacterial activity, according to the zone of inhibition, and this is because it contains bioactive substances such as phenols, tannins, flavonoids, and terpenoids [31]. A previous study [32] showed that the extracts of *P. americana* leaves have antimicrobial activity against all tested organisms, with inhibition zones ranging from 10.27 mm to 34.20 mm, which is in agreement with the results of the current study. Gram-positive and gram-negative bacteria have different responses to *P. americana* leaves extracts. Their cell wall structure is the primary factor in their variance. One layer is all that gram-positive bacteria have on their cell walls; the other, gram-negative, has many layers and an outside cell membrane [33]. According to

some reports, the difference in cell wall transparency between bacteria and fungi makes bacteria more sensitive to antimicrobials [34]. The demonstrated relative efficacy of plant extracts in comparison to commercial medications may imply a greater benefit when both are taken synergistically [35].

Table 4. Antimicrobial activity of ethanol and ethyl acetate extracts of *P. americana* leaves

sample	Zone of inhibition (mm)					
	Gram positive bacteria		Gram negative bacteria		Fungi	
	Bacillus Subtilis	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus fumigatus*
Gentamycin	25±0.29[b]	15±0.17[c]	17±0.06[b]	22±0.12[b]	21±0.23[c]	15±0.12[b]
Ethanol extract	30±0.35[a]	22±0.29[b]	21±0.23[a]	24±0.29[a]	25±0.17[a]	18±0.29[a]
Ethyl acetate extract	22±0.40[c]	25±0.12[a]	20±0.46[a]	20±0.46[c]	22±0.29[b]	16±0.40[b]
LSD 5%	1.21	0.71	1.04	1.11	0.82	1.02

Values are mean ± SE (n=3). The mean values within a column indicate significant differences ($p<0.05$). LSD is the least significant difference.

3.6. Cytotoxic activity

The cytotoxic activity of *P. americana* leaf extracts (EE and EAE) was tested using the MTT protocol against human lung fibroblast normal (Wi38), human lung carcinoma (A549), and human liver carcinoma (HepG2) cell lines and compared to untreated cells (control) and drug doxorubicin at different concentrations (31.25, 62.5, 125, 250, 500, and 1000 ppm. In Table (5), the toxicity percent and IC_{50} of Wi38, A549, and HepG2 were presented. *P. americana* leaves significantly increased their toxicity percentages (Wi38, A549, and HepG2) with increasing concentrations of EE and EAE. The results revealed that the highest toxicity percentage of Wi38 cells was obtained from EE and EAE (96.03%±0.13 and 95.66%±0.08), respectively, at a concentration of 1000 µg/ml, the highest toxicity percentage of A549 cells was obtained from EE and EAE (95.36%±0.08 and 95.27%±0.08), respectively, at a concentration of 1000 µg/ml, and the highest toxicity percentage of HepG2 cells was obtained from EE and EAE (95.15%±0.09 and 95.06%±0.09), respectively, at a concentration of 1000 µg/ml. When compared to EE, which gave IC_{50} values of 171.47 µg/ml in Wi38 cell line, 104.68 µg/ml in A549 cell line, and 101.83 µg/ml in HepG2 cell line, EAE showed stronger cytotoxic effects with IC_{50} values of 90.52 µg/ml in Wi38 cell line, 44.28 µg/ml in A549 cell line, and 88.91 µg/ml in HepG2 cell line. IC_{50} values revealed that the EE has the highest cytotoxic effect on the HepG2 cell line (101.83 µg/ml) followed by the A549 cell line (104.68 µg/ml) and the Wi38 cell line (171.47 µg/ml), while EAE has the highest cytotoxic effect on the A549 cell line (44.28 µg/ml) followed by the HepG2 cell line (88.91 µg/ml) and the Wi38 cell line (90.52 µg/ml). IC_{50} values proved that the anticancer activity of EAE was higher than EE against Wi38, A549, and HepG2 cell lines. The reason for that is that EAE contains cinnamic acid, which was determined in this study, and studies have shown that cinnamic acid has anticancer properties [36, 37]. The results of this study corroborate those of a recent study [38], which demonstrated the cytotoxicity of EAE of *P. americana* leaves against HeLa cells with an IC_{50} value of 132.7 µg/ml.

Table 5. Toxicity percent of ethanol and ethyl acetate extracts of *P. americana* leaves on human lung fibroblast cell line (Wi38), human lung carcinoma cell line (A549), and human liver carcinoma cell line (HepG2)

Samples	Concentration (µg/ml)	Wi38 cells		A549 cells		HepG2 cells	
		Toxicity %	IC_{50} (µg/ml)	Toxicity %	IC_{50} (µg/ml)	Toxicity %	IC_{50} (µg/ml)
Control	0	0		0		0	
Doxorubicin	31.25	4.66 ± 0.94^{g}	92.05	46.03 ± 2.60^{e}	26.86	50.45 ± 1.67^{f}	20.09
	62.5	26.32 ± 2.09^{f}		73.02 ± 2.29^{c}		69.01 ± 2.36^{de}	
	125	75.42 ± 1.26^{c}		86.79 ± 1.82^{b}		83.31 ± 1.86^{c}	
	250	88.43 ± 1.10^{b}		96.94 ± 0.10^{a}		90.63 ± 2.40^{b}	
	500	96.72 ± 0.67^{a}		97.17 ± 0.15^{a}		97.37 ± 0.10^{a}	
	1000	97.39 ± 0.09^{a}		97.28 ± 0.10^{a}		97.60 ± 0.13^{a}	
ethanol extract	31.25	0.96 ± 1.30^{h}	171.47	0.00 ± 1.69^{h}	104.68	0.71 ± 1.77^{i}	101.83
	62.5	1.76 ± 0.89^{gh}		29.45 ± 1.99^{g}		21.08 ± 2.02^{h}	
	125	38.78 ± 2.04^{e}		60.76 ± 1.98^{d}		66.75 ± 1.30^{e}	
	250	77.70 ± 1.84^{c}		86.83 ± 1.69^{b}		94.18 ± 0.46^{ab}	
	500	95.66 ± 0.27^{a}		95.19 ± 0.14^{a}		94.62 ± 0.38^{ab}	
	1000	96.03 ± 0.13^{a}		95.36 ± 0.08^{a}		95.15 ± 0.09^{a}	
ethyl acetate extract	31.25	0.96 ± 1.33^{h}	90.52	38.14 ± 0.89^{f}	44.28	2.38 ± 2.26^{i}	88.91
	62.5	40.25 ± 2.07^{e}		75.45 ± 1.92^{c}		40.12 ± 1.91^{g}	
	125	71.15 ± 1.86^{d}		90.55 ± 0.88^{b}		72.93 ± 1.54^{d}	
	250	95.36 ± 0.22^{a}		95.02 ± 0.08^{a}		93.83 ± 0.49^{ab}	
	500	95.51 ± 0.15^{a}		95.19 ± 0.14^{a}		95.24 ± 0.00^{a}	
	1000	95.66 ± 0.08^{a}		95.27 ± 0.08^{a}		95.06 ± 0.09^{a}	
LSD 5%		3.59		3.95		4.18	

Values are mean ± SE (n=3). The mean values within a column indicate significant differences ($p<0.05$). LSD is the least significant difference. The IC_{50} values were determined from dose – effect curves by linear regression.

4. Conclusions

The current study's goal was to determine the total phenolic and flavonoid content, as well as antioxidant, antibacterial, and anticancer activity of *P. americana* leaf extracts. The ethanolic extract has the highest total phenolic and flavonoid content, as well as extremely high concentrations of chlorogenic acid, naringenin, rutin, ferulic acid, syringic acid, gallic acid, coumaric acid, caffeic acid, methyl gallate, vanillin, and ellagic acid, indicating that it has the highest antioxidant activity. It also possesses the highest antibacterial activity of all the microorganisms tested. Against A549, HepG2, and Wi38, the ethyl acetate extract had the most antitumor action.

5. Conflicts of interest

The authors stated no possible conflicts of interest.

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