Fungicidal, Molluscicidal and Phytocidal Activity of Avocado, *Persea americana* Mill Industrial Wastes

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ABSTRACT

The avocado fruits were divided into peel, flesh, and seeds. Each part was individually extracted and checked for its phytocidal effects against wheat (*Triticum aestivum*) and squash (*Cucumber pepo*) plants at different stages; its fungicidal effects against *Botritis cenerea* and *Fusarium oxysporum* fungi; and its molluscicidal effects against *Theba pisana*. The peel, flesh and seed extracts inhibited the growth of *B. cenerea* and *F. oxysporum* with EC_{50} values of >1000, > 1000; 816, 616 and 1077 and 471 µg/ml, respectively. The remained seed powder killed the treated snail increasingly after 4, 8, and 12 days of exposure, with 58.6, 37.9 and 32.3 % LC_{50} values in the same array, while its extract exhibited LC_{50} values of > 1.0%, 1.08%, and 0.31%, respectively, within the exposure time. All extracts inhibited the germination of seeds and the growth of the germinated seedlings. The inhibition effect was higher on squash seeds than wheat grains. On wheat seedlings, the seed extract exhibited the highest effect inhibiting the root and shoot systems, with EC_{50} values of 29 and 170 µg/ml, respectively. The seed extract was identified for its bioactive compounds through GC-MS analysis. Its active biochemical compounds were identified to be rutin (quercetine-3-glucoside-rhamnoside) and quercetin-3-O-arabinosyl-glucoside as phenolic derivatives (flavonol derivatives), carotenoids (lutein, zeaxanthin and lycopene) and procyanidin dimer B type.

Keywords: Avadoco, phytocidal, molluscicidal, fungicidal, biological activity, extraction, GC-MS.

INTRODUCTION

Currently conventional pesticides used in agricultural crops caused allergic diseases in children and adolescents (Rodrigues *et al*., 2022), decreased sperm count, and damage to the ecosystem due to their persistence (Knapke *et al*., 2022). The persistent compounds go through biotic and abiotic transformation processes, resulting in derivatives that are more toxic than the original compounds. Therefore, it is necessary to get new alternatives to these commercially conventional synthetic pesticides as organic products due to their biodegradability. So plants have been attracting the attention of several investigators for their importance in plant protection (Abde-Aty, 2018; Abdel-Aty & Abdel-Megeed, 2015 and Abdel-Aty & Al-Solami, 2020). Several studies have reported that the avocado (*Persea americana* Mill.) crop is highly demanded internationally because of its great social and economic importance (FAO, 2018). The avocado fruit weighed 150-400 g approximately consisting of the exocarp (skin), mesocarp (pulp), endocarp, and seed. The mesocarp accounts for 52.9- 81.3% of the fruit mass, however, the shell and seed (21-30%) are not used in the food industry, so they become an excessive amount of waste, which ultimately pollutes the environment (Mora-Sandí *et al*., 2021). It is an economically and environmentally advantageous idea to use these waste by-products (Bangar *et al*., 2022). Leite *et al*. (2009) showed that the hexane and methanol extracts from avocado seeds killed the *Aedes aegypti* larvae, with LC_{50} values of 16.7 and 8.87 mg/ml, respectively. The ethanolic extracts of avocado seed at higher than 500 µg/ml presented a moderate activity against *Trypanosoma cruzi*, the etiologic agent of Chagas disease, one of the most serious protozoal diseases in Latin America (Abe *et al*., 2005). Ethanolic and aqueous extracts of *P. americana* seeds, regardless the cultivar used, showed promising insecticidal activity against whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) biotype B nymphs and became an eco-friendly solution for agriculture (de Carvalho *et al*., 2021). Also, Leite *et al*. (2009) showed that avocado seeds organic extracts inhibited *Candida* spp., *Cryptococcus neoformans*, and *Malassezia pachydermatis* as well as bacteria including *Staphyllococcus aureus, S. pyogenes, Candida ulcerans, C. albicans, Escherichia*

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coli, and S. typhi. Methanol and chloroform extracts of avocado seed exhibited antifungal potential against *Cryptococcus neoformans*, while petroleum ether extracts inhibited *S. aureus* activity (Falodun *et al*., 2014). Jiménez-Arellanes *et al*. (2013) observed the anti-parasitic activity of seeds on *E. histolytica*, and *G. lamblia*. Chemical profiling of volatile compounds indicated that avocado seed contains sesquiterpenoids, poly, and unsaturated fatty acid esters with antimicrobial activities (Soledad *et al*., 2021). Avocado seed extract is a rich source of acetogenins, which have strong antimicrobial, antifungal, and insecticidal properties (Salazar-López *et al*., 2020). The extract inhibited *Listeria monocytogenes* completely and showed antibacterial activities against several Gram-positive bacteria, including *Bacillus subtilis, S. aureus, Clostridium perfringens, C. sporogenes,* and *Alicyclobacillus acidocaldarius* (Villarreal-Lara *et al*., 2019). Paternina-Ricardo *et al*. (2022) pointed out that the herbicidal activity of avocado seed extracts has not yet been explored, however, the bioactive components present in the seed identified to date are associated with herbicidal activities, so it is important to identify the phytotoxic bioactive components in avocado seeds.

`So in this research, the peel, flesh, and seed of the avocado fruits were investigated. Each part was individually extracted and checked for their phytocidal effects against a narrow-leaf (wheat, *Triticum aestivum*) and a broad-leaf (squash, *Cucumber peppo*) examples at different stages; their fungicidal effects against plant pathogenic air-born *Botritis cenerea* and soil-born *Fusarium oxysporum* fungi and their molluscicidal effects against the plant attacking dangerous snail, *Theba pisana*. The most active extract was identified for its containing constituents using the gas chromatography mass spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

- Preparation of the tested extracts

The ripe fresh fruits of avocado (*Persea americana* Mill.) (1.05 Kg) were purchased from a local market in Alexandria, transferred to the laboratory in plastic sucks, washed several times and air dried. The cleaned fruits were separated into three parts; the dark green outer fruit peel (171.38 g), the light green fruit flesh (756.77 g) and the dark brown fruit seed (114.25 g). Each plant origin (peel, flesh or seed) was separately frozen, mashed and individually soaked twice in acetone at room temperature in the dark for a week. The resulted acetone extract of each part was filtered under reduced pressure and concentrated under vacuum at < 50 ^{O}C to complete elimination of the organic solvent. The concentration of the extracted solid materials in each extract was calculated for their biological uses. The obtained three extracts; peel, flesh, and seed powders after extractions were dark green solution, 62.4 g (0.028 g) solid materials/ml), light green solution, 70.2 g (0.1 g solid materials/ ml) and light red solution, 67.0 g (0.05 g/ ml), respectively, achieving 2.37 g, 7.02 g, and 3.35 g oven dried extract, respectively. The extraction and separation procedure is based on Abdel-Aty (2018).

I. Fungicidal Activity

Tested fungi

Botrytis cenerea and *Fusarium oxysporum* were tested as economical plant pathogenic fungi damaging several crops. These pathogenic fungi were provided from the Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt.

Bioassay measurement:

The pathogenic fungi were grown on potato dextrose agar (PDA) medium for seven days before use. Measurements were carried out using the radial growth test (Torgeson, 1967). A definite volume of the well-known potato dextrose agar medium containing agar (4.5 g/100 ml of water) was used under sterilized conditions. The tested extracts in dimethyl-sulfoxide (DMSO) were used at 50, 100, 200, 500, 750, and 1000 µg/ml. Three replicates were considered as one treatment. After solidification, the inoculum disc (7 mm in diameter) of each treated fungus was located in the center of the petri-dish. Control in the presence of DMSO as high as 1 % was concurrently conducted. When the untreated fungi completely covered the surface of petri-dishes, the hyphal growth in each replicate was measured. The inhibition percent of the hyphal growth were calculated according to Topps and Wain (1957).

II. Molluscicidal activity

Treated animals

The white garden snails, *Theba pisana* (Müller) were collected from the farm of the Faculty of Agriculture, Alexandria University, at Abbis area, Alexandria, Egypt and were kept for adaptation under laboratory conditions for two weeks.

Toxicity measurements

The bait used was prepared on the wheat bran according to Miller *et al*. (1988). Water was added at 20% of the prepared bait. Five grams of the tested bait were introduced in a Petri dish (9 cm) to ten snails in each replicate. The Petri dish was placed in a plastic pot covered with a piece of fixed cloth. The cloth pieces were daily moistened. Three replicates were used for each treatment, and control was concurrently carried out. The tested plant debris after extraction were preliminarily examined at 1/6, 2/6, 3/6, and 4/6 ratios in gram of plant debris to the total weight of the toxic bait, while the other liquid extracts were tested

at 0.1, 0.2, 0.4, 0.5 and 1% of the total prepared bait as non-choice food. The number of dead snails was recorded and excluded at different times (4, 8, and 12 days). Mortality percent, lethal concentration that caused 50% mortality (LC_{50}), and lethal time needed to cause 50% lethality (LT_{50}) were calculated for each tested material (plant debris or the liquid extracts) according to Lokma and Al-Harpy (1999).

Phytocidal activity

Seed Treatment

The extracts were tested for their phytotoxicity on wheat and squash seeds using the cotton plug technique (Grodzinsky and Grodzinsky, 1973) at 50, 100, 250, 500, 1000, 2000, and 5000 µg/ml. They were dissolved in DMSO at a concentration as high as 1% of the tested solution volume. Thirty wheat seeds were used in each replicate. Three replicates were considered a treatment. Control was concurrently conducted. After 10 days, the number of germinated seeds was counted and the height of the grown seedlings was measured. The inhibition percent in both seed germination and the produced seedling growth were calculated and compared with control. The effective concentration that caused 50% inhibition of the treated population (EC_{50}) was also calculated.

Seedling treatment (Plain agar technique)

Phytocidal effects of the extracts were individually tested against wheat seedlings (*T. aestivum*) and squash seedlings (*C. pepo*) using the plain agar technique (Zemanek, 1963). The extracts were mixed with the medium at 250, 500, 1000, 2000, and 4000 µg/ml in test tubes. Pre-germinated seeds were sown in the solidified agar. Three replicates were used for each treatment. Control was concurrently done under the same conditions. The seedlings were watered until the roots reached the bottom of a test tube. The length of both root and shoot systems was measured and the inhibition percentages were calculated. The EC_{50} values were compared among the tested extracts.

III. Identification of *Persea* armeniaca seed kernel extract by GC-MS

GC–MS analysis was done in the Central Laboratory of the Faculty of Agriculture, Alexandria University, Egypt. The extract of the most active constituent (seed kernel extract) was identified using GC-MS analysis under the following conditions:

The used GC-MS spectrometer was a Thermo Scientific ISQ single quadruple GC/MS. The column, a TG-5SILMS capillary column (30 m, 0.25 mm, 0.25 µm film thickness; Agilent) was used as the stationary phase. Helium served as a mobile phase with 1.0 ml/min flow rate. Injection temperature 300°C. Detector: MS-Ion source temperature 300°C. A sample in acetone (1µl of 0.5 mg/ml) was performed in a split ratio (1:10) mode. Column Oven Temp.: 80.0°C. The applied program of oven temperature included an initial step for 3 min at 80°C, temperature ramped to 180° C with 5°C /min, the temperature was held for 2 min, 200°C to 280°C with 4°C /min, and the temperature was held for 5 min, 280°C to 300°C with 8°C /min, the temperature was held for 4 min. All mass spectra were recorded in electron impact ionization (EI) at 70 electron volts. Peak area percent was used for obtaining quantitative data with the Excalibur 2.0 Software (Thermo Technologies) without using response factor correction. The compounds in each sample were identified by comparison of their mass spectral pattern and their linear retention indices (RIs) based on a homologous series of alkanes (C_8-C_{24}) with those of authentic references and the MS libraries (NIST and Wiley) database under identical GC-MS conditions.

IV. Statistical analysis

Mortality percentages were analyzed using the analysis of variance (ANOVA). The EC_{50}, LC_{50} and LT_{50} values with 95% confidence limits were determined using probity analysis (Finney, 1971).

RESULTS AND DISCUSSION

1. Fungicidal Activity

The treated fungi hyphal growth was inhibited differently as a function of the treated extract, the tested concentration and the treated fungus. *B. cenerea* and *F. oxysporum* hyphal growth was inhibited by the tested extracts in systemic arrangement with their tested concentrations. The peel extract caused inhibition ranged between 15.3 – 43.8% and 0 - 47.7% against *B. cenerea* and *F. oxysporum*, respectively, with EC_{50} value more than 1000 µg/ml (Table **1**). The flesh extract inhibited the hyphal growth with 0 – 53.4% and 6.3 – 62.5% inhibition against *F. oxysporum* and *B. cenerea* with EC_{50} values of 816 and 616, respectively. The seed extract achieved 18.8 – 68.8% and 0 – 52.4% inhibition against *B. cenerea* and *F. oxysporum*, respectively with 471 and 1077 µg/ml, respectively. The results revealed that the seed extract was more effective against the *B. cenerea* hyphal growth, while the flesh extract appeared more effective against the *F. oxysporum* hyphal growth (Table 1).

II. Molluscicidal Activity

The treated terrestrial snail was affected by the organic extracts and the remained plant debris after extraction in different degrees. The mortality of the treated snail, *T. pisana* increased with increasing the tested concentration in general. Introducing the remained fruit flesh powder (after extraction) as a

poison bait with wheat bran caused the least effect among the tested powders with lethality ranged from 0 - 4.8%, 0 - 9.5%, and 0 - 23.8% after 4, 8, and 12 days of exposure, with LC_{50} values > 66% in all cases. The peel powder appeared more effective than the flesh powder as it caused lethality ranges of 0 – 23.8, 0 – 28.6, and 0 – 38.1 after 4, 8 and 12 days of exposure, respectively, with LC_{50} values > 66% in all cases. After the extraction of the fruit seeds, the remaining seed powder overcame the other two powders (flesh and peel) in their effect. Mortality of snails increased with increasing the time of exposure and the tested concentration, where mortality ranged from 0 – 57.1, 4.8 – 100, and 9.5 – 100% after 4, 8, and 12 days of exposure with LC_{50} values of 58.6, 37.9 and 32.3 %, respectively. On the other hand, the seed powder mortal effect reached 50% mortality after 12.3, 4.3 and 2.7 days when applied (mixed with the wheat bran) at 1:3, 1:1 and 2:1 (W/W) ratio, respectively (Table 2).

Table 1. Effect of the avocado extracts on the growth of *Botrytis cenerea* and *Fusarium oxysporum*

Tested Extract	The treated fungi					
	Air-born fungus			Soil-born fungus		
	B. cenerea			F. oxysporum		
	EC50 (µg/ml) 95% C.L.	Slope ±SE	χ^2	EC50 (µg/ml) 95% C.L.	Slope ±SE	χ^2
Peel	>1000			>1000		
Seed	471 371 – 598	1.34±0.032	0.5	1077 853 – 1361	2.29± 0.082	5.4
Flesh	616 497 -764	1.69±0.038	2.3	816 665 – 1003	2.09±0.054	2.7

C.L.= Confidence limit; SE= Standard error and χ^2= Chi squared

Table 2. Toxicity of the avocado fruit originated powders after extraction against the land snail, *Theba pisana*; shown as average percent mortality ± SD and LC_{50} values

Fruit Part Used	Exposure Time (days)	Plant material % in a poison bait					LC_{50} 95% CL	Slope ±SE	χ^2
		0	17	33	50	66			
Flesh powder	4	0	0	0	0	4.8±1.2	> 66%		
	8	0	0	0	4.8±1.2	9.5±2.3	> 66%		
	12	0	0	0	14.3±3.1	23.8±5.2	> 66%		
Peel powder	4	0	0	0	9.5±2.3	23.8±4.3	> 66%		
	8	0	0	4.8±1.2	14.3±4.3	28.6±2.2	> 66%		
	12	0	0	9.5±2.3	19.1 ±4.1	38.1±3.1	> 66%		
Seed powder	4	0	0	19.1±4.1	38.1±4.1	57.1±5.3	58.6 53 – 64.5	4.1± 0.23	2.4
	8	0	4.8±0.8	33.4±4.3	71.4±6.3	100	37.9 35.8 – 40	6.8± 0.29	10.3
	12	0	9.5±1.3	57.1±9.3	80.9±8.2	100	32.3 30.3 –34.3	6.1± 0.24	8.2
	LT_{50} values at different ratios								
	Ratio	0		1:3	1:1	2:1			
	LT_{50}	>14 days		12.3	4.3	2.7			
	95% CL			9.2 – 16.4	3.4 – 5.4	2.1 – 3.4			
	Slope±SE			1.54±0.08	1.81±0.07	2.65±0.13			
	χ^2			1.94	0.48	6.6			

C.L.= Confidence limit; SE= Standard error and χ^2= Chi squared

The obtained organic extracts originated from the avocado fruit peel, flesh, and seed revealed varying degree of effect. All extracts caused their lethal effect increasingly with increasing both the time of exposure and the tested concentration against *T. pisana* (Table 3). The extract originated from the flesh appeared the least effective among the tested extracts with mortality rates of 0, 13.3, and 23.3% at the highest concentration after 4, 8, and 12 days of exposure, respectively, exhibiting a LC_{50} value > 1.0% at all periods. The peel extract caused 30, 40, and 53.3 mortality percentages at the highest concentration after 4, 8, and 12 days of exposure, respectively, achieving a LC_{50} value > 1.0% after 4 and 8 days of exposure, while it exhibited a 1.08% LC_{50} value after 12 days of exposure. The seed extract appeared the most effective one, exhibiting mortality of 66.7, 86.7 and 100% after 4, 8, and 12 days of exposure to the treated snail population, achieving a LC_{50} value of 0.73, 0.49 and 0.31%, which is an impressive effect against this snail pest that is too difficult to control. At the same time, it is well known that the avocado seed is considered as waste material in avocado industry, so it is very good to use it for killing such a stubborn pest as one of the most dangerous pests threatening the agriculture productivity with no high cost.

III.Phytocidal Activity

It was noticed that all the extracts inhibited the germination of wheat (*T. aestivum*) and squash (*C. peppo*) seeds and the growth of the germinated seedling, which originated from the treated seeds as a function of the tested concentration and the tested extract. The inhibition effect increased with increasing the tested concentrations (Table 4).

Table 3. Effect of the avocado organic extracts on *Theba pisana* snails; shown as average percent mortality ±SD and LC_{50} values

Tested Extract	Time days	Mortality % at different concentrations (%)						LC_{50} 95% CL	Slope ±SE	χ^2
		0	0.1	0.2	0.3	0.5	1.0			
Flesh powder extract	4	0	0	0	0	0	0	>1.0		
	8	0	0	0	3.3 ±1.8	7. 7 ±2.5	13.3 ±3.8	>1.0		
	12	0	0	0	6. 2 ±1.5	23.3 ±2.8	15.3 ±4.5	>1.0		
Peel powder extract	4	0	0	4.3 ±2.8	8.7 ±1.5	16.2 ±2.5	30.0 ±3.3	>1.0		
	8	0	6.7 ±2.6	6.7 ±2.5	12.2 ±2.7		40.0 ±3.9	>1.0		
	12	0	3.3 ±0.9	6. 7 ±1.5	13.3 ±2.8 11.2 ±1.8	16.7 ±3.2	53.3 ±4.2	1.08 0.84 – 1.4	2.11 ±0.06	7.0
Seed powder extract	4	0	0	6.7± 1.2	10.0 ±0.8	26.7±2.8	66.7 ±3.8	0.73 0.60 – 0.89	1.98 ±0.45	9.7
	8	0	6.7 ±0.9	12.0 ±3.1	20.0 ±3.1	50.0±2.1	86.7 ±5.8	0.49 0.44 – 0.54	2.96 ±0.06	12
	12	0	9.0 ± 1.0	23.3 ±5.8	30.0 ±5.2	73.3±6.1	100	0.31 0.28 – 0.34	3.60 ±0.07	19

C.L.= Confidence limit; SE= Standard error and χ^2= Chi squared

Table 4. Effect of the avocado extracts on wheat (*Triticum aestivum*) and squash (*Cucumber peppo*) seeds; shown as inhibition% ±SD and EC_{50} values

Treated Plant	Extract	Effect on	Inhibition% at different concentrations (µg/ml)								EC_{50} 95% CL	Slope ±SE	χ^2
			0	50	100	250	500	1000	2000	5000			
T. aestivum	Peel	Germ	0	0	4.8± 1.2	14.4± 0.21	28.6± 0.17	42.8± 2.7	52.4± 4.2	57.1± 3.2	2113 1608-2781	1.13±0.009	10
		Shoot	0	8.3± 2.2	20± 3.2	33.3± 3.1	41.7± 4.2	50± 2.2	53.3± 5.2	66.7± 4.2	1208 880-1663	0.81±0.095	5
	Seed	Germ	0	4.8± 2.1	19.1± 5.3	28.6± 3.3	47.6 ±5.2	57.1± 14.3	66.6± 6.3	85.7± 8.3	707 578-866	1.2±0.079	5
		Shoot	0	16.7± 2.3	33.3±3. 2	41.7± 4.1	58.3± 5.3	66.7± 4.8	83.3± 5.1	91.7± 6.2	317 244 - 410	1.08±0.011	3
	Flesh	Germ	0	4.8± 1.2	19.1± 6.3	19.1± 8.3	28.6± 4.2	33.4± 6.5	38.1± 4.3	42.9± 8.3	>5000		
		Shoot	0	0	16.7± 2.1	23.3± 3.1	33.3± 4.2	36.7± 3.2	41.6± 5.3	41.6± 4.2	>5000		
C. peppo	Peel	Germ	0	0	6.7± 1.9	8.3± 2.9	13.3± 4.8	21.7± 5.8	30± 3.2	36.7± 2.9	>5000		
		Shoot	0	0	0	25± 3.2	30± 4.2	30± 3.2	35± 6.1	30± 5.2	>5000		
	Seed	Germ	0	0	10	16.6± 2.9	23.3±5.8	30± 4.1	43.3± 5.8	47.7± 6.3	>5000		
		Shoot	0	0	0	10± 3.2	25± 4.2	60± 3.6	65.0± 5.2	70± 6.2	1285 1072-1540	1.53±0.014	21
	Flesh	Germ	0	0	6.7± 2.9	13.3± 3.8	13.3± 5.4	30.0± 6.2	33.3± 2.7	36.7± 9.5	>5000		
		Shoot	0	0	0	0	0	0	0	0	>5000		

C.L.= Confidence limit; SE= Standard error and χ^2= Chi squared

The inhibition in seed germination and seedlings growth was higher on the squash seeds than the wheat grains. The seed extract was more effective than the peel extract and then the flesh extract against seeds germination as they achieved EC_{50} values of 707, 2113, and > 5000 µg/ml on wheat seeds germination, respectively. The corresponding effect on the squash seeds germination was less (EC_{50} values of > 5000 µg/ml). These extracts inhibited seedling growth with EC_{50} values of 317, 1208, and > 5000 µg/ml, compared with 1285, > 5000, and > 5000 µg/ml on wheat and squash, respectively. From the results obtained, it could be said that the active compounds contained are more effective on narrow-leaf plants (monocotyledons) than on broad-leaf plants (dicotyledons).

On the other hand, the pre-germinated seeds of the two treated plants were differently affected with the tested extracts (Table 5). Worth mentioning, deformation in roots in all treatments was noticed at higher concentrations more than in shoot systems. Both seed and peel extracts were more effective against the squash seedlings than the flesh extract. The flesh extract appeared ineffective within the tested concentrations with EC_{50} values > 4000 µg/ml on both root and shoot systems. The fruit seed extract overcame the peel extract in its effect, as they achieved EC_{50} values of 1138 and 1607 µg/ml on the root system growth and 857 and 1326 µg/ml on the shoot system growth, respectively. It was noticed that the effect on the root system was lower than on the shoot system growth.

Regarding the effect on the wheat seedlings, all tested extracts inhibited both root and shoot systems in a systematic manner with increasing concentrations (Table 5). Both seed and peel extracts were also more effective than the flesh extract. Their inhibitory effect on the root system appeared higher than on the shoot system in all cases. The peel extract was more effective than the flesh extract in its effect as it inhibited both root and shoot systems, with EC_{50} values of 471 and 1170 µg/ml compared with 919 and 1456 µg/ml, respectively. Avocado seed extract exhibited the highest effect in inhibiting the root and shoot system, with EC_{50} values of 29 and 170 µg/ml, respectively.

IV: Identification of *Persea armeniaca* seed kernel extract by GC-MS

Based on the obtained molecular weights of the eluted compounds at each retention time and the literature cited about the avocado seeds contents, each active compound was identified.

At a retention time of 10.54 min, a compound with a molecular weight equal to 611 was referred to be rutin (quercetine-3-glucoside- rhamnoside) as a phenolic derivative (flavanol derivative). These data agreed with Rosero *et al*. (2019), as they detected the phenolic compound quercetin-3-O-rutinoside in avocado seed with the characteristic ion fragments at m/z 301, 271, 243, 199, and 107 of aglycon quercetin. The high area% (22.66) reflected the presence of other isomers of quercetin-glucoside-rhamnoside compound (the same molecular weight and different substitution positions of the two sugar moieties). The ion fragment at m/z 301 in the quercetin-3- quercetin-3-O-rutinoside mass spectrum corresponds to the loss of one hexose and one rhamnose moieties $[M - 162–146 - H]^+$ (Table 6).

Table 5. Effect of the avocado extracts on wheat (*Triticum aestivum*) and squash (*Cucumber peppo*) seedlings; shown as inhibition% ±SD and EC_{50} values

Tested Extract	Effect on	Mono-cotyledons (T. aestivum)			Dicotyledons (C. peppo)		
		EC50 95% CL	Slope ±SE	χ^2	EC50 95% CL	Slope ±SE	χ^2
Peel powder extract	Root	471 386 - 576	1.6±0.03	10.1	1607 1326 - 1947	1.53±0.024	7.4
	Shoot	1170 1026 - 1334	2.3±0.03	11.4	1326 1104 - 1593	1.53±0.020	20
Seed powder extract	Root	29 6.8 - 117.7	1.0±0.05	3.3	1138 943 - 1374	1.44±0.02	3.4
	Shoot	170 107 - 271	1.24±0.03	13.8	857 692 - 1062	1.23±0.021	12
Flesh powder extract	Root	919 750 - 1124	1.33±0.021	15.7	>4000		
	Shoot	1456 1255 - 1689	2.0±0.03	26.6	>4000		

C.L.= Confidence limit; SE= Standard error and χ^2= Chi squared

Table 6. GC-MS identification of *Persea armeniaca* seed extract constituents

No	Rt (min.)	Area %	MW	Compound	Remarks
1	10.54	22.66	612	Quercetin-3-O-rutinoside (rutin) $C_{27}H_{30}O_{16}$	A rutinoside that is *quercetin* with C3-OH substituted with glucose and rhamnose sugar groups.
2	28.14	5.23	568	Lutein (zeathanthin) $C_{40}H_{56}O_2$	Lutein and zeaxanthin are part of carotenoids.
3	28.17	3.55	536	Lycopene $C_{40}H_{56}$	Non-provitamin A carotenoid
4	28.20	14.41	552	Lycoxanthin $C_{40}H_{56}O$	It is a carotenol (psi,psi-carotene. It derives from a hydride of a lycopene.
5	33.33	28.91	596	quercetin 3-O-arabinosyl-glucoside $C_{26}H_{28}O_{16}$	Phenolic compound (flavonol) derivatives (Psuedotannins)
6	37.13	25.25	578	Procyanidin dimer B $C_{30}H_{26}O_{12}$	Procyanidin dimer B proanthocyanidin consisting of epicatechin-(4, 8' or 4-6')-catechin
Total		100.01			

At a retention time ranged from 28.14 to 28.20 min, the identified biochemical compounds were cleared to be carotenoids with little shift in their retention times according to their chemical structural differences. At a retention time of 28.14 min, a compound with a 568 molecular weight was identified to be $C_{40}H_{56}O_2$**,** which refers to lutein or its isomer, zeathanthin, as part of carotenoids. The probability of containing both lutein and zeathanthin increased the percentage of area to 5.23%. These data agreed with Tabeshpour *et al*. (2017).

Another carotenoid compound with a molecular weight of 536 was eluted at 28.17 min with a 3.55% area. This compound was identified as lycopene ($C_{40}H_{56}$), a non-provitamin A carotenoid, while the other carotenoid compound identified as lycoxanthin ($C_{40}H_{56}O$) with a molecular weight equals 552 with an area of 14% was eluted at a retention time of 28.20 min. It is well known that lycoxanthin is a carotenol that is carotenol psi,psi-carotene substituted by a hydroxy group. It derives from a hydride of a lycopene, which increased its presence, so it occupies 14% of the area (Table 6).

The next identified compound was eluted at 33.33 min retention time with a high area% equaled 28.91. It has a 596 molecular weight and identified as quercetin 3-O-arabinosyl-glucoside ($C_{26}H_{28}O_{16}$). Its high percent may be due to its isomers, as this structure has many isomers of substituted quercetin molecules substituted with arabinosyl and glucosyl substituents in other positions on it, as for example, Quercetin-3-arabinoside-7-glucoside ($C_{26}H_{28}O_{16}$), and so several molecules are found leading to this high percent (Kosińska *et al*., 2012). The ion fragment at m/z 301 in the quercetin-3-O-arabinosyl-glucoside mass spectrum corresponds to the loss of one hexose and one pentose moieties $[M–162–132-H]^+$.

Worth mentioning is that all quercetin containing identified compounds, ion fragments at m/z 271, 243,199, and 107, are produced from the qercetin molecule itself through its fragmentations. Fragment at m/z 271 $[301- CH_2O]^+$ resulted from the loss of a formaldehyde molecule, which loses a carbon monoxide to give m/z 243 $[271-CO]^+$, the successive loss of carbon dioxide $[243-CO_2]$ gives the fragment ion at m/z 199, however, the m/z 107 fragment ion corresponds to the A and B rings loss from quercetin.

At 37.13 min retention time, a compound with a 578 molecular weight was eluted with an area of 25.25% and identified to be a procyanidin dimer B type ($C_{30}H_{26}O_{12}$). In fact, the high percent refers to some isomers of procyanidins that were previously identified. Besides the parent molecular weight of the dimer, fragment ions at m/z 425, 407, 289, and 245were previously detected. In each isomer, fragment at m/z 425 $[M-152-H]^+$ corresponds to retro-diels–alder (RDA) fission of the heterocyclic rings of the procyanidins dimers; fragment ion at m/z 407 is generated by RDA fission in the C ring, with a subsequent elimination of a water molecule; fragment

at m/z 289 $[M–288-H]^+$ is originated as a consequence of a methyl-quinone break of the interflavan bond, with the loss of an epicatechin molecule, subsequent elimination of CO_2, generates the fragment ion at m/z 245. Li & Deinzer (2007) and López-Cobo *et al*. (2016) identified four procyanidin B dimer isomers in avocado seeds. The identified catechin unit with m/z 289 may be produced through the hydrolysis of a procyanidin trimer. On the other hand, the procyanidins trimmers type A and B differed according to the avocado varieties. All the identified compounds are shown in Figure (1).

From the obtained results, avocado seed extract has surpassed other fruit extracts (peel and flesh) as a good natural source of biologically active fungicides, phytocides and molluscicides, which promises to develop novel products with added value and a safe alternative to synthetic compounds. Ecologically, besides the delicious taste, rich nutrient composition, and several health-promoting bioactivities in the human system like a strong antioxidant (Soledad *et al*., 2021), anti-effect against hypercholesterolemia (Uchenna *et al*., 2017), microbes (Villarreal-Lara *et al*., 2019), cancer (Lara-Márquez *et al*., 2020), obesity, inflammation (Dabas *et al*., 2019), diabetes (Tremocoldi *et al*., 2018), neuro generative agent, as well as improving eye function, preventing or slowing macular degeneration and skin lightening potential (Laksmiani *et al*., 2020). Avocado seed residue is a healthy ingredient in the food industry as its chemical composition (content of protein, carbohydrates, fiber, and fat) and physicochemical properties make it a valuable additive to cereal snacks. Its addition at 6% promoted five-fold increase in the polyphenol content and four-fold higher antioxidant potential of the snacks, in addition to increasing the dietary fiber content (Siol and Sadowska, 2023).

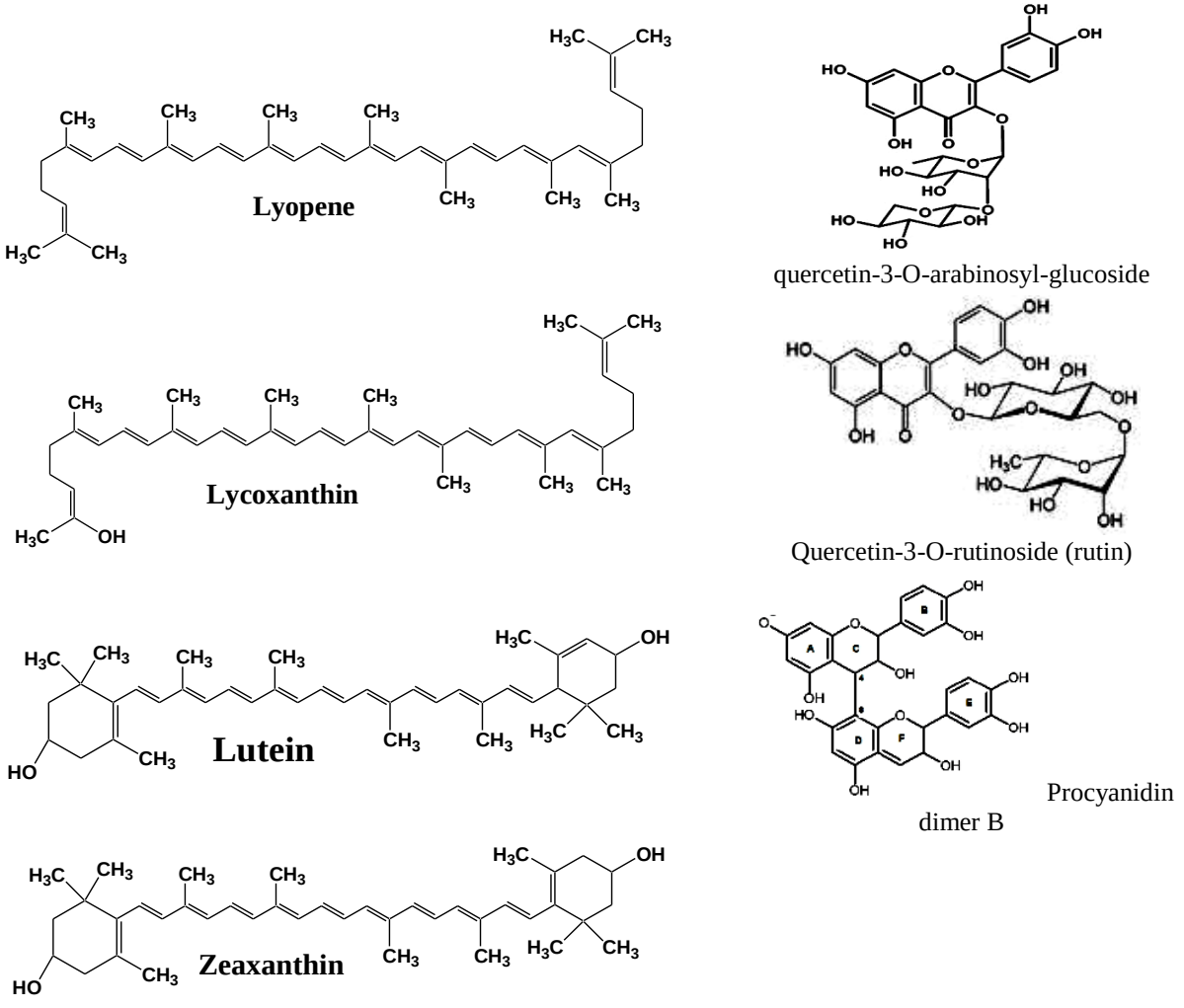

Figure 1. The identified compounds in the active *P. armeniaca* seed extract

In conclusion, our study can demonstrate diverse and effective uses of avocado seeds, resulting in reducing pollution without ending up as agricultural and food industry waste, and will provide nutritional and health benefits along with economic gains, as well as allowing the development of eco-friendly organic biopesticides.

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الملخص العربي

النشاط الإبادى على الفطريات والقواقع والنباتات لمخلفات صناعة الأفوكادو

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تم تقسيم ثمار الأفوكادو إلى القشرة الخارجية ولب الثمار والبذور، تم إستخلاص كل جزء على حده واختبار المستخلصات الناتجة على نباتى القمح والكوسة كمثال للنباتات رفيعة الأوراق وعريضة الأوراق على التوالى في مراحل مختلفة، تم اختبار نشاطها الإبادى على فطريات الـ *B. cenerea* و*F. oxysporum* وايضا اختبار هذه المستخلصات المتحصل عليها وكذلك الجزء المتبقى بعد الإستخلاص على قوقع التيبا الأرضى *T. pisana*. أدت مستخلصات القشرة الخارجية ولب الثمار والبذور إلى تثبيط نمو فطريات الـ *B. cenerea* و *F. oxysporum* بتركيز مؤثر على 50% من العشيرة المعاملة قدره >1000 ، > 1000 و816، 616 و1077، 471 ميكروجرام/مل على الترتيب. تم قتل القوقع المعالج بالبذور المتبقية بعد الإستخلاص بشكل متزايدة بعد 4، 8، 12 يوم من التعرض بتركيز لازم لقتل 50% قدره 58.6، 37.9 و32.3%، فى حين أظهر مستخلصها تركيز لازم لقتل 50% من العشيرة المعاملة قدره >1.0%، 1.08% و0.31% خلال فترة التعرض المختلفة على الترتيب. أدت جميع المستخلصات الى تثبيط إنبات البذور والنمو الخضرى الناتج من البذور المعاملة (البديرات). كما أن التاثير المثبط كان أعلى فى بذور الكوسة منه فى حبوب القمح. وفي شتلات القمح، أظهر مستخلص البذور الأكثر فعالية فى تثبط نمو المجموع الجذرى والخضرى بتركيز مؤثر على 50% من العشيرة قدره 29 و170 ميكروجرام/ مل على الترتيب. تم التعرف على المركبات الفعالة فى مستخلص البذور النشطة بيولوجية من خلال استخدم جهاز الكروماتوجرافى الغازى المتصل بجهاز مطياف الكتلة(GC-MS). تم تحديد مركباته البيوكيميائية النشطة على أنها rutin (quercetine-3-glucoside-rhamnoside) and quercetin-3-O-arabinosyl-glucoside as phenolic derivatives (flavonol derivatives), carotenoids (lutein, zeaxanthin and lycopene) and procyanidin dimer B type.