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Research Article

Comparative study of Biological activity and chemical composition of Methanolic and Ethanolic plant extracts of *Persea americana leaves in-vitro*

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Abstract

The aim of this study was to evaluate and compare biological activity, chemical composition, antioxidant and antimicrobial activity of *Persea Americana* mill refluxed in various solvents, ethanol and methanol respectively. *Persea Americana* is a plant that is quite new to the turkish medicine and found its way to Turkey as it is little over 10 years old in use. It has been used to treat certain skin problems anddiahrea. The plant extraction was done with various solvents and comparison was done between the extracts produced by ethanol solvent and that of methanol solvent. Chemical constituents of the leaf extract were analyzed using techniques of gas chromatography/mass spectrometry (GC/MS). The methods employed for in vitro antimicrobial screening were the broth microdilution assay and Kirby bauer assay. The technique 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging was applied for antioxidant activity. The ethanol aqueous extract (EAE) IC50 value of 2,4 mg / mL and methanol aqueous extract (MAE) IC50 value was 2,51 mg/ml respectively. According to literature the lesser the IC50 value the more potent the molecule is in the DPPH assay, therefore the results of the IC50 for could be viewded as a powerful antioxidant thus it is highly potent. Scavenging activity for the MAE and EAE were analyzed. Scavenging/inhibition activity increases as concentration increases. The antibacterial activities of the various solvent extract were screened and compared against to *Bacillus cereus*, *Escherichia coli*, *Staphylococcus Aureus* and *Salmonella typhrium*. The aqueous extracts showed a favourable amount of microbial activity, especially on *S. Aureus* and *E. coli.* Persea Americana in both extract conditions showed strong antioxidant ability, strong antimicrobial activity and gave an impressive amount of chemical components which reveal the compounds responsible for its biological activities.

Keywords: *Persea Americana*; methanol- ethanol extract; GC- MS; antimicrobial effect, antioxidant effect.

Persea americana Metanolik ve Etanolik Bitki Ekstraktlarının *in vitro* Biyolojik Aktivite ve Kimyasal Bileşiminin Karşılaştırmalı Çalışması

Öz

Bu çalışmanın amacı, çeşitli çözücülerde, etanol ve metanolde geri akıtılan *Persea Americana* bitkisinin biyolojik aktivitesini, kimyasal bileşimini, antioksidan ve antimikrobiyal aktivitesini değerlendirmek ve karşılaştırmaktır. *Persea Americana*, Türk tıbbı için oldukça yeni olan ve 10 yaşından küçük olduğu gibi Türkiye'de görülmeye başlayan bir bitkidir. Bazı cilt problemlerini ve diyareyi tedavi etmek için kullanılmaktadır. Çalışmamızda, bitki özütlemesi için çeşitli çözücüler kullanılmış ve etanol çözücüsü ile üretilen özütler ve metanol çözücüsü ile karşılaştırılmıştır. Yaprak ekstraktının kimyasal bileşenleri, gaz kromatografisi / kütle spektrometrisi (GC / MS) tekniği kullanılarak analiz edilmiştir. Broth mikrodilüsyon ve Kirby bauer teknikleri kullanılarak *in vitro* antimikrobiyal analizler gerçekleştirilmiştir. Antioksidan aktivite için 2,2-difenil-1-pikrilhidrazil (DPPH) serbest radikal temizleme tekniği uygulanmıştır. Etanol

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sulu ekstrakt (EAE) IC50 değeri 2.4mg/mL ve metanol sulu ekstrakt (MAE) IC50 değeri sırasıyla 2.51mg/ml olarak saptanmıştır. Literatüre göre, IC50 değeri ne kadar düşükse, molekül DPPH testinde o kadar güçlüdür, böylece IC50'nin sonuçları güçlü bir antioksidan olarak görülebilir, bu nedenle oldukça güçlüdür. MAE ve EAE için antioksidan aktivitesi incelenmiştir. Antioksidan aktivitesinin konsantrasyon arttıkça arttığı gözlenmiştir. Çeşitli çözücü ekstraktlarının antibakteriyel aktiviteleri incelenmiş ve *Bacillus cereus, Escherichia coli, Staphylococcus Aureus* ve *Salmonella typhrium* bakterileri ile karşılaştırılmıştır. Sulu ekstraktların özellikle *S. Aureus* and *E. Coli* bakterilerin de uygun miktarda mikrobiyal aktivite gösterdiği saptanmıştır. Her iki ekstrakt koşulunda *Persea Americana*, güçlü antioksidan ve güçlü antimikrobiyal aktivite gösterdiği, biyolojik aktivitelerinden sorumlu bileşikleri ortaya çıkaran etkileyici miktarda kimyasal bileşeni olduğu bulunmuştur.

Anahtar Kelimeler: *Persea Americana*; Metanolik – Etanolik bitki ekstraktları; GC- MS; Antimikrobiyal etki, antioksidan etkisi

1. Introduction

Persea americana which is called avocado; is a fruit originalily grown in tropical and subtropical regions that is greatly known around the world. The avocado has gained large stride in worldwide popularity. It has been exposed to intense marketing and an extensive distribution, largely promoted by its health benefits (Araújo et al., 2018). Persea americana is cultured in the southern coastal region of Turkey due to its commercial importance. The leaves are widely used for pass kidney stone and against the urinary tract infections as therapeutic among the people in Turkey (Kendir & Köroğlu, 2018).Currently Persea Americana is gradually finding its relevance in turkish medicine since its arrival a little over 10 years ago. Avocado belongs to the plant kingdom, and of the Lauracea family under the order *Laurales*, and genus *Persea*, and specie *P. Americana* (Zafar & Sidhu, 2011). The whole avocado fruits are rich in lots of bio-compounds (pulp, seed and peel) and has enormous adavantages, amongst which include antioxidant and anticancer activities as well as skin exfoliation uses (Araujo et al.,2018). Research has shown that avocado seeds whether toasted or dried can be used to treat rashes, diahrea amongst other common household diseases (Jiménez-Arellanes et al., 2013). Ancient and cultural folklore in mexico explain that avocado seeds whether toasted/dried can be used to treat rashes, diahrea amongst other common household diseases(Jiménez-Arellanes et al., 2013). The antioxidant activity of avocado fruit as a whole is a phenomena to reckon with in the ongoing cancer research around the globe as there is the existence of radicals such as superoxides and hydroxyl which is a large scale problem to human health. (Antasionas et al., 2017). Antioxidants are substances that are capable of adequately reducing the effects of the free radicals and oxidative reactions of this macromolecules (Antasionas et al., 2017). Inorganic sources of antioxidants amidst their commendable performance also pose disadvantages that are gradually outweighing their advantage. Research therefore is gradually moving towards greener and natural sources from living things especially plants. Plants therefore have established a widely and far more commendable stride as they feature for therapeutic agents for treatment of diseases. Avocado pears are rich source of soluble phenolics, flavonoids and also retain some therapeutic activity against certain strains of bacteria (Ogundare & Oladejo, 2014). The complement system is an significant buffer of the frequently experienced inflammatory process, so an active study of the biological activity of molecules that can add to this scheme, and more specifically, prevent the activation of this process, is of excellent significance to different research advancement. Compounds showing both complement-inhibiting property and antioxidant activity could be considered prospective therapeutic agents in this note (Yamasaki, 2018).Previous studies (Ogundare & Oladejo, 2014); (Osuntokun et al., 2017) have extensively described the antimicrobial activity of *Persea Americana* against *Salmonella typhi*, *Escherichia coli*, *klebsiella pneumonia* amongst others. The aim of this study was to identify the chemical compounds within the *P. Americana* (avocado) leaves from Yeşilırmak Region and to extract with various solvents, and after which to monitor the effect of the solvent on the *in-vitro* biologic activity of *Persea americana* relative to its antioxidant ability and antimicrobial activity.

2. Material and Method

2.1 Chemicals and reagents

Mateials used in the experiments encompassing reagents and chemicals, were anaytical grade and obtained from Merck (Darmstadt, Germany).

2.2 Plant sample collection

Avocado *(Persea Americana)* was collected in February 2019 from the region of Yeşilırmak of Northern Cyprus (35° 08' 40" N;32° 43' 21" E). Avacado leaves were washed with deionized water and dehydrated at room temperature for about 10 days and weighted. Theprocess of drying the Persea americana leaves was carried out until they were kept at a steady weight and free of moisture. Then *Persea americana* leaves were grinded to fine powder using a simple laboratory mill. Botanical identification was done in the Faculty of Agricultural Sciences and Technologies by Prof. Dr. İbrahim Baktır (Dean) from Cyprus International University.

2.3 Preparation of fruit extract

Persea americana methanol and ethanol extract was prepared according to (Ribeiro et al., 2013). Dried *P. Americana* leaves were grinded by a miller at the laboratory. To 2 aliquot of 20g ground leaves of *P. Americana*, 200 ml of 95% ethanol and methanol HPLC grade was added and allowed to shake for 48 h using an overhead shaker. After 48hrs the methanol aqueous extract (MAE) and the ethanol aqueous extract (EAE) were strained with the use of a Whatman filter paper. The extract was collected by the use of rotary evaporator at 40rpm at a temperature of 50°C for 1hr. Dry mass of extracts were stored for further use.

2.4 Phytochemical Analyses

2.4.1. Determination of total phenolic content (TPC)

The TPC values were established by applying the Folin-Ciocalteu colorimetric assay described previously by Büyüktuncel et al, 2014. The preparation of calibration curve was done by obtaining 0.1 mL volume of various w/v concentrations of 50, 100, 150, 200, 250 and 300 mg/L gallic acid solutions.0.1 mL 20-times dilution samples of *P. Americana* (diluted with 13% (w, v) ethanol and methanol) were transfered into a glass test tube and then 2 mL of 2% disodium carbonate was added. After incubation for 2 min, 0.1 mL of Folin-Ciocalteu's reagent was added. Sequel to this, at a 760 nm wavelength the absorbance was detected by using a UV Visible Spectrophotometer (Shimadzu UV-2450). The amount of phenols was shown as mg of gallic acid equivalent per gram of extract (mg GAE/g). Spectrophotometric determination of the TPC was carried out with Folin-Ciocalteu method as adapted for *P. Americana* leaves extract analysis, standardised with gallic acid. The method works by the principle of reduction of a phosphotungstenphosphomolybdate complex by phenols present to blue colored products.

2.4.2. Determination of total flavonoid content (TFC)

The TFC data were analysed with the aluminum chloride colorimetric method as discussed by Marinova et al., (2005). Absorbance measured at a wavelength of 510 nm. The total amount of flavanoids was quantified in mg (weight) of quercetin equivalents per gram of extract (mg QE/g). All tests were performed in three sets.

2.5 Gas chromatography-mass spectrometry (GC-MS)

The different solvent extracts were analyzed by gas chromatography–mass spectrometry (GC–MS) according to a certain conditions stated by (Ukwubile et al., 2019). The GC or GC–MS conditions were optimized based on the property of the various solvent extract. A 30 m×0.25 mm, (thickness of film) 0.25 μm, HP-5 fused silica capillary column was utilized. The carrier gas, Helium (purity 99.999%), had a flow rate of 0.9 mL/min. The oven temperature of the column was programmed from 50 ◦C (hold 1 min) to 240 ◦C (hold 10 min) at a 5 ◦C/min rate. EAE and MAE was dispersed in methanol and ethanol respectively introduced in an Agilent 7890A GC system joined with an MS (Agilent technologies) by author injection at the environmental laboratory, Cyprus international university. The operating conditions of the GC–MS used in the study were as follows: temperature of oven was set at 50°C for 2 min then 100°C with the rate of 10°C/ min and steadily increased to 200°C and held at a constant temperature for a period of 10 min. The quantity of the sample was 2 μL and the carrier gas (helium) was 1 mL / min. 70 eV was used to ionize the sample parts. The methanol and ethanol P. Americana extracts were analyzed for a time period of 24.50 min.

The ethanol extract was analysed by the following GC conditions stated by (Abdel-Naime et al., 2019). 1 μL of the sample was injected and the extract analysis was done as described by a flame ionization detector programmed temperature method. Initial temperature was set as 70 °C, and rate of increase was arranged as 5 °C/min to 220 °C. The total time using technique was 60 min and the split ratio was 1:50. Carrier gas; Helium with a flow rate of 0.8 mL/min. To identify the compounds, a comparison of their mass spectra ws done against the database the (NIST library) and Wiley library 9.

2.6 Antioxidant activity by DPPH scavenging assay

The antioxidant activity was deterrmined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) as a scavenging radical on ethanolic and methanolic extracts of P. Americana respectively with a few modifications. DPPH ethanolic and methanolic solution (1mM) were prepared respectively. 3 mL of solution separately added to 100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively and kept in the dark. This was done for both the EAE and MAE. Absorbance values of the DPPH ethanolic and methanolic solutions at different periods were established at 517 nm by a spectrophotometer (Shimadzu UV-2450).

The follow equation was utilised to determine the %DPPH Scavenging activities of examined samples.

$\%DPPH = [1 - (As/Ac)] \times 100$

Where: As (tested sample) and Ab (blank sample) is the absorbance of the the sample with no reagent within.

2.7Antibacterial activity

2.7.1 Broth Microdilution

Four strains of bacteria were used to test the inhibitory ability of the ethanolic and methanolic extract. *Bacillus cereus (ATCC 7064)*, *Escherichia coli* (932), *Salmonella typhyrium (*B-4420*)* and *Staphylococcus aureus (*6538 P*)* were choosen to test as microorganisms. Microbial strains obtained from the Biotechnology Research Center, Cyprus International University. The suspensions of microbial cultures were serially diluted until the resultant concentration was at par with the mcfarland turbidity standard (1.5 × 10^8 CFU/mL). The stock solution concentration was 1g of EAE and MAE were dissolved in 35% DMSO to give a 10ml (w/v) solution. To the labelled microliter plate containing nutrient Broth (NB), EAE and MAE at different volumes of the stock solution was added to the well plates ranging from 100ul, 50ul, 25ul, 12.5ul, 6,25ul respectively. Sterilized culture medium including tested samples DMSO was adopted as negative controls. The well concentrations where no turbidity was noted in the wells after a 24hour were stated to be the MIC of the ethanolic and methanolic extracts. The potency of bacteria in the culture of Mueller Hinton Broth (MHB) was further established by transferring a volume of the microbial suspension to a petri dish containing Muller Hinton Agar (MHA), following which incubation at a stable temperature of 37 ° C. The MBC were determined as the least concentration of ethanolic and methanolic *P. Americana* extract required to totally restrict the growth of the microorganisms after 24 h. All analysis was carried out twice on two separated tests.

2.7.2 Disk diffusion (Kirby Bauer) assay

The protocols in this study was according to (Umar et al., 2019) with slight modifications. Briefly, each sterile Petri dish with a diameter of 9 cm was prepared with 20 mL of Mueller–Hinton medium. Standard quantity of a bacterial suspension (108 CFU / mL) was dispersed on the plates after solidification. A sterile paper filter disk (6 mm) with 20 μL of P. americana was put on the plate surface after 5 min. To accelerate extact diffusion into the agar, the plates were incubated at 4 °C for 1 h and then incubated at 37 °C, for 24 to 48 h. Bactericidal areas (mm) diameters were evaluated, including control disks' diameter. Gentamycin (30 µg / disk) was used as positive controls for gram-positive fungi and chloroamphenicol (30 µg / disk). All experiments were carried out in duplicates.

2.8 Statistical analysis

All experiments were carried out parallely duplicated with three replicates. Graphs and peaks were plotted using the Origin Pro.

3. Results and Discussion

This study conducted to identify the chemical compounds within the *P. Americana* (avocado) leaves and to extract with various solvents, and after which to monitor the effect of the solvent on the *in-vitro* biologic activity of *P. Americana* relative to its antioxidant ability, physicochemical compositionand antimicrobial activity. Total phenols and flavannoid are presented in Table 1.

3.1 Total phenols and total flavannoid content

Table 1. Physicochemical composition for *Persea Americana* leaves

Plant	Sample	Total Phenolics* (mg GAE/g of extract)**	Total Flavonoids* (mg QE/g of extract)***
***Persea Americana* leaves**	Ethanol	82.48 ± 4.33	44.32 ± 2.85
	Methanol	91.72 ± 3.42	71.49 ± 3.07

*Each value is expressed as mean ± standard deviation (SD) (n = 3). Values in the column followed by a different letter superscript are significantly different ($p \leq 0.05$).

**Total amount of phenols was expressed as Gallic acid equivalents (mg GAE/g of extract).

***Total number of flavonoids was expressed as Quercetin equivalents (mg QE/g of extract).

The extraction method of *Persea americana* (avocado) leaves using 95% HPLC grade ethanol and methanol as a universal solvent for refluxing is quite capable of dissolving polar compounds, so that multiple polar and non-polar compounds, such as certain bioactive elements contained in avocado leaves, can then be attracted to the solvent, although study has shown that ethanol is less effective in screening antioxidants (Rahman et al., 2018). Methanol in comparison to ethanol has proven to be a better solvent for extraction of bioactive and antioxidant but the use of ethanol is safer compared to methanol (Rahman et al., 2018). The Table 1 shows the total phenolic content of *Persea Americana* extracted by both 95% methanol

and ethanol respectively. It wasobserved that for the total phenolics in the methanol extract appeared quite higher than that of ethanol extract.

P. Ameriana's total phenolic content and avocado's antioxidant potential were influe nced by the type of avocado and solvent extraction. By donating hydrogen atoms or by transferring single electrons, flavonoids can immobilise free radicals. Flavanoids are abundant in fruits and vegetables, but they form an significant cereal and pulse gro up. (Saharan et al., 2017). The flavanoid content for the methanol extract of *P. Americana* was 71,49 mg QE/gof extract and that of ethanol extract was which can be compared to that of Rahaman et al., 2017 where values of flavanoid in avocado leaves showed to be strongly positive which encourages the result of a high amount og of flavanoid observed in this study.

3.2 GC-MS analysis of bioactive compounds

The Table 2 and Figure 1. showed occurrence of the various bioactive compound as observed by different organic solvents. Some compounds were in increased occeurence depending on the solvent of choice.

Table 2. GC-MS profile of *P. Americana for* methanolic extract

S/N	Chemical compound Name Methanolic extract	Retention times	Molecular formula	Molecular weight g/mol
1	Disiloxane	5.542	H_6Si_2O	78.217
2	1,1'-bibicyclo (2.2.2) octyl-4-carboxylic acid	7.767	$C_{11}H_{16}O$	164.248
3	Salicylic acid, bis-TMS	9.108	$C_7H_6O_3$ OR HOC_6H_4COOH	138.122
4	Palmitic acid	9.608	$C_{16}H_{32}O_2$	256.43
5	Cyclopentasiloxane	10.342	$H_{10}O_5Si_5$	230.5
6	Orcinol	11.575	$C_7H_8O_2$	
7	Diterpenes	11.775	$C_{34}H_{47}NO_{11}$	645.746
8	Cyclomethicone 6	13.000	$C_{10}H_{30}O_5Si_5$	370.77
9	2,3-dehydro-4-oxo-. beta.-ıonol	14.083	$C_{13}H_{18}O_2$	206.285
10	Cholestreyl oleate	16,292	$C_{45}H_{78}O_2$	651.117
11	Diethyl o phthalate	17.008	$C_{12}H_{14}O_4$	222.24
12	d-Nerolidol	24.000	$C_{15}H_{26}O$	222.372

S/N	Chemical compound Name Ethanolic extract	Retention times (min)	Molecular formuala	Molecular weight g/mol
1	Vanillylmandelic acıd (trimethylsilyl derivative)	14.167	$C_{18}H_{34}O_5Si_3$	414.72
2	Solvanol	15.717	$C_{12}H_{14}O_4$	222.24
3	Allethrine	16.892	$C_{19}H_{26}O_3$	302.414
4	Palatinol C	17.817	$C_{16}H_{22}O_4$	278.348
5	Provitamin D4	20.042	$C_{28}H_{46}O$	398.675
6	Isochiapin B	27.550	$C_{19}H_{26}O_6$	350.173
7	Globulol	29.058	$C_{15}H_{26}O$	222.372
8	5.alpha.-Ergost-8(14)-ene	34.900	$C_{28}H_{48}$	384.692

Table 3. GC-MS profile of *P. Americana* for Ethanolic extract

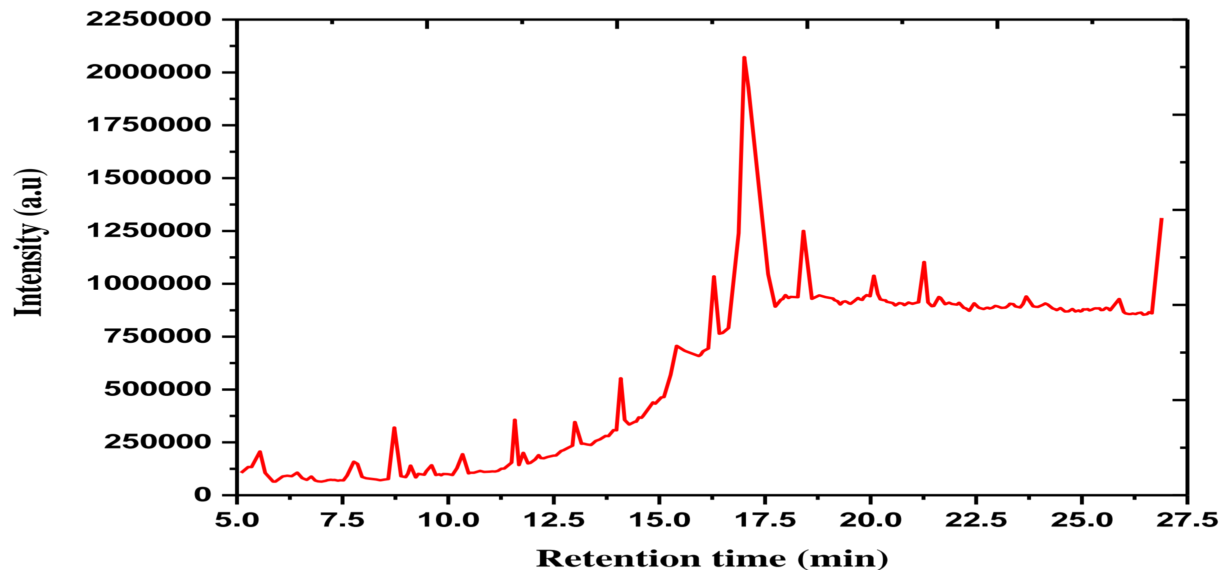

Figure 1. Chromatogram showing the *P. Americana* extracted by Methanol

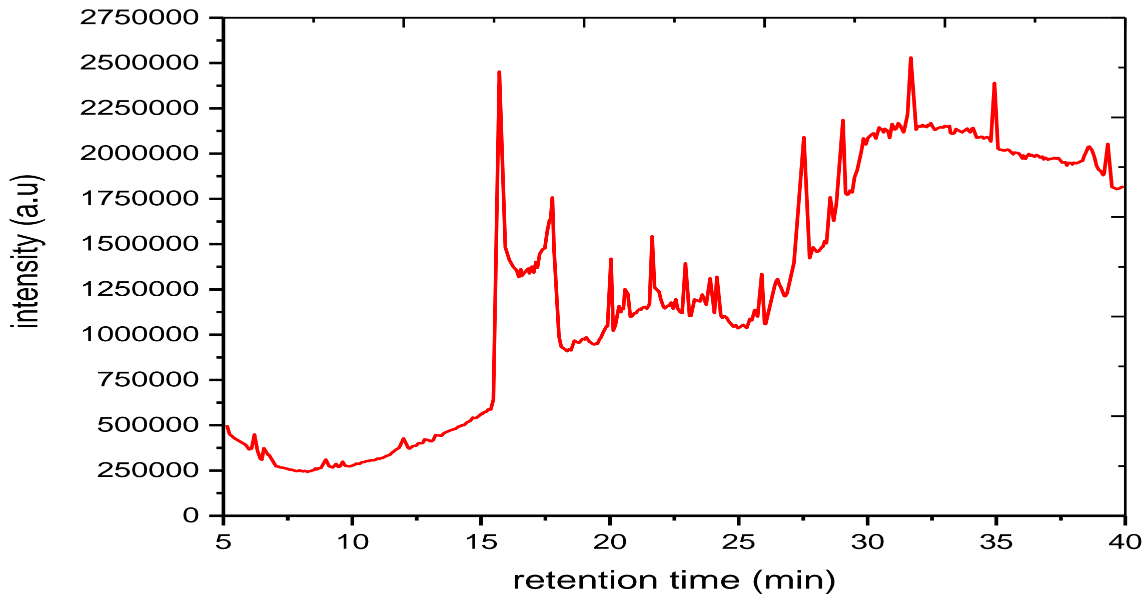

Figure 2. Chromatogram showing the *P. Americana* extracted by Ethanol

It was gathered by observation that the qualitative extraction of *P. Americana* using methanol and ethanol showed the presence of varying chemical components such as Globulol which is one of the compounds responsible for antimicrobial activity according to (Manliang et al., 2007; Manliang 2008). Nerolidol also known as peruviol was present in the ethanolic extract of *P. Americana*; Nerolidol has a floral odor, a type of alcohol and exists as a vital bioactive component in some plants including *P. Americana* and possess strong anticancer and antitumor ability (Chan et al., 2016). In another study, they found some of the chemical compounds like palmitic acid, isochiapin B which were part of the components observed in the profiling carried out. Besides these aforementioned most of the represented components are not yet reported or accessible (Senthilkumar et al., 2012).

3.3 Antioxidant activity

Table 4. Various extracts and standards showing IC50 values

Samples analyzed	IC50 values
Ethanolic extract (EAE)	2,5 mg/ml
Methanolic extract (MAE)	3,2 mg/ml
Ascorbic acid standard	5 mg/ml

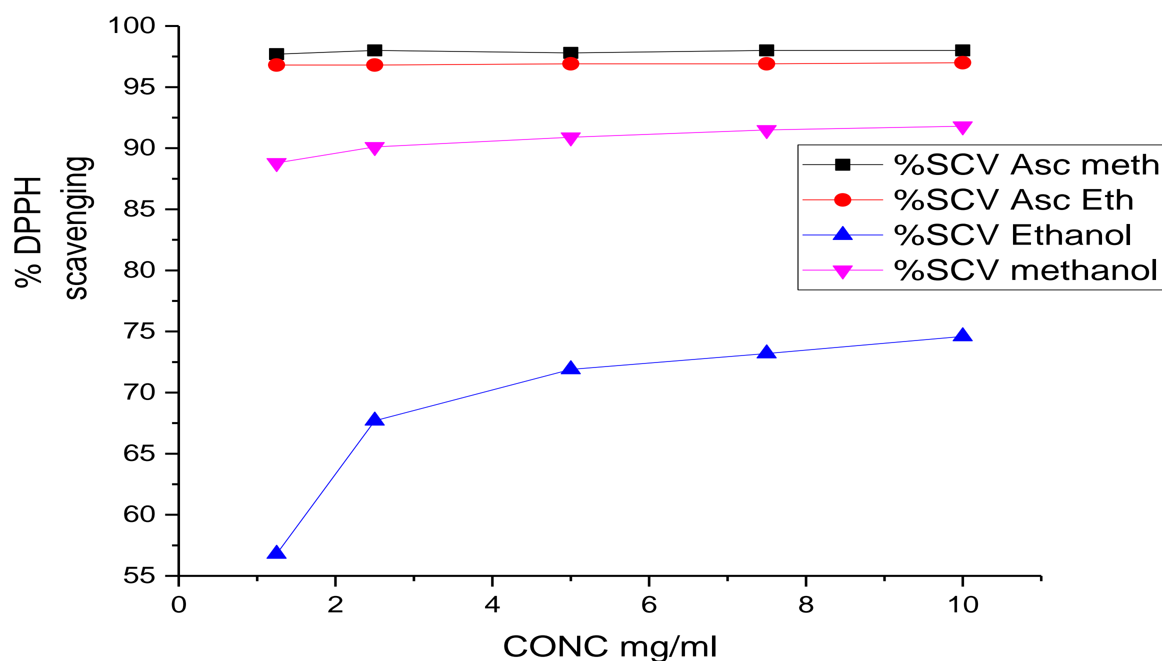

Figure 3. Showing the comparison between the various solvent % scavenging activity in relation to ascorbic acid Standard SCV; Percentage of scavenging activity.

In human's research has shown that oxidative stress results as a result of increased oxygen radicals which could affect the function of some bio-molecules (Nathaniel et al., 2016). In the *In vitro* antioxidant assay, ascorbic acid was utilized as a standard control. Antioxidants yields from food or natural sources, has a strongly correlation with various extraction parameters like solvent concentration and physiochemical characteristics (Rodríguez-Carpena et al., 2011). Ascorbic acid was weighed anddissolved in both methanol and ethanol respectively**.** These methods were done to determine the antioxidant potential of *P. Americana* ethanolic and methanolic extracts and the interactions with free radicals. *In vitro* antioxidant assays have the potential to give a certain level of information that could contribute and give insight as to what happens in *in vivo* assay. Ascorbic acid has very high standard antioxidant activity, the percentage DPPH scavenging activity appeared to increase as concentration increased for both the standard and the other solvent extracts of consideration. The absorbance appeared to reduce as concentration increased and it correlated with what (Rahman et al., 2018) reported in a similar research. The varying of of IC_{50} value of *P. Americana* extract in ethanol and methanol is worthy of note. Compared to the IC50 values of Ascorbic acid which was 5mg/ml, methanolic extract had a lower IC50 value is 3.2mg/ml which according to previousliterature is a good one given that lower IC50 values indicate great antioxidant activity (Rahman et al., 2018). Ethanolic plant extract appeared to have the lowest IC50n value amongst the three of them 2.5 mg/ml which was lower that both the methanolic extract and the standard.

Table 5: Showing the comparison between other authors findings and this present research

Specimens/extract of *Persea americana*	Antioxidant Scavenging activity (%)	Authors
Ethanolic extract **Methanolic extract**	74.6 91.8	Present study Present study
Ethanolic extract **Methanolic extract**	70 88	(Kingne et al., 2018)
Ethanolic extract	66.7	(Fatmawaty, Anggreni, Fadhil, & Prasasty, 2019)
Methanolic extract	80	(*Asaolu et al.*, *2010*)
Ethanolic extract	48.3	(Gbadamosi & Kalejaye, 2017)
Methanolic extract	91.7	(Salim, 2013)

This research recorded a highest antioxidant activity of 74.8 % for *Persea Americana* ethanolic extract and for *P. Americana* methanolic extract 91.8%. It's safe to say that increasing percentage of scavenging activity will mean a reducing IC50 value. In comparison to other authors, it is worthy of note that the *P. Americana* ethanolic extract considered within this study had a higher antioxidant activity than other studies expressed in the Table 5. Similar to most studies, it has been observed that ethanolic extracts of *P. Americana* leaves have relatively low antioxidant activities in comparison to the counterpart methanolic extracts of the same plants. Further observation and comparison of the methanolic extract showed that across the authors evaluated, methanol extracts of *P. Americana* leaves appear to have higher antioxidant activities. The antioxidant activity recorded for this study appeared to be higher than the studied authors. This variances might be as a result of many factors like human and equipment errors and maturity of the leaves in question. Some research have reported that maturity of the *P. Americana* leaves used for the extract could affect the result of the antioxidant activities thus younger leaves are more likely to have higher antioxidant activity than mature leaves (Kingne et al., 2018).

3.4 Antibacterial activity

Table 6: Minimum inhibitory concetrations(MIC), and minimum bactericidal concentrations(MBC)

Values are expressed as the mean ± SD (n = 3)

Microorganisms	*E. coli* O157:H7 (932)					*S. typhimurium* (B-4420)					*B. cereus* (ATCC 7064)					*S. aureus* (6538 P)				
Concentration (mg/ml)	100	50	25	12.5	6.25	100	50	25	12.5	6.25	100	50	25	12.5	6.25	100	50	25	12.5	6.25
Ethanolic extract (EAE)	-	+	+	+	+	-	-	-	-	+	-	-	-	-	+	-	+	+	+	+
Methanolic extract (MAE)	-	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	-	+	+	+

EAE= Ethanol Extract; MAE= Methanol Extract

Negative Control = NB: Nutrient Broth; Positive Control = NNB: Nutrient Broth with Bacteria

(+): Shows growth of Bacteria; (): Shows NO growth of Bacteria

The minimum inhibitory concentration is the weight per unit volume of a drug capable of inhibiting the growth of microorganisms. The MIC for this research was determined as the lowest concentration that didn't have color change after inoculating the microorganism with the similar concentration as the Mc farland standard. In this research, the various wells were plated out for better observation. Keen observation of the interaction between the microrganisms and the plant extracts helped to assess the viability and antimicrobial activity of *P. Americana* (Chakraborty, M., & Mitra, 2008). *E. coli* and *S. aureus* had a minimum inhibitory concentration (50mg/ml) as the ethanolic extract was able to inhibit its growth at the said concentration, while *E. coli* and *S. aureus* MIC for methanolic extract was 25mg/ml. This explains that for the *E. Coli* and *S. aureus*, the methanolic extract has a lower MIC and thus was more effective than the ethanolic extract. The S. typhirium and *B. cereus* had very low MBC an MIC because the plant exract had the ability to completely inhibit the proliferation of the said bacteria. In the metanolic extract of *P. Americana*, on an average a lower MIC was observed for all the microorganisms. A few factors may have been responsible for the different MIC of *P. Americana* in various solvents like polarity which might be as a result of the solvent of extraction and or maybe thepolarity of the solvent. For *E. Coli* and *S. aureus* the MIC was 50mg/ml with the ethanolic exract while that of the methanolic extract was 25mg/ml. The *P. americana* ethanolic and mehanolic extract was more effective on *S. typhirium and B. cereus* repectively. The efficacy of various extracts can be described by the polarity of the solvents which might affect how certain compounds within the samples dissolve or interact with the solvent (Cardoso et al., 2016).

4. Conclusions and Recommendations

The research intends to compare activities of *Persea Americana* in two different solvents with respect to the antioxidant activity, chemical composition and antimicrobial analysis. From the antioxidant test, we observe different % scavenging activity and IC50 between the solvent extracts; for the microbial test some microorganisms were more sensitive to one of the extracts than the other; for the chemical composition certain compounds were more visible with significant amount in methanol that in ethanol and vise versa. In other words it is safe to say that the solvent of choice for extraction plays a role in the bioactivity of a given plant material as different solvents interact differently with certain components of this biological material. As a result, *Persea Americana* in both extract conditions showed strong antioxidant ability, strong antimicrobial activity and gave an impressive amount of chemical components which reveal the compounds responsible for its biological activities.

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References

Abdel-Naime, W. A., Fahim, J. R., Fouad, M. A., & Kamel, M. S. (2019). Antibacterial, antifungal, and GC–MS studies of Melissa officinalis. *South African Journal of Botany*, *124*, 228–234. https://doi.org/10.1016/j.sajb.2019.05.011

Antasionas, I., Riyanto, S., & Rohman, A. (2017). Antioxidant Activities and Phenolics Contents of Avocado (Persea americana Mill.) Peel in vitro. *Research Journal of Medicinal Plants*, *11*(2), 55–61. https://doi.org/10.3923/rjmp.2017.55.61

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science and Technology*, *80*(August), 51–60. https://doi.org/10.1016/j.tifs.2018.07.027

Asaolu. Evaluation of in.pdf. (n.d.).

Büyüktuncel, E., Porgalı, E., & Çolak, C. (2014). Comparison of Total Phenolic Content and Total Antioxidant Activity in Local Red Wines Determined by Spectrophotometric Methods. *Food and Nutrition Sciences*, *05*(17), 1660–1667. https://doi.org/10.4236/fns.2014.517179

Cardoso, P. F., Scarpassa, J. A., Pretto-Giordano, L. G., Otaguiri, E. S., Yamada-Ogatta, S. F., Nakazato, G., … Vilas-Boâs, G. T. (2016). Actividad antibacteriana de extractos de aguacate (Persea americana Mill.) sobre Streptococcus agalactiae. *Phyton*, *85*, 218–224.

Chan, W. K., Tan, L. T. H., Chan, K. G., Lee, L. H., & Goh, B. H. (2016). Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. In *Molecules* (Vol. 21). https://doi.org/10.3390/molecules21050529

Fatmawaty, F., Anggreni, N. G. M., Fadhil, N., & Prasasty, V. D. (2019). Potential In Vitro and In Vivo Antioxidant Activities from Piper Crocatum and Persea Americana Leaf Extracts. *Biomedical and Pharmacology Journal*, *12*(2), 661–667. https://doi.org/10.13005/bpj/1686

Gbadamosi, I. T., & Kalejaye, A. O. (2017). Comparison of the antioxidant activity, phytochemical and nutritional contents of two antihypertensive ethnomedicinal plants. *Ife Journal of Science*, *19*(1), 147. https://doi.org/10.4314/ijs.v19i1.15

Jiménez-Arellanes, A., Luna-Herrera, J., Ruiz-Nicolás, R., Cornejo-Garrido, J., Tapia, A., & Yépez-Mulia, L. (2013). Antiprotozoal and antimycobacterial activities of Persea americana seeds. *BMC Complementary and Alternative Medicine*, *13*. https://doi.org/10.1186/1472-6882-13-109

Kendir, G., & Köroğlu, A. (2018). Evaluation of avocado (Persea americana mill.) leaves in terms of public health. *Marmara Pharmaceutical Journal*, *22*(3), 347–356. https://doi.org/10.12991/jrp.2018.74

Kingne Kingne, F., Doungue Tsafack, H., Teboukeu Boungo, G., Mboukap Ntongme, A., Solefack Nguepi, E., Edie Kinge, E., … Afeanyi Azia, T. (2018). Phenolic Content and Antioxidant Activity of Young and Mature Mango (Mangifera indica) and Avocado (Persea americana) Leaves Extracts. *Journal of Food Stability*, *1*(1), 14–27. Retrieved from www.foodstability.com

Marinova, D, Ribarova, F, Atanassova, & M. (2005). Total phenolic and total flavanoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, *40*(3), 255–260.

Nathaniel, O. B., Selina, A. S., John, K. M., Mercy, B., Sylvester, A. A., & Michael, B. M. (2016). Phytoconstituents, antimicrobial and antioxidant properties of the leaves of Persea americana Mill cultivated in Ghana. *Journal of Medicinal Plants Research*, *9*(36), 933–939. https://doi.org/10.5897/jmpr2015.5902

O, O. A., & O, O. B. (2014). Antibacterial Activities of the Leaf and Bark Extract of Persea americana. *American Journal of Ethnomedicine*, *1*(1), 64–071. Retrieved from www.ajethno.comhttp://www.ajethno.com

Osuntokun, O., Ukwuoma, P., Yusuf-Babatunde, A., & Ige, O. (2017). Evaluation of Antimicrobial Activity, Phytochemical Screening and Gas Chromatography-Mass/ Spectrophotometric Profile of Essential Oil from Persea americana & Citrus sinensis. *Asian Journal of Medicine and Health*, *5*(1), 1–17. https://doi.org/10.9734/ajmah/2017/33766

Rahman, N., Dewi, N. U., & Bohari. (2018). Phytochemical and antioxidant activity of avocado leaf extract (Persea americana Mill.). *Asian Journal of Scientific Research*, *11*(3), 357–363. https://doi.org/10.3923/ajsr.2018.357.363

Saharan, P., Sadh, P. K., & Singh Duhan, J. (2017). Comparative assessment of effect of fermentation on phenolics, flavanoids and free radical scavenging activity of commonly used cereals. *Biocatalysis and Agricultural Biotechnology*, *12*(October), 236–240. https://doi.org/10.1016/j.bcab.2017.10.013

Salim, D. M. (2013). *in Vitro Cyt Otoxic and Ant Iox Id Ant Activ Ities Extracts on D Ifferent Hum an C an Cer Cell Lines of Som E*. *9*(1), 137–144.

Senthilkumar, N., Murugesan, S., & Vijayalakshmi, K. B. (2012). GC-MS-MS analysis of Trichilia connaroides (Wight & Arn .) Bentv (Meliaceae): A tree of ethnobotanical records. *Asian Journal of Plant Science and Research*, *2*(2), 193–197.

Tan, M., Zhou, L., Huang, Y., Wang, Y., Hao, X., & Wang, J. (2008). Antimicrobial activity of globulol isolated from the fruits of Eucalyptus globulus Labill. *Natural Product Research*, *22*(7), 569–575. https://doi.org/10.1080/14786410701592745

Ukwubile, C. A., Ahmed, A., Katsayal, U. A., Ya'u, J., & Mejida, S. (2019). GC–MS analysis of bioactive compounds from Melastomastrum capitatum (Vahl)Fern. leaf methanol extract: An anticancer plant. *Scientific African*, *3*, 10–17. https://doi.org/10.1016/j.sciaf.2019.e00059

Umar, H., Kavaz, D., & Rizaner, N. (2019). Biosynthesis of zinc oxide nanoparticles using albizia lebbeck stem bark, and evaluation of its antimicrobial, antioxidant, and cytotoxic activities on human breast cancer cell lines. *International Journal of Nanomedicine*, *14*, 87–100. https://doi.org/10.2147/IJN.S186888

Yamasaki, R. (2018). Connexins in health and disease. *Clinical and Experimental Neuroimmunology*, *9*, 30–36. https://doi.org/10.1111/cen3.12433

Zafar, T., & Sidhu, J. S. (2011). Avocado: Production, Quality, and Major Processed Products. In *Handbook of Vegetables and Vegetable Processing* (pp. 525–543). https://doi.org/10.1002/9780470958346.ch26.