

## Chemical analysis and GC-MS characterization of ether fraction from *persea americana* seed

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### Abstract

The use of herbal medicines as alternative to orthodox medicines have given rise to many research methods of evaluating the medicinal values of most plant kingdoms so that their safety profiles can be ascertained. Hence, this study investigates phytochemical screening and isolation of alkaloid from the methanol extract of *Persea americana* (Avocado pear) seed. The dried seeds were pulverized and mixed with methanol solvent in a soxhlet extractor to obtain crude extract that was subjected to phytochemical screening using standard methods while a portion of it was subjected to physical separation to isolate the basic alkaloid fraction. The results indicated the presence of alkaloid, flavonoids, glycosides, terpenoids, steroids and phenolics. The GC-MS indicated hexadecamide (Rt: 26.43 31.21%), 9-octadecamide (Rt: 28.21; 17.80%) and oleic ester (Rt: 30.80; 26.78%) as major components. Minor components detected were oleic acid (Rt: 26.00; 0.29%), 1, 15-pentadecandiol (Rt: 29.86, 0.5%), a long chain alcohol. The presence of these phytochemicals suggests that the seed of *Persea americana* when subjected to pharmacological studies will be of immense medicinal value

## 1. Introduction

Avocado tree is botanically referred to as *Persea americana* and belong to the family of *Lauraceae*. It is an evergreen tree of height 40 – 80 feet widely grown in tropical and mediterranean climate of the world which includes Mexico, Central America, West Indian, West, East and Central Africa [1]. The plant leaves are 3-10 inches long, elliptically shaped, its fruits are round, pear shaped with green-yellow outer epicarp which turn bright yellow when ripen [2]. Plants in all facet of life have served as valuable starting materials for drugs development [3]. The medicinal values of these plants have been found to lie in their phytochemical constituents such as alkaloid, tannins, steroids, terpenoids, carotenoids, flavonoids, phenols and glycosides which produce definite physiological action in living systems [4,5,6].

Many different parts of *Persea americana* serve as sources for preparing different herbal medicines in the treatment of different disorders [7]. Isolation of bioactive phytoconstituents of the leaves of *Persea americana* (*Lauraceae*) yielded isohamnaetin, luteolin, rutin, quercetin and apigenin, isohamnaetin [6]. These compounds were evaluated for their ability to scavenge free radical using DPPH<sup>+</sup> and H<sub>2</sub>O<sub>2</sub> systems [6]. The peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activities of these compounds were in decreasing order of quercetin>rutin>isohamnaetin>luteolin>apigenin. This study indicates that the leaves of *P. americana* contain anti-oxidant activity which might be helpful in preventing the progress of various oxidative stress related diseases in rats [6]; thus, most ethno-medicine

practitioners use *P. americana* to lower body cholesterol. Administered 260 mg/kg/day x 100 days of aqueous seed extract milled from *Persea americana* were pretreated on native rats with the mean arterial pressure (MAP) and heart rate (HR) determined, the effects showed that 10 days' pretreatment significantly reduced the MAP and HR; also, this might be reasons for its use by herbalists for the management of hypertension [8]. The effects of the leaf extracts and active fractions of *P. americana* against mycobacterium tuberculosis and staphylococcus aureus indicated that the growth of these bacteria was significantly inhibited and as such this study might validate the use of *Persea americana* for the treatment of cough and diarrhea respectively [9].

The rats treated with aqueous seed extract of *Persea americana* significantly reduced the glucose level in them hence most trado-herbalists use it as blood sugar lowering agent [6]. The larvicidal activity and anti-fungal potential of hexane and methanol extracts of avocado seeds against *Aedes aegypt* and strains of *candida* spp., *Cryptococcus neoform* and *malassezia pachydermatis* indicated that both extracts had inhibitory effects on the growth of the larva and fungi and thus these plants can be used as larvicida and antifungal agent [11]. The treatment of seed extract of *Persea americana* mill on lipid profile in hypertensive rats reported that the aqueous seed extract of *P. americana* reduced cholesterol concentrations in the treated centration [12]. The carotenoid content may play significant role in cancer risk reduction [13], wound healing [14] and such properties have been ascribed to its oil (fatty acid) constituent proximate analysis conducted on the seeds [15]. This study involves the phytochemical screening and GC-MS analysis of the alkaloid fraction of the seed extract.

## **2. Methodology**

### **2.1. Material Collection and Preparation of Samples**

Avocado pear fruits were harvested from a bush in Isiohor, Ovia North East Local Government Area of Edo State, Nigeria. The fruits were identified by Prof. J. F. Bamidele, a taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The seeds were isolated from the fruits, washed with distilled water, air-dried under shade in the laboratory for four weeks and pulverized to powdered form. Extraction was done in soxhlet extractor using methanol as solvent. The crude extract was dried using  $\text{Na}_2\text{SO}_4$  and concentrated in a rotary evaporator (model: RE, 200).

### **2.2. Phytochemical Screening of Methanol Seed Extract**

Phytochemical screening was done to find the presence of the active chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, phenols, and eugenols by using the standard procedures [16,17,18].

### **2.3. Isolation of Alkaloid Fraction Seed Extract**

The methanol seed extract was treated with mineral acid (2M HCl) and the aqueous layer (lower layer in the separatory funnel) which contains the organic bases was separated and treated with 30mL  $\text{Na}_2\text{CO}_3$  to release the soluble bases as insoluble precipitate. The precipitate (which should contain basic fraction) was re-extracted with ether and dried for Infra-Red (I.R) analysis.

## 2.4. GC-MS Analysis

The analysis was carried out on a GC-MS Spectrometer filled with an HP-5 MS (5% phenylsiloxane) column at a temperature programme of 70°C (2 minutes) increase at 10°C/min to 280°C and held for 7 minutes. The carrier gas was nitrogen and flow rate, 1.80mL/min.

## 3. Results and Discussion

Results of the phytochemical screening and GC-MS analysis of the alkaloid fraction of the *P.americana* seeds extract are shown below. *P.americana* serves as sources for preparing different herbal medicines in the treatment of different disorders.

Table 1: Phytochemical screening of methanol extract of *P. americana* Seed.

S/N	Phytochemical Constituents	Name of Test	Methanol Extract
1	Glycoside	General test	+
2	Saponin	Foam test	++
3	Flavonoid	Lead acetate test	++
4	Phenolics	Ferric chloride	+
5	Tannin	Ferric chloride	-
6	Eugenol	KOH/HCl	+
7	Steroid	Acetic acid/H <sub>2</sub> SO <sub>4</sub>	+
8	Terpene	Salkowski test	-
9	Alkaloids	Picric acid test	++

Key: + = present, ++ = largely present, - = absent.

In Table 1 the qualitative phytochemical analysis shows that saponin, flavonoid, and alkaloids were largely present in the extracts, low steroid present and absent of tannin and terpene.

### 3.1. GC-MS Analysis

The GC-MS chromatogram of the isolated brown alkaloid fraction given in Figure 1 showed 14 peaks, indicating from the search list of the chemical abstract service as fourteen compounds identified in the alkaloid fraction is presented in Table 2.

The phytochemicals are bioactive readily synthesized by the plants as secondary metabolites which when ingested by man act as defensive mechanism against microorganisms [6]. In Table 1, glycoside, phenolics, eugenol, and steroids were present while saponin, flavonoid and alkaloids were largely present. Phenolics and alkaloids in most plants leaves, bark, fruits and roots have been indicated as useful bioactive agents with high physiological effect in man [16] being that they used as antioxidant, antimicrobial and anti-diabetic active agents.

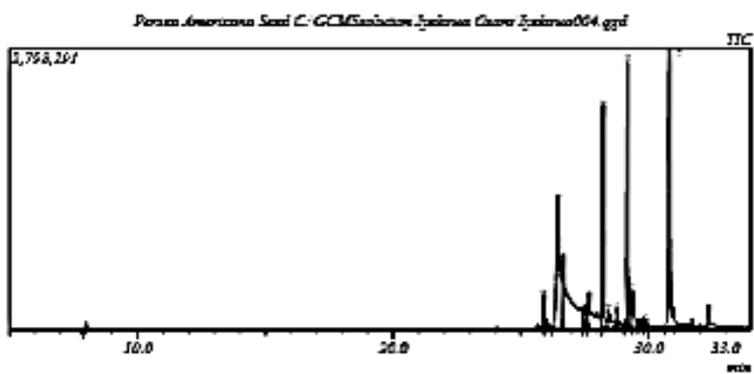
From Table 2, major components detected from the alkaloid fraction of *P. americana* are hexadecanamide (Rt: 26.43, 31.21%), 9-octadecenamide (Rt: 28.21, 17.80%); 2, 3-dihydroxypropylester 9-octadecenoic acid (Rt: 30.80; 26.78%) and adipic acid (Rt: 29.16; 13.80%). while minor components among others includes oleic acid (9-octdecenoic acid) (Rt: 26.00; 0.29%). The result also suggested presence of amide group which formed component of amide salt and confirmed basic alkaloid fraction of the isolated compound. The amide constituents recorded high percentage at Rt: 26.43; 31.21% and at Rt: 28.76; 17.80% for hexadecanamide and 9-octadecenamide respectively. This analysis conducted characterized and identified the large percentage of amide group among other antioxidants contain in alkaloid extract from *Persea americana* seed as promising active agent against bacterial activity.

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Sample Information  
 Analyzed by : Sibus 05  
 Analyzed : 12/11/2015 07:24:34  
 Sample Type : Unknown  
 Label # : 1  
 Sample Name : Unknown  
 Sample ID : Persea Americana Seed  
 ID Account : 1091  
 Sample Amount :  
 Dilution Factor : 1  
 Vial # : 4  
 Injection Volume : 1.00  
 Data File : C:\GC\Software\System1\ChemStation\1\4\1091\_001.D  
 Exp Data File : C:\GC\Software\System1\ChemStation\1\4\1091\_001.D  
 Method File : C:\GC\Software\System1\ChemStation\1\4\1091\_001.D  
 Exp Method File : C:\GC\Software\System1\ChemStation\1\4\1091\_001.D  
 Report File :  
 Template File : C:\GC\Software\System1\ChemStation\1\4\1091\_001.D  
 Modified by : Admin  
 Modified : 12/11/2015 20:01:28



Peak	Retention Time (min)	Area	Area (%)	Height	Height (%)	Width	Width (%)	Label	Name
1	7.90	1.00	0.11	0.02	0.02	0.02	0.02	1	7-Octadecenoic acid methyl ester
2	25.90	1.13	1.13	0.05	0.05	0.05	0.05	2	10-methyl, octadecenoate
3	26.00	0.29	0.29	0.02	0.02	0.02	0.02	3	9-Octadecenoic acid (E)-
4	26.43	31.21	31.21	0.15	0.15	0.15	0.15	4	Hexadecanamide
5	27.43	1.13	1.13	0.05	0.05	0.05	0.05	5	13-Octadecenoic acid
6	27.61	0.48	0.48	0.02	0.02	0.02	0.02	6	15-Hydroxypentadecanoic acid
7	28.21	17.80	17.80	0.08	0.08	0.08	0.08	7	9-Octadecenamide
8	28.42	0.60	0.60	0.03	0.03	0.03	0.03	8	Decanamide
9	28.76	0.80	0.80	0.04	0.04	0.04	0.04	9	2,3-Dihydroxypropyl ester 9-Octadecenoic acid
10	29.16	13.80	13.80	0.06	0.06	0.06	0.06	10	adipic acid
11	29.40	5.04	5.04	0.02	0.02	0.02	0.02	11	2,3-Dihydroxypropyl ester hexadecanoic acid
12	29.62	0.74	0.74	0.03	0.03	0.03	0.03	12	oleic acid, methyl ester
13	29.86	0.50	0.50	0.02	0.02	0.02	0.02	13	1, 15 pentadecandiol
14	30.80	26.78	26.78	0.12	0.12	0.12	0.12	14	2,3-Dihydroxypropyl ester 9-Octadecenoic acid (oleic ester)

Library Figure 1: Alkaloid fractions

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Table 2: GC-MS analysis of ether fraction of *Persea Americana* Seed

Peak No	Retention time (Rt.)	Name of compound	Area (%)	Mol. Formula	Mol. Wt.
1	7.90	7-octadecenoic acid methyl ester	0.11	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
2	25.90	10-methyl, octadecenoate	1.13	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
3	26.00	9-octadecenoic acid	0.29	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
4	26.43	Hexadecanamide	31.21	C <sub>16</sub> H <sub>33</sub> NO	255
5	27.43	13-octadecenoic acid	1.13	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
6	27.61	15-hydroxypentadecanoic acid	0.48	C <sub>15</sub> H <sub>30</sub> O <sub>3</sub>	258
7	28.21	9-octadecenamide	17.80	C <sub>18</sub> H <sub>35</sub> NO	281
8	28.42	Decanamide	0.60	C <sub>10</sub> H <sub>21</sub> NO	171
9	28.76	2,3-dihydroxypropyl ester 9-octadecenoic acid	0.80	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356
10	29.16	adipic acid	13.80	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	282
11	29.40	2,3-dihydroxypropyl ester hexadecanoic acid	5.04	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330
12	29.62	oleic acid, methyl ester	0.74	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
13	29.86	1, 15 pentadecandiol	0.50	C <sub>15</sub> H <sub>32</sub> O <sub>2</sub>	244
14	30.80	2,3-dihydroxypropyl ester 9-octadecenoic acid (oleic ester)	26.78	C <sub>19</sub> H <sub>48</sub> O <sub>4</sub>	
Total			100.00		

#### 4. Conclusion

The phytochemical screening and GC-MS study on *Persea americana* extract has shown presence of medicinal bioactive agents and that the alkaloid fraction contain unsaturated fatty acid (oleic acid) which is necessary in human diet. Furthermore, this work corroborates the traditional claims of the use of the seed in traditional medicine due to the bioactive constituents detected in the seed extract.

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