Chemical Compounds Identification and Phytochemical Screening of *Persea americana* (Avocado) Root Extract

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Abstract: Members of *Persea* are typically medium-size trees, 15-30 meters tall at maturity with leaves that are simple, lanceolate to broad and flowers arranged in short panicles, with six small greenish-yellow perianth segments 3-6mm long, nine stamens, and an ovary with a single embryo. The aim of this work is to identify the chemical compounds in dichloromethane and methanol (2:1) and screened for phytochemicals in *Persea americana* root extracts. Solvent extraction was used to extract crude extracts from the root and the crude extracts were subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) analysis and phytochemical screening. The result showed that hundred chemical compounds were identified but eleven are the major which are correlated with NIST library in which the correlation percentage (%) was taken from 18-100%. The compound identified were Carbondioxide (0.94%), Nitrous Oxide (3.78%), Ethylene Oxide (3.62%), Ethyne Fluoro (0.68%), Acetonitrile Hydroxy (1.04%), 1-Heptanamine (3.37%), 1-Decanamine (3.37%), Cyclobutanol (1.05%), 1-Heptadecanamine (2.46%), 5-Aminovaleric acid (2.46%), Acetaldehyde (2.09%). Ethanolic extract of the phytochemical revealed the presence of all screened phytochemical in the work, dichloromethane and methanol revealed the presence of all screened phytochemical except mayer (one of the test in alkaloid) which is absent, aqueous extracts revealed the presence of only wagner which is some of the test in alkaloid and the rest of the test were absent. The presence of phytochemicals in the ethanoic, dichloromethane and methanol extracts of *Persea americana* root which include alkaloid, saponin, steroid, tannin, triterpenoid and phenol can be used as a herbal medicine and in pharmaceutical industries in the production of drugs and other medical formulations.

Keywords: *Persea americana* Root, Chemical Compounds Identification, Phytochemical Screening.

Introduction

The *Persea americana* is a tropical and sub-tropical fruit tree, originated in central America, adjoining regions of north and south America. It has now spread to much of the near tropical world. The *Persea americana* is limited especially by its climatic requirements, with their race differences. It is also highly susceptible drought injury. The tree is widely cultivated in tropical and subtropical areas (Lu *et al*., 2005). *Persea americana* are part of the laurel family, Lauraceae, which comprises a group of flowering plants included in the order Laurales. The *persea americana* is the best-known member of the genus

Persea, which is comprised of about 150 species of evergreen trees. Members of *Persea* are typically medium-size trees, 15-30 meters tall at maturity, with leaves that are simple, lanceolate to broad lanceolate, and flowers arranged in short panicles, with six small greenish-yellow perianth segments 3-6mm long, nine stamens, and an ovary with a single embryo. The *Persea americana* grows to 20 meters (65 feet), with alternately arranged, evergreen leaves, 12–25centimeters long. The pear shaped fruit is botanically a berry. It typically measures 7 to 20 centimeters in length and weighs between 100 and 1000 grams. The *Persea americana* fruit also has one large central seed, 3 to 5 centimeters in diameter. The *Persea americana* is a climatic fruit, which means that it matures on the tree but ripens off the tree. An average *Persea americana* tree produces about 120 avocados annually. Commercial orchards produce an average of seven metric tons per hectare each year, with some orchards achieving 20 ton per hectare (Whiley, 2007). The common names "avocado pear" or "alligator pear" for the fruit are due to its shape and rough green skin. It is speculated that the *Persea americana* fruit's poisonous pit was once dispersed through the excretion of an animal with which it co-evolved. However, since the disappearance of its propagating partner, human cultivation seems to have unobliged further seed dispersal-driven evolution. Previously, *Persea americana* had a long-standing stigma as asexual stimulant and were not purchased or consumed by any person wishing to preserve a chaste image.

The fruit is not sweet but fatty, almost distinctly, yet subtly flavored, and of smooth, almost creamy texture. *Persea americana* fruits in many countries such as Mexico, Brazil, South Africa and India are frequently used for milkshakes and occasionally added to ice-cream (Zeldes, 2010). While several works had been reported on the chemical characterization of phyto-constituents of *Persea americana* fruit, there is still limited information on its potential use in the management/prevention of degenerative diseases associated with oxidative stress (USDA, 2011). *Persea americana* are a farm-to-market food; they require no processing, preservatives or taste enhancers. The *Persea americana*

natural skin eliminates the need for packaging and offers some disease and insect resistance, which allows them to be grown in environmentally sustainable ways. The *Persea americana* leaf, stem, fruit and peel have biological activities scientifically proven (Gomez-Flores *et al*., 2008; Castro *et al.*, 2010; Rodríguez-Carpena*et al*., 2011). Studies with seed demonstrated antioxidant activity and antimicrobial activity against Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Pseudomonas spp. and Yarrowia lipolytica. The Gram-positive bacteria are more sensitive than Gram-negative bacteria (Rodríguez-Carpena *et al*., 2011).

Aim and Objectives: To identify the chemical compounds in dichloromethane and methanol (2:1) and screened for phytochemicals in *Persea americana* root extracts.

Methodology

Plant Sample Collection

Persea americana root was gotten from Oremeji, Ore ring road Ondo, Ondo State Nigeria.

Plant Sample Preparation

Persea americana root was washed and air dried in the laboratory at room temperature and was reduced to smaller size using mortar and pestle.

Solvent Extaction of *Persea americana* Root

30g of the ground *Persea americana* root were soaked with 250ml of Ethanol, Methanol and Dichloromethane (2:1) and distilled water for 72hours. The solvents were decanted and concentrated at about 37°C using rotary evaporator. After concentration, the extracts were placed under the fume cupboard to ensure that remaining solvents evaporate into the atmosphere.

Phytochemical Screening of *Persea americana* Root Extract

Phytochemical analysis was carried out on the extracts obtained to screen for the presence of different classes of plant on secondary metabolites. The screening tests are triterpenoid, saponin, tannin, sterold, alkanoid, phenols

Test for Saponin (Foam Test)

5ml of extract was shaked with 2ml of water. If the foaming produce persists for ten minutes, it indicates the presence of saponin.

Test for Tannin (Ferric Chloride Test)

1ml of the extract boiled in 2ml of distilled water in a test tube and 4ml of filter after with few drops of 10% ferric chloride was added and the solution was observed from green to brownish green.

Test for Steroid

1ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. Reddish upper layer and yellowish sulphuric layer with green fluorescence indicate the presence of steroids.

Test for Triterpenoids

3mL of sample was measured into a test tube and 2mL of chloroform and 3mL of concentrated tetraoxosulphate (VI) acid was added. The presence of a reddish brown colouration at the interface shows positive results for the presence of triterpenoids.

Test for Alkaloid

2ml of extract stirred with 1ml HCL and 1ml ammonia in a water bath was filtered, 2ml of filtrate was divided into three test tubes, to one of the test tube, it was treated with few drops of mayer's reagent without a precipitate and to the second test tube containing the filtrate was treated with wagner reagent which gives a reddish brown precipitate coloration with little precipitate and to the third test tube was treated with Hager reagent.

Test for Phenols (Ferric Chloride Test)

Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The GC-MS analysis of extract was carried out by employing 1 µl of sample. The GC-MS analysis was carried out using Agilent Technologies 7890 A equipped with 5975 MSD with HP-5ms Capillary column (30 m length, 0.32 mm internal diameter, 0.25 µm thickness). Helium gas was used as carrier gas at constant flow rate of 1mL per minute. Injector temperature was set at 250^{0}C. The oven temperature was programmed from 90^{0}C for 0 minutes then at 3^{0}C per minute to 180^{0}C for 10 minutes. Identification of the compounds was carried out by comparing the spectral data of sample with reference spectra in spectral libraries (NIST).

Results and Discussion

Table 1: Show The Percentage Yield of *Persea americana* Dichloromethane and Methanol (2:1), Ethanol and Aqueous Root Extract

S/N	Extract	%Yield
1	PARDM	3.71%
2	PARE	1.94%
3	PARA	2.65%

Keys:

PARDM: *Persea americana* Methanol and Dichloromethane (2:1) Extract

PARE: *Persea americana* Ethanol Extract

PARA: *Persea americana* Aqueous Extract

Percentage Yield of *Persea americana* Root Extracts

Root Extract of Dichloromethane and Methanol (2:1)

Weight of empty container = 64.56g

Weight of container + extract =65.672g

Total weight = (65.672-64.56) g =1.112g

Weight of extract = 1.112g

Weight of sample = 30g

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100$$

$$\frac{1.112}{30} \times 100 = \frac{111.2}{30} = 3.71\%$$

Root Extract of Ethanolic

Weight of empty container = 71.843g

Weight of container + extract =72.325g

Total weight = (72.325-71.843) g =0.582g

Weight of extract = 0.582g

Weight of sample = 30g

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100$$

$$\frac{0.582}{30} \times 100 = 1.94\%$$

Root Extract of Aqueous

Weight of empty container = 61.566g

Weight of container + extract =62.297g

Total weight = (62.297-61.566) g =0.531g

Weight of extract = 0.531g

Weight of sample = 20g

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100$$

$$\frac{0.531}{20} \times 100 = 2.65\%$$

Table 2: Chemical Compounds Identified In *Persea americana* Dichloromethane and Methanol (2:1) Root Extract

S/N	Chemical Compounds	Correlation%	%Area
1	Carbon dioxide	25.91	0.94
2	Nitrous Oxide	23.78	3.78
3	Ethylene Oxide	100.00	3.62
4	Acetonitrile Hydroxy	28.82	1.04
5	Acetaldehyde	57.82	2.09
6	1-Heptanamine	93.10	3.37
7	1-Decanamine	93.10	3.37
8	Cyclobutanol	29.12	1.05
9	Ethyne Fluoro	18.72	0.68
10	1-Heptadecan-amine	68.01	2.46
11	5-Aminovaleric	68.01	2.46
	Total		24.86%

Discussion

Chemical compounds identified in *Persea americana* root extract of dichloromethane and methanol (2:1) were listed in table 2 which are in order of elution with their retention time on the agilent column (HP-5MS), calculated kovat and area in percentage were also recorded. NIST library was also used to confirm the identity of the chemical compounds. Figure1

represent *Persea americana* root extract of methanol and dichloromethane (2:1) of GC chromatograms.

Hundred chemical compounds were detected but eleven are the major which are correlated with NIST library in which the correlation percentage (%) was taken from 18-100%. The compound detected were Carbondioxide (0.94%), Nitrous Oxide (3.78%), Ethylene Oxide (3.62%), Ethyne Fluoro (0.68%), Acetonitrile Hydroxy (1.04%), 1-Heptanamine (3.37%), 1-Decanamine (3.37%), Cyclobutanol (1.05%), 1-

Heptadecanamine (2.46%), 5-Aminovaleric acid (2.46%), Acetaldehyde (2.09%) (See appendix for the mass spectra) with the total of 24.86% for the percentage area. This work is compared with the work done by Jos**é** *et al*., 2009 in which they identified stigmastan-3,5-diene as compound isolated from organic phase of root extracts of *Persea americana* and realized that it is completely different. To my knowledge, this is the first report on dichloromethane and methanol crude extract of *Persea americana* root.

Table 3: Phytochemical Screening of *Persea americana* Root Extract

Phytochemical screening	PARE	PARDM	PARA
Saponin	+	+	-
Tannin	+	-	-
Triterpenoid	+	+	-
Steroid	+	+	-
Phenol	+	+	-
Alkanoid (wagner)	+	+	+
Mayer	+	-	-
Hager	+	+	-

Keys:

PARE: *Persea americana* ethanoic extract

PARDM: *Persea americana* dichloromethane and methanol extract

PARA: *Persea americana* aqueous extract

+ Means Phytochemical Present

– Means Phytochemical Absent

Discussion

Phytochemical analysis conducted on the *Persea americana* root extracts revealed the presence of constituents which are known to exhibit medicinal properties as well as physiological activities. Phytochemical screening of *Persea americana* ethanol root extract revealed the presence of Phytochemicals such as tannin, saponin, phenol, triterpenoid, steroid and alkaloid (Wagner, Mayer, Hager). Phytochemical screening of *Persea americana* dichloromethane and methanol root extract revealed the presence of tannin, saponin, phenol, triterpenoid, steroid and alkaloid (Wagner, Hager) and the absence of Mayer which is one of the test in alkaloid i.e not all the three test in alkaloid is present. Phytochemical screening of *Persea americana* aqueous root extract revealed the absence of tannin, saponin, phenol, triterpenoid, steroid and alkaloid (Mayer, Hager) and the presence of wagner which is one of the test in alkaloid i.e not all the three test in alkaloid were present (Table 3). Comparing the three extracts; root extract with ethanol, root extract with dichloromethane & methanol, root extract with distilled water. Root extract with ethanol as the most active compound because most of the phytochemicals are present in it.

Conclusion

Three compounds have the correlation percentage between 90 to 100, another three compounds between 50 to 68.01 and the last five between 18 to 29.12 which make it eleven compounds taken from 18 to 100.

The presence of phytochemicals in the ethanoic, dichloromethane and methanol extracts of *Persea americana* root which include alkaloid, saponin, steroid, tannin, triterpenoid and phenol can be used as a herbal medicine and in pharmaceutical industries in the production of drugs and other medical formulations.

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APPENDICES

The structure of the compounds Identified in *persea Americana* root extract of dichloromethane and methanol (2:1)