https://dx.doi.org/10.4314/dujopas.v9i1a.18
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490

Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 9 No. 1a March 2023*

Determination of Bioactive Constituents and Mineral Contents of Avocado Pear Seed Oil

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Abstract
Edible oils of plant origins are fundamental components of human diet, and are essential for various industrial applications, such as in food, cosmetics, and pharmaceutical industries. Of increasing interest is the utilization of edible oils obtained from plant parts such as peels, seeds, rinds, leaves, flowers, and fruits, which are usually regarded as wastes. This study investigated the usability of edible oil extracted from avocado pear seed by assaying its bioactive constituents and mineral composition. The bioactive compounds in the oil were determined by Gas Chromatography Mass Spectroscopy (GC-MS), while the mineral content was assayed using Atomic Absorption Spectrometry (AAS). Results of the GC-MS analysis showed the presence of twenty nine compounds, with 18-Nonadecen-1-ol (13.20%) being the most predominant, and 3,7,7-Trimethyl-1,3,5-cycloheptatriene (0.24%) the least. Compounds such as 10-Undecyn-1-ol, 7-Hexadecenoic acid methyl ester, 14-methyl Pentadecanoic acid methyl ester, and Linolelaidic acid, methyl ester, identified in the avocado seed oil, have previously been reported to possess various biological activities, including antifungal, antioxidant, antimicrobial, hypocholesterolemic, antiandrogenic, hemolytic, anti-cancer and anti-inflammatory activities. Results of the mineral analysis showed that potassium (13.09 ± 0.22 mg/100 g) was the major element, while copper (0.06 ± 0.11 mg/ 100 g) was the least; cadmium and lead were not detected in the oil. The presence of pharmacologically important phytochemicals and essential minerals in avocado seed oil suggests its potential as a nutraceutical substance, with applications in food and drug preparations.

Keywords: minerals, avocado pear, seed, seed oil, bioactive constituents

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INTRODUCTION

Plants and their derived phytochemicals have a rich history of promoting health and well-being. For centuries, they have been utilized for their medicinal properties, and contemporary studies have validated the vast array of health benefits provided by various phytochemicals, including vitamins, minerals, antioxidants, and anti-inflammatory compounds. The rich pharmacological properties of these substances, such as their antioxidant, antimicrobial, anti-inflammatory, and anti-cancer effects, have greatly contributed to the development and creation of new drugs. As a result, many people are turning to plant-based diets and supplements to improve their overall health and vitality. According to the World Health Organization (WHO), over 80 % of the population in developing countries rely on medicinal plants for primary health care needs. This is mainly attributed to their low cost, high accessibility, and relatively low toxicity. Extracts from different parts of plants such as seeds, roots, leaves, stems, and peels, have been reported to possess various biological activities. Avocado pear (*Persea americana*) is one such plant from which edible oil can be obtained and it is considered a climacteric fruit, which means it ripens after being picked from the tree. It changes from green to dark purple as it matures and ripens, and consists of 15 % peel, 65 % flesh, and 20 % seed (Tan *et al*., 2017; Bahru *et al*., 2019). Avocado oil is commonly extracted from the flesh of the fruit and is a rich source of healthy fats, including monounsaturated fatty acids, oleic acid, and polyunsaturated fatty acids, like linoleic acid. It is also a rich source of energy and its low sugar content makes it suitable for diabetic diets (Adaramola *et al*., 2016). Apart from its peel, avocado fruit seed is often regarded as non-edible and hence regarded and discarded as waste. However, avocado seeds and peels are also rich in fibre, phenolic compounds, and have antioxidant and antimicrobial properties (Satriana *et al*., 2018). Moreover, the seed contains mostly monounsaturated fatty acids, which have anti-inflammatory and cardiovascular benefits (Carvajal-Zarrabal *et al*., 2014). Although not considered a primary oil source, avocado oil is becoming increasingly popular in human nutrition and cosmetics due to its appreciable qualities. Its therapeutic properties are attributed to bioactive compounds such as oleic acid, palmitic acid, tocopherols, squalene, beta-sitosterol, campesterol, and cycloartenol acetate (Santos *et al*., 2014). Most of the oil is contained in the mesocarp of the fruit (60 %) while only 9 % is obtained from the seeds (Adaramola *et al*., 2016; Tan *et al*., 2017). Despite this low oil yield, avocado seeds have been reported to contain antibacterial compounds (Temitope *et al*., 2017), antioxidants, phenolic compounds, and essential minerals such as calcium, phosphorus, and potassium (Maitera *et al*., 2014). Because of the relatively low oil yield from the avocado seeds, not much has been done in evaluating the properties of the oil. Therefore, this study was carried out to determine the bioactive and mineral constituents of edible oil extracted from avocado pear seed, with the aim of ascertaining its potential pharmacological importance.

MATERIALS AND METHODS

Collection and preparation of sample

The avocado pear seeds needed for oil extraction were obtained by purchasing mature avocado pear fruits from a local market in Ilishan-Remo, Ogun State Nigeria. After washing them to remove impurities, the fruits were left to ripen for four days in a dark, room-temperature environment. Then, the seeds were manually removed, cut into pieces, dried in an oven at 50 °C for 48 h, and pulverized in a LEXUS MG-2053 OPTIMA laboratory blender. The resulting powder was used for oil extraction, with 50 g of it placed in a thimble and extracted with n-hexane for 16 h using a Soxhlet apparatus. Afterward, the n-hexane was removed from the oil *in vacuo* with a rotary evaporator (Eyela N-1001) at 40 °C, and the oil was then stored in an airtight glass bottle.

Mineral analysis
The mineral content of the avocado seed oil was analyzed using the wet digestion method. 0.5 g of the sample was measured into a 100 mL micro digestion tube, to which 6 mL of concentrated sulfuric acid and 4 mL of hydrogen peroxide were carefully added. The tube was heated in a pre-heated digester block (270 °C) until a clear solution was obtained, indicating complete digestion. After cooling, the resulting digest was filtered and a reagent blank was concomitantly prepared with a mixture of 6 mL concentrated sulfuric acid and 4 mL hydrogen peroxide, but without the oil sample. Nine mineral elements (Ca, Mg, K, Na, Fe, Cu, Zn, Cd, and Pb) in the digest solution and blank were determined using an Atomic Absorption Spectrophotometer (Buck Scientific Model 2010 VGP) calibrated with the standard solution of each element. Phosphorus was determined with a UV-visible spectrophotometer (LabMed SPECTRO SC Spectrophotometer). All analyses were conducted in triplicate, with the data reported as mean ± SD of three replicates.

GC-MS Analysis
The bioactive compounds in the avocado seed oil were analyzed using a Gas Chromatograph interfaced with a Mass Spectrometer (GC-MS) equipment; (Schimadzu GCMS-QP2010 PLUS). The GC-MS was equipped with a split injector and an ion-trap mass spectrometer detector, and used a fused-silica capillary column with a thickness of 1.00 μm, dimension of 30 mm × 0.25 mm, and a programmed temperature range from 60 °C to 250 °C at a rate of 3.0 °C/min. The injector temperature was set at 250 °C, ion-source temperature 230 °C and the detector temperature was 200 °C, with helium as the carrier gas flowing at a rate of 1.58 mL/ min and a sample injection volume of 1 μL. The oven temperature was programmed from 80 °C (isothermal for 1 min), with an increase of 10°C/minute, to 200 °C, then 10 °C/minute to 280 °C, ending with a 5 min isothermal at 280 °C. Mass spectra were taken at 70 eV with a scan interval of 0.5 s. Total run time was 28 min. The relative percentage of each identified compound was determined by comparing its average peak area to the total area. The identification of components was done through computerized matching of their spectra with known compounds from the NIST spectra library (2009). The fragmentation patterns of the eluted compounds were identified by comparing them with known data from the database.

RESULTS AND DISCUSSION
Many industries, such as food, cosmetics, and pharmaceuticals, utilize edible oils as a crucial component in their production processes. Of utmost interest, however, are those obtained from waste materials such as avocado seed used in this present study. In order to maximize economic value and reduce waste loads in the environment, it is essential to explore the potential uses of non-edible and under-utilized materials such as avocado pear seeds, which are currently regarded as wastes. This study investigated the bioactive compounds and mineral content of oil extracted from avocado pear seed.

Bioactive Compounds
The bioactive constituents present in the avocado pear seed oil were determined by gas chromatography mass spectrometry. The GC-MS chromatogram (Fig. 1) shows the presence of twenty nine bioactive compounds in the oil sample and the identity of these bioactive compounds is presented in Table 1. From the results, the most prominent compound in the oil was 18-Nonadecen-1-ol (13.20 % area) and closely followed by 3-Ethyl-3-pentanol (13.12 %) and 10-Undecyn-1-ol (11.69 %) while the least was 3,7,7-Trimethyl-1,3,5-cycloheptatriene (0.24 %). Other major compounds identified in the oil sample are Linolelaidic acid, methyl ester (9.64 %), 1,2-15,16-Diepoxyhexadecane (6.25 %), 9-Octadecenoic acid, methyl ester (5.70 %), 2-pentadecyl-1,3-dioxane (5.70 %), Pentadecanoic acid, 14-methyl-, methyl ester (5.41 %), 9-Octadecen-1-ol (2.62 %), 2,3-dimethyl-5,8,10-Undecatrien-3-ol (2.62 %), Oxirane [(dodecyloxy) methyl] (2.51 %), 1,2,4-Trimethylbenzene (2.38 %), 1,2-Epoxyhexadecane (2.04

%), and Pentacosanoic acid, 4-methyl-, methyl ester (2.01 %). Biological activities of some of the compounds identified in the avocado seed oil have been reported in literature. One of the major bioactive components of the oil, 10-Undecyn-1-ol, has been found to exhibit antifungal activity (Neoh *et al.*, 2008). Devi and Serfoji (2018) have reported the antioxidant, hypocholesterolemic, antiandrogenic, hemolytic and alpha reductase inhibitory activities of 7-Hexadecenoic acid, methyl ester. Additionally, 14-methyl Pentadecanoic acid, methyl ester possesses antioxidant, antifungal, antimicrobial activities (Bashir *et al.*, 2012), Linolelaidic acid, methyl ester has been reported to have antifungal and antioxidant activities (Chandrasekaran *et al.*, (2011) while 9-Octadecenoic acid, methyl ester have anti-inflammatory, antiandrogenic, anticancer, dermatitigenic, hypocholesterolemic, anemiagenic, and 5-alpha reductase inhibitory activities (Rajeswari and Rani, 2015). Eicosanoic acid, methyl ester has also been reported to act as an alpha-glucosidase inhibitor (Duke, 1992). The presence of these bioactive compounds in avocado seed oil suggests the pharmacological potential of the seed oil and its potential applicability in the food, cosmetic and pharmaceutical industries.

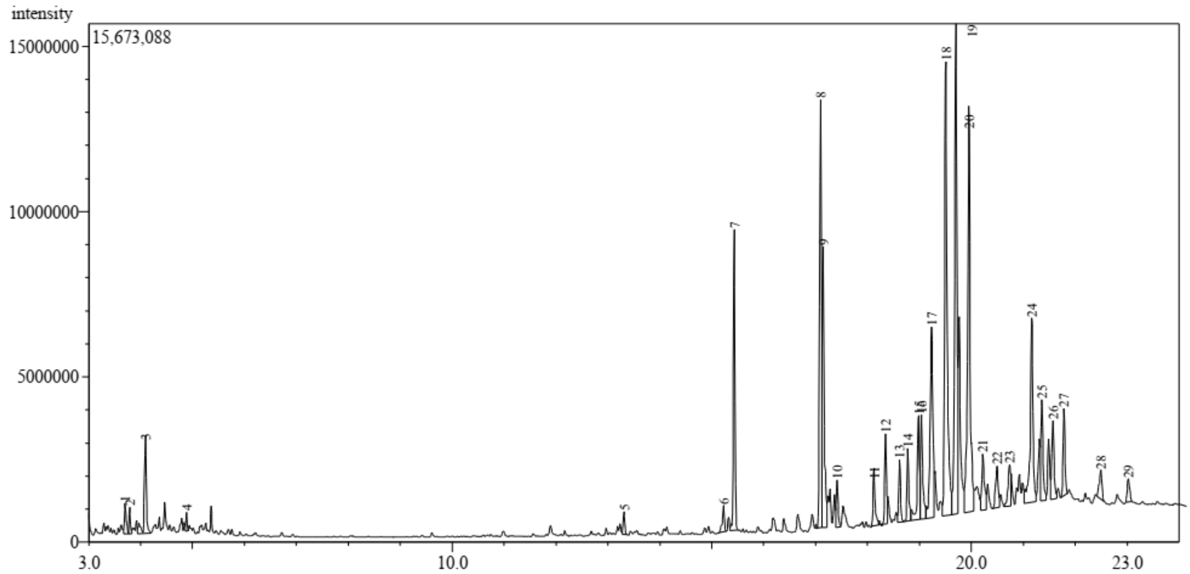

Fig. 1 GCMS chromatogram of avocado seed oil

Table 1. Bioactive compounds identified in avocado seed oil

S/N	RT	Compound name	Molecular formula	Molecula Weight (g/mol)	Peak area (%)	Nature of compound
1.	3.700	1-ethyl-2-methylBenzene	(C_9H_{12})	120	0.62	Aromatic compound
2.	3.792	1,2,3-Trimethylbenzene	(C_9H_{12})	120	0.37	Aromatic compound
3.	4.100	1,2,4-Trimethylbenzene	(C_9H_{12})	120	2.38	Aromatic compound
4.	4.883	3,7,7-Trimethyl-1,3,5-cycloheptatriene	($C_{10}H_{14}$)	134	0.24	Aromatic compound
5.	13.308	Decanoic acid methyl ester	($C_{11}H_{22}O_2$)	186	0.40	Saturated fatty acid methyl ester
6.	15.225	7-Hexadecenoic acid, methyl ester	($C_{17}H_{32}O_2$)	268	0.60	Unsaturated fatty acid methyl ester
7.	15.433	14-methyl Pentadecanoic acid, methyl ester	($C_{17}H_{34}O_2$)	270	5.41	Saturated fatty acid methyl ester .
8.	17.092	Linolelaidic acid, methyl ester	($C_{19}H_{34}O_2$)	294	9.64	Unsaturated fatty acid methyl ester
9.	17.142	9-Octadecenoic acid, methyl ester	($C_{19}H_{36}O_2$)	296	5.70	Unsaturated fatty acid methyl ester

10.	17.408	4-Methyl-4-heptanol	($C_8H_{18}O$)	130	1.01	Alcohol
11.	18.133	1,3,5-triazinane-2,4,6-trione	($C_3H_3N_3O_3$)	129.07	1.33	Triazine
12.	18.342	1,2-Epoxyhexadecane	($C_{16}H_{32}O$)	240	2.04	Epoxide
13.	18.617	1-Tetradecyne ($C_{14}H_{26}$)		194	1.30	Aliphatic alkyne
14.	18.775	2-Heptyl-1,3-dioxane	($C_{11}H_{22}O_2$)	186	1.57	Ether
15.	18.983	9-Octadecen-1-ol	($C_{18}H_{36}O$)	268	2.62	Unsaturated fatty alcohol
16.	19.033	2,3-dimethyl-5,8,10-Undecatrien-3-ol	($C_{13}H_{22}O$)	194	2.62	Unsaturated fatty alcohol
17.	19.233	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254	6.25	Epoxide
18.	19.508	3-Ethyl-3-pentanol	($C_7H_{16}O$)	116	13.12	Alcohol
19.	19.700	18-Nonadecen-1-ol	($C_{19}H_{38}O$)	282	13.20	Unsaturated fatty alcohol
20.	19.958	10-Undecyn-1-ol	($C_{11}H_{20}O$)	168	11.69	Unsaturated fatty alcohol
21.	20.217	12-Methyl-E,E-2,13-octadecadien-1-ol	($C_{19}H_{36}O$)	280	1.70	Unsaturated fatty alcohol
22.	20.492	4-Methyl-2-pentadecyl-1,3-dioxolane	($C_{19}H_{38}O_2$)	298	1.27	Ether
23.	20.733	2,3,4-Trimethyl-3-pentanol	($C_8H_{18}O$)	130	1.22	Alcohol
24.	21.158	2-Pentadecyl-1,3-dioxane	($C_{19}H_{38}O_2$)	298	5.70	Ether
25.	21.358	Oxirane [(dodecyloxy)methyl]-	($C_{15}H_{30}O_2$)	242	2.51	Ether
26.	21.567	Pentacosanoic acid, 4-methyl-, methyl ester	($C_{27}H_{54}O_2$)	410	2.01	Saturated fatty acid methyl ester
27.	21.783	11,12-Epoxytetradecan-1-ol	($C_{14}H_{28}O_2$)	254	1.96	Alcohol
28.	22.492	Eicosanoic acid, methyl ester	($C_{21}H_{42}O_2$)	326	0.88	Saturated fatty acid methyl ester
29.	23.017	Linoleoyl chloride	($C_{18}H_{31}ClO$)	298	0.65	Acyl halide

Mineral Contents

The pharmacological potential of plants is established not only by phytochemicals, but also by the presence of essential minerals. Both micro and macro minerals are essential for good health considering their vital roles in ensuring proper functioning of all body systems. The results of mineral analysis (Table 2) show the presence of both macro and micro minerals in avocado seed oil. It is clear from the results that the most prevalent mineral in avocado seed oil was potassium (9.65 ± 1.13 mg/ 100 g), followed by magnesium (2.50 ± 0.11 mg /100 g) and the least was copper (0.06 ± 0.11 mg/ 100 g). Others were calcium (1.12 ± 0.03 mg /100 g), sodium (1.77 ± 0.05 mg /100 g), iron (0.09 ± 0.05 mg /100 g), zinc (0.13 ±0.10 mg /100 g) and phosphorus (0.62 ±0.04 mg /100 g). Meanwhile, cadmium and lead were not detected in the oil sample. The results also show high potassium to sodium ratio; suggesting the beneficial effect of the oil in preventing or ameliorating cardiovascular issues such as hypertension and arteriosclerosis (Saupi *et al*., 2015). In our previous study, we have reported the presence of these important minerals in the seed oils of other fruits such as watermelon and pawpaw (Adaramola and Onigbinde, 2016). In a previous study by Atasie *et al*. (2009), potassium has been reported as the most prominent mineral in groundnut seed oil as well as high potassium to sodium ratio. Reports have shown that the minerals present in the avocado pear seed oil possess therapeutic properties required for proper growth, development and functioning of the body (Folashade *et al*., 2018). They are crucial in supporting many biochemical reactions including enzymatic reactions, transportation of gases, muscle contraction, transmission of nerve impulses and utilization of nutrients from foods (Prashith *et al*., 2014). The presence of minerals such as calcium, magnesium and phosphorus in the oil indicates that the consumption of avocado seed oil will support bone formation and bone health. Also, the calcium to phosphorus ratio obtained in this study is greater than one; indicating that the oil is a good source of calcium, since a Ca:P less than 0.5 is indicative of poor calcium source (Audu *et al*., 2019). This is attributed to the fact that phosphorus-rich diets may enhance calcium loss through the urine (Shills and Young, 1988). The results of this study suggest that oil from avocado seed could serve as a cheap source of important minerals such as potassium, magnesium, sodium, calcium, and phosphorus.

Table 2 Mineral composition of avocado pear seed oil

S/N	Mineral	Concentration mg/100 g
1.	Calcium	1.12 ± 0.03
2.	Magnesium	2.50 ± 0.11
3.	Potassium	13.09 ± 0.22
4.	Sodium	1.77 ± 0.05
5.	Iron	0.09 ± 0.05
6.	Copper	0.06 ± 0.01
7.	Zinc	0.13 ± 0.10
8.	Phosphorus	0.62 ± 0.04
9.	Cadmium	0.00 ± 0.00
10.	Lead	0.00 ± 0.00

CONCLUSION

This study revealed that avocado seed oil is packed with beneficial bioactive principles and essential minerals, making it a viable nutritional and pharmacological option. The presence of biologically active principles such as 10-Undecyn-1-ol, 7-Hexadecenoic acid methyl ester, 14-methyl Pentadecanoic acid methyl ester, Linolelaidic acid methyl ester, 9-Octadecenoic acid methyl ester, and Eicosanoic acid methyl ester, in addition to essential minerals like potassium, magnesium, sodium, and zinc, suggests that this oil can be an important factor in maintaining good health and preventing/solving various health issues. The findings of this study also indicate that avocado seed oil is a highly nutritious and functional ingredient; suggesting that the oil holds significant promise as a raw material in various applications, including animal feed, cosmetics, and pharmaceutical products.

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