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Isolation, Purification and Characterization of Herbal-Based Ligands Extracted from Avocado (*Persea americana*) Leaves in Jos North Local Government Area of Plateau State, Nigeria

Musa S.[1]; Lohdip Y. N.[2]; Kagoro M. L. P.[3]

[1,2,3] Department of chemistry University of Jos, Nigeria
Corresponding Author: Musa S.*

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Abstract: This research evaluates the bioactive compounds in Avocado, aiming to utilize them as a potential herbal source for developing metal complexes. *Persea americana* is a tropical plant commonly found in the southeastern part of Nigeria. The bulk of the fruit is utilized as a food product. The fruits, leaves, and seeds are utilized in traditional herbal medicine for treating diverse health issues. The plant material was obtained through solvent extraction with water. Phytochemicals were quantitatively assessed, FT-IR and UV-Vis spectroscopy were carried out following previously defined procedures. A phytochemical study on avocado leaf extract revealed the detection of flavonoids (2.09 ± 2.00 mg/L), saponins (8.54 ± 0.02 mg/L), steroids (0.67 ± 0.04 mg/L), alkaloids (4. 01 ± 1.60 mg/L), Phenoids (0.99 ± 0.08 mg/L), Terpenoids (0.76 ± 0.05 mg/L), carbohydrate (0.85 ± 0.08 mg/L) and glycosides (0.95±0.08 mg/L) while Anthraqunones were absent. The FT-IR results revealed the presence of C-O stretching vibrations for both alcohol and ester groups in both crude and isolated samples. The study's findings revealed that the avocado extract harbors notable bioactive compounds capable of functioning as herbal ligands in the formation of metal complexes.

Keywords: *Avocado, Phytochemicals, Herbal-Based Ligands, Metal Complex.*

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I. INTRODUCTION

The avocado pear (*Persea americana*) is packed with nutritional benefits, containing substantial quantities of vitamins, minerals, proteins, and fibers, along with generous amounts of unsaturated fatty acids, which are advantageous for health. Moreover, avocado pear seeds are rich in numerous bioactive phytochemicals, including phenolic acids, condensed tannins, and flavonoids, like procyanidins, flavonols, hydroxybenzoic, and hydroxycinnamic acids [1]. These bioactive compounds display a range of biological activities, such as antioxidant and anti-inflammatory properties. Phenolic compounds have notable anti-inflammatory properties because they can neutralize oxidative radicals, which is essential for preserving cellular and oxidative equilibrium [2]. For millennia, plants have been employed in traditional healing practices. These components can be obtained from different parts of the plant, including bark, leaves, flowers, roots, fruits, seeds, and peels. In other words, any part of the plant may possess bioactive compounds. Exploring the plant's chemical makeup is crucial as it facilitates the synthesis of intricate chemical substances [3]. Studies suggest that the leaves possess anti-inflammatory and pain-relieving properties [4]. The seeds of *Persea americana* are employed in diverse ethno-medicinal practices, with some studies suggesting their use in treating conditions like diarrhea, dysentery, toothache, intestinal parasites, skin issues, and even for beauty treatments. [5]. Previous studies in Nigeria reveal that avocado tree leaves (*Persea americana*) possess a range of pharmacological properties, including cough reduction (anti-tussive), seizure prevention (anti-convulsant), blood vessel relaxation (vasorelaxant), pain relief (analgesic), inflammation inhibition (anti-inflammatory), blood sugar control (antidiabetic), blood sugar reduction (hypoglycaemic), blood pressure reduction

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(hypotensive), and high blood pressure management (antihypertensive). [6]. The current research underscores the use of qualitative, quantitative, and spectroscopic techniques to identify, isolate, purify, and measure the bioactive compounds within avocado leaf extracts, potentially as herbal ligands.

II. MATERIALS AND METHODS

A. Collection and Extraction of Plant Material

Avocado leaves, belonging to the *Persea americana* species, were collected from Jos North Local Government Area in Plateau State. The leaves were carefully washed with tap water and then cut into smaller portions. Leaves that were allowed to dry in the shade and free from moisture for a duration of seven days. The sample was ground into a fine powder using a mortar and pestle. The procedure was carried out using the cold maceration method. A 100g portion of the powdered plant was submerged in 500ml of distilled water contained within a conical flask. The substance was periodically agitated for 72 hours under normal environmental conditions. The sample was processed through a filtration system using Whatman No. Certainly. A filter paper and the filtrate were concentrated by heating the mixture in a water bath at temperatures from 60 to 650 degrees Celsius to remove excess solvent. The substance was termed a crude extract for use in subsequent research endeavors.

B. Qualitative Phytochemical screening of Avocado Leave Extract

- *Test for Tannins*

0.1 g of extract was stirred with 10 ml of distilled water and filtered; a few drops of 1% ferric chloride solution were added to 2 ml of each filtrate. The observation of a blue-black hue indicated the presence of tannins.

- *Test for Alkaloids*

0.1 g of the extract was dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagent, formation of red precipitate, which signified the presence of alkaloids.

- *Test for Saponins*

0.1 g of the extract was boiled with 5 ml of distilled water and filtered. To each filtrate, about 3 ml of distilled water was added and vigorously mixed for about 5 minutes. The frothing that persisted after heating was seen as evidence of the presence of saponins.

- *Test for Glycosides*

0.1 g of the extract was mixed with 30 milliliters of distilled water and heated on a water bath for five minutes. To each filtrate, introduce 5 milliliters of the solution. A mixture of two milliliters of Fehling's solution A and B was prepared by combining them until the solution reached a basic state. The remedies were placed in a water bath for 7 minutes. A reddishbrown material, referred to as a precipitate, indicated the presence of a glycoside.

- *Test for Terpenoids*

0.1 g was dissolved in ethanol. Acetic anhydride 1 ml was added, followed by the addition of concentrated H_2SO_4. A transition from pink to violet suggested the presence of terpenoids.

- *Test for Flavonoids*

0.1 g was dissolved in water and filtered. For every filtrate, 3 milliliters of lead ethanoate solution were added to 5 milliliters of each. The sight of a light-brown solid hinted at the presence of flavonoids.

- *Test for Steroids*

To 0.1 g of extract, 2 ml of acetic acid was added. The solution was efficiently cooled with ice, and subsequently, concentrated acid was incorporated. Exercise caution when dealing with tetraoxosulphate (vi) (H_2SO_4). The change from violet to blue or bluish green hues indicates the presence of a steroid ring.

- *Test for Phenols*

0.1 g extract was boiled with distilled water and then filtered. A small amount of a 10% ferric chloride solution was subsequently added to the filtrate. A shade of green-blue or violet indicated the presence of a phenolic hydroxyl group.

C. Quantitative Phytochemical Concentration of Avocado Leave Extract

The research project measured the chemical compounds in *Persea americana* leaves through gas chromatography, utilizing the BUCK M910 system with a flame ionization detector. The RESTEK 15-meter MXT-1 column is 15 meters long, having a diameter of 250 micrometers and a wall thickness of 0. A measurement of 15 micrometers was utilized. The injector temperature climbed to 280 °C, utilizing a splitless injection of 2 µl of the sample, with a linear velocity of 30 cms−1 and helium as the carrier gas at a flow rate of 5. A sample is dispensed at a rate of 40 milliliters per minute. The oven increased from 200 °C to 330 °C at a rate of 3 °C per minute. A temperature reduction of 0 degrees Celsius per minute is being measured. The temperature was maintained for five minutes, and the detector operated at a temperature of 320°C. Phytocompounds were quantified by calculating the ratio of the area under the peak of the internal standard to its mass, then comparing it to the area under the peaks of the identified phytocompounds.

D. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FT-IR analysis was performed using a Cary 630 FTIR instrument, which is manufactured by Agilent Technologies, Inc., and is housed in a postgraduate research laboratory at the University of Jos, Nigeria. Samples of 0. A quantity of five grams of fresh pulp was placed on a diamond crystal of the

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FTIR system, and the infrared spectra were captured within the 4000-650 cm−1 range at a resolution of 4 cm−1, with each sample being scanned four times.

E. UV Spectroscopy of Pear Avocado Leave Extract

In this research, the UV-Vis spectrum was captured using a Thermo Scientific GENESYSTEM 8 spectrophotometer from England.

III. RESULTS

Table 1. Study of Phytochemical Levels in Avocado Leaf Extract.

S/No.	Composition	Qualitative	Quantitative of Constituents (mg/L)
1	Alkaloid	+	4. 01 ± 1.60
2	Anthraqunones	-	ND
3	Flavonoid	+	2.09 ± 2.00
4	Phenoids	+	0.99 ± 0.08
5	Saponins	++	8.54 ± 0.02
6	Steroid	+	0.67 ± 0.04
7	Terpenoid	+	0.76 ± 0.05
8	glycoside	+	0.95 ± 0.08
9	Carbohydrate	+	0.85 ± 0.08

Key: - = Not Detected, + = Detected

Table 2. Analysis of the Avocado Leaf Extract Using Fourier-Transform Infrared Spectroscopy

Class of Compounds	Wave Number (cm^{-1})	Intensity	Assignment
Aromatics /Alkenes	779.0	Medium	C-H bending
Alcohol/Esters	1019.4	Strong	C-O stretching
Alcohol/Ethers	1155.5	Strong	C-O stretching
Methyl/Methylene	1317.6	Weak-medium	C-H bending
Amines	1407.1	Medium	C-H bending
Aromatics rings/Alkenes	1545.0	Medium	C-N stretching
Aromatics	1604.6	Strong	C=C stretching or N-H bending
Alkenes	2849.5	Medium-strong	C-H stretching

Table 3. FT-IR Analysis of Individual Phytochemicals Extracted from Avocado Leaves

Class of Compounds	Wave Number (cm^{-1})	Intensity	Assignment
Alcohol/Alkenes	2920	Strong	C-H Stretching
Esters/Ketones	1740	Strong	C=H Carbonyl Stretching
Alkenes	1460	Medium	$CH2/CH_3$ Bending Vibration
Esters/ether	1250	Weak	C-O single bond stretching
Alkenes	1120	Weak	Long-chain alkenes

Table 4. Evaluation of Avocado Leave Extract Through Ultraviolet Spectroscopy Analysis

Compound	Wavelength (nm)	Absorbance (A)	Assignment
Polyphenols	280 – 320	3.441	Strong
Flavonoids	240 – 280	3.440	Strong
	280 – 300	3.442	

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IV. DISCUSSION

The phytochemical study of avocado leaf extract (Table 1) indicated the detection of flavonoids (2.09 ± 2.00 mg/L), saponins (8.54 ± 0.02 mg/L), steroids (0.67 ± 0.04 mg/L), alkaloids (4. 01 ± 1.60 mg/L), Phenoids (0.99 ± 0.08 mg/L), Terpenoids (0.76 ± 0.05 mg/L), carbohydrate (0.85 ± 0.08 mg/L) and glycosides (0.95±0.08 mg/L) were found, yet anthraqunones were absent. These phytochemicals exhibit varied pharmacological and biochemical actions when ingested by animals. Plants used in medical treatments are thought to contain bioactive compounds that have biological effects. These compounds often give plants unique smells, flavors, and colors, while some also possess culinary, medicinal, or toxic properties. Studies suggest that flavonoids and phenolics function as potent antioxidants, neutralizing free radicals and safeguarding cells from oxidative damage. [7]. Saponins have the capacity to induce the clumping and aggregation of red blood cells [8]. Cardiac glycosides are a vital class of naturally occurring drugs that significantly contribute to the treatment of congestive heart failure [9].

Table 2 demonstrates the fundamental structural elements of avocado leaves as analyzed through FT-IR spectroscopy. The functional groups in both the crude and isolated samples were identified by comparing the vibrational frequencies in wave numbers from the spectrograph of the sample obtained from an FT-IR spectrophotometer with those from an IR correlation chart. A prominent absorption peak at 2849 cm−1 is detected in the FT-IR spectrum of the crude extract, the absorption band 2849.50 cm^{-1} medium (m), indicated the C-H stretch vibration in of alkenes, while that at 1604.60 cm^{-1} (s), result from N-H bending of aromatics, the frequencies at 1545.0 cm^{-1} (m) and 1407.10 cm^{-1} medium (m) indicate C-N stretching and C-H bending for aromatic rings and amines respectively. The absorption band at 1155.50 cm^{-1} (s) showed the presence of C-O stretching of alcohol, the absorbance at 1019.40 cm^{-1} (s) showed the presence of C-O stretch of esters. Absorption band that appeared at 779.0 cm^{-1} (m) indicated C-H bending of aromatic. Despite being separated, the sample displayed the following absorption peaks: 2920.0 cm^{-1} (s) and 1740 cm^{-1} (s) corresponding to C-H stretching and C=O carbonyl stretching of alcohol and esters. 1460.0 cm^{-1} (m) indicated CH_2/CH_3 bending vibration of alkenes. The frequencies at 1250.0 cm^{-1} (w) showed the presence of C-O single bond stretching of esters while that at 1120.0 cm^{-1} (w) indicated long-chain alkenes. The appearance of peak at 2849.50-1545.0 cm^{-1} confirms the presence of polyphenolics/flavonoids.

Table 4 demonstrates the UV-Visible absorbance of avocado leaf extract, showing that all these compounds exhibit peak absorption around 240-280 nm, due to the presence of saponins, alkaloids, tannins, reducing sugars, flavonoids, and polyphenolic compounds (catechins, hydroxyl benzoic acids, and hydroxyl cinnamic acids) respectively.

V. CONCLUSION

The study indicated that the avocado leave extract harbors potent bioactive compounds (herbal-based ligands) that may be employed to create metal complexes with potential biological efficacy for combating microbial infections.

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