



# NUTRITIONAL COMPOSITION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS OF METHANOL LEAF EXTRACT OF Terminalia ivorensis

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# ABSTRACT

Terminalia ivorensis (TI) is used in West African ethnomedicine for the treatment of ulcers, malaria, inflammation, and wounds. Despite its widespread use, its nutritional status remains largely undetermined. This study was undertaken with the aim to evalute the nutritional and phytochemical composition of TI. Proximate analysis of the methanol leaf extracts of TI was carried out using the AOAC methods. Elemental analysis of the methanol leaf extracts of the plant was also carried out using an Atomic Absorption Spectrometer (AAS). Identification and quantification of the bioactive components were determined by Gas Chromatography-Mass Spectrometry (GC-MS) single-phase ion mode. Spectra from detected compounds were matched with known compounds of the National Institute of Standards and Technology (NIST). Proximate analysis reveals that it is rich in primary nutrients such as carbohydrates (37.70±0.05%), proteins (10.94±0.03%), fats (7.97±0.12%), fibre (0.95±0.01%), ash (1.42±0.01%) and moisture content (41.02±0.21%). Mineral analysis of the extract revealed the presence of essential minerals such as calcium (641.2±1.20 mg/100g), copper (6.28±0.02 mg/100g), magnesium (186.16±0.29 mg/100g), iron (17.13±0.06 mg/100g), manganese  $(3.47\pm0.01 \text{ mg/100g})$ , and zinc  $(3.82\pm0.01 \text{ mg/100g})$ , which are essential for the metabolic processes and pregnancy. Forty-Seven (47) phytochemical compounds were detected by GC-MS including 1, 2, 3- Benzenetriol (26.31%), Diethyl Phthalate (14.93%), D-Allose (6.96%), Anhydro-.alpha-dgalactofuranose (5.09), Glycerin (6.28%) as the most predominant. The study revealed that Terminalia ivorensis could be a useful source of nutrients, minerals, and several helpful bioactive compounds that may serves as potential drug target needed for drug development.

Keywords: Terminalia ivorensis, Nutritional, Phytochemical, Gas Chromatography, Proximate

# INTRODUCTION

Phytomedicine, also known as herbal medicine or botanical medicine or phytotherapy, is the use of plant-based substances for therapeutic and healing purposes (Mohd, 2019). This practice has ancient origins and has been an integral part of human healing traditions across cultures for thousands of years. The use of plants for medicinal purposes dates to prehistoric times. Archaeological evidence suggests that early humans relied on plants for food and discovered their healing properties through trial and error. Ancient civilizations, including the Sumerians, Egyptians, and Chinese, developed extensive herbal knowledge, recording their findings on clay tablets and scrolls. The earliest known herbal texts, such as the Sumerian "Pen Ts'ao" and the Egyptian "Ebers Papyrus," provide insights into the plants used for healing ailments. and a variety of ailment such as malaria, asthma, diabetes, diarrhoea, fever, hypertension, and even constipation have been reported to be treated using various herbal plants, which are usually administered as poultices (a mass of soft moist herbs, applied to the body to treat sores, aches or inflammation), concoctions of various plant, infusions of teas and tinctures (a medicine made by dissolving a drug in alcohol) or as part of ingredients in porridge and soups (Doughari, 2012). Terminalia ivorensis is a large tree known as Ivory Coast almonds that is mainly found on the coasts of West Africa). Studies have shown that this plant is known to possess antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory, wound healing, anti-psychotic, trypanocidal activities (Ben-Azu et al., 2016, Akinyemi et al., 2006, Agbedahunsi et al., 2006, Claudine et al., 2017, Kipré et al., 2023, Annan et al., 2012). Terminalia ivorensis is reported to be useful in the treatment of ulcers, cuts, sores, wounds, general body pains, hemorrhoids, diuresis, malaria

and yellow fever (Burkill, 1985, Ouattara *et al.*, 2013). Despite its widespread use, the phytochemical profile and bioactive components of TI remain largely undetermined. Although the composition of the nutritional component varies in plants, a study has shown that plants have the right quantity to meet human nutritive requirements (Suroowan, *et al.*, 2019). This study investigated the nutritional composition and elemental composition of the methanol leaf extract of *Terminalia ivorensis*.

# MATERIALS AND METHODS Collection of plant materials

The fresh leaves of *Terminalia ivorensis* were pruned from the trees of *Terminalia ivorensis* at the open field of Department of Pharmacy, University of Benin, Benin City, Edo state, Nigeria. The leaves of *Terminalia Ivorensis* were identified by Professor Akinibosun of the Botany Department, Faculty of Life Sciences, University of Benin, Benin-city, Edo state, Nigeria, with the voucher number, UBH-T2161.

## **Preparation of the Extracts**

Freshly collected leaves of *Terminalia Ivorensis* were dried under the sun for 14 days and milled with a Bench-top milling machine in the Pharmacognosy Department, Faculty of Pharmacy, University of Benin into fine powder (1100 g) and soaked in 15 L of methanol and stirred for 3 days. The methanol solution was filtered thrice using several folded clean pieces of cheesecloth during each filtration process to remove debris. The mixture was concentrated using a rotary evaporator set at  $60^{\circ}$ C and Freeze-dried to a constant weight with a freeze drier. The dried extract gave a yield of 31.14% (w/w) and was stored in an air-tight container in a refrigerator at about 4°C until when needed.

### **Proximate Analysis**

Proximate analysis was carried out on the methanol extract of the leaves of *Terminalia Ivorensis* to ascertain its nutritional composition. Analysis of the sample was carried out using the method of association of official analytical chemists, (AOAC, 1990).

## Ash Content

When organic matter is burnt in a furnace for three hours at a temperature of  $600^{\circ}$ C, the amount of inorganic residue left over is measured as the sample's ash content. The sample's ash content was determined using the AOAC (1990) method, which involved loading 2g of the sample into an empty, preweighed crucible (w<sub>0</sub>), which was then heated in a muffle furnace set to 600°C for three hours. The ash was then allowed to cool in a desiccator before being reweighed (w<sub>2</sub>). By comparing the weight of the sample before and after ashing, it was possible to determine the weight of the ash.

Percentage ash was obtained by  $\%Ash = \frac{(W_1 - W_2) \times 100}{(W_1 - W_0)}$ 

Where:  $w_0$ =Weight of the empty crucible, g  $w_1$ = Weight of crucible + sample, g  $w_2$ = Weight of crucible + ashed sample, g

## Crude fibre determination

This was done following the AOAC (1990) protocol. A 1 liter conical flask (w<sub>0</sub>) was filled with 2 g of the samples. The flask was filled with 200 ml of 1.25% H2SO4 acid, which was then allowed to boil for 30 minutes while being constantly monitored by cooling fingers and then filtered using a poplin cloth. Using a spatula, the residue was transferred back to the flask, and 200 ml of 1.25% NaOH (produced by dissolving 100g of NaOH pellets in 1 liter of distilled water) was added. The mixture was then allowed to boil for an additional 30 minutes while being constantly agitated with cooling fingers. A poplin cloth was used to filter the material, and the residue was properly cleaned with hot distilled water before being thoroughly rinsed twice with industrial methylated spirits, acetone, or ethanol. Rinse three times with petroleum ether (BP 40-60 °C) to finish. Heat dry overnight at 105°C in the oven, allow it to drain completely and scrape the residue into a crucible. Once removed, it was chilled in a desiccator. In a muffle furnace, the weighed sample (w1) was ashed for 90 minutes at 550 °C. In the end, it was dried in a desiccator and weighed once more (w<sub>2</sub>).

The percentage crude fibre was calculated thus; Where:

%of crude fibre = 
$$\frac{(W_1 - W_2) \times 100}{W_0}$$
  
W<sub>0</sub> = Weight of sample, g  
W<sub>1</sub> = Weight of dried sample, g

 $W_1 =$  weight of ash sample, g

### Crude protein determination

For the purpose to determining crude protein, the AOAC (1997) employed was a modified version of the micro-Kjeldahl method.

A small amount of anti-bumping granules was added to a micro-Kjeldahl digestion flask along with three grams of each of the defatted samples that were individually weighed on preweighed scales. Each flask received two grams of the catalyst solution (CuSO4: Na2SO4: SeO2, 5:1:02 w/w), along with 10 ml of nitrogen-free concentrated H2SO4. The flasks were set up on a heating mantle in a fume cupboard at an angle. After the foaming stopped, the temperature was raised to 50°C for another 30 minutes, then to full heating (100°C), where it remained until a clear solution was achieved. In order to achieve thorough digestion and nitrogen conversion to ammonium sulphate, simmering was maintained below boiling point for an additional 30 minutes. Samples were transferred quantitatively into 100 mL volumetric flasks after washing and cooling to room temperature once digestion was finished. With distilled water, volumes were adjusted to the proper levels.

A 10 ml pipette was used to transfer 10 ml of the digest filtrate into a 25 ml standard flask. Alkaline phenate (2 ml) was added, and the mixture was agitated to ensure good mixing. After properly shaking, 2ml of sodium hypochlorite was added, followed by 6ml of sodium potassium tartrate. With the use of a UV/visible spectrophotometer, the solution's absorbance at 630 nm was measured after being diluted with distilled water to the 25 ml mark. The sample and the nitrogen standards were handled in the same manner. *calculation* 

%N

= Instrument Reading × Slope Reciprocal × Color Vol.× Digest Vol. Weight of Sample × Aliquot Taken × 10000

% Crude Protein = % Nitrogen×6.25

## **Crude Fat**

AOAC (1973)' s soxhlet extraction method was used to estimate the crude lipid. Prior. Befor weighing the empty 250 ml extraction flask, dry it in the oven at 110°C, then let it cool in the desiccator. A porous thimble with a label was filled with cotton wool, and the folded filter paper (w<sub>0</sub>) containing 2 grams of the sample was placed inside. In the dry 250-ml extraction flask, add about 200 ml of petroleum ether. Construct the apparatus by inserting the cover porous thimble into the condenser. 5-6 hours should be used for the extraction. Carefully remove the porous thimble, then retrieve the petroleum ether and store it in the top container (tube) for future use. Drying takes place in ovens at 1050°F-1100°F for a period of one hour. Take the weight after cooling in the desiccator. When the petroleum ether is nearly completely gone, remove the extraction flask from the water bath. Calculation:

Weight of empty porous thimble  $=W_0$ Weight of empty thimble + ground sample  $=W_1$ Weight of ground sample  $=W_1$ -  $W_0$ Weight of empty extraction flask  $=W_2$ Weight of empty extraction flask + ether  $=W_3$ Weight of ether (fat or oil)  $=W_3 - W_2$ 

weight of ether (lat of oil) =  $W_3 - W_2$  $W_3 - W_2$ 

$$\%Fat = \frac{W_3 W_2}{W_1 - W_0 \times 100}$$

### Moisture content

This represents the amount of moisture that has evaporated after drying at  $105^{\circ}$ C in an oven. A pre-weighed (w<sub>0</sub>) crucible (w<sub>1</sub>) was filled with one (1) gram of the sample. The crucible containing the weighed sample was kept in a  $105^{\circ}$ C oven for three hours. After being taken out of the oven, the crucible was placed in a desiccator where it was permitted to cool. The weight was taken after cooling. Repeating the drying, cooling, and weighing steps led to the achievement of a consistent weight (w<sub>2</sub>). The percentage of moisture was obtained by the equation;

%moisture = 
$$\frac{(W_1 - W_2) \times 100}{(W_1 - W_0)}$$

w<sub>0</sub>= Weight of the empty crucible, g

 $w_1$  = weight of sample + empty beaker, g

W<sub>2</sub> =weight of dried sample + empty beaker, g

# Determination of soluble carbohydrate (nitrogen-free extractive)

# Test method no.: qal/am/po6

The nitrogen free extractive (N.F.E.), also known as soluble carbohydrate, is obtained as a difference between crude protein and the total of ash, protein, crude fat, and crude fiber rather than being directly assessed.

N.F.E. 100-(%Ash +% crude fibre + % crude fat + crude protein)

N.F.E. is a general term that refers to all starches and sugars, a small amount of hemicellulose, and various amounts of lignin. It does not refer to any specific compound or collection of substances.

### **Elemental analysis**

Nitric-Perchloric acid digestion via the Atomic Absorption Spectroscopy (AAS) technique was used to analyze specific trace minerals. About 50 mg of dried methanol leaf extract of *Terminalia ivorensis* was weighed into a 250 ml conical flask with 5 ml of Nitric-Perchloric acid mixture and allowed to stand for 24 hours. After digestion of the dried extract, known elemental and blank samples were also prepared. The digested samples and blank were run on the AAS to obtain the absorbance values. Concentrations of the elements in the samples were calculated from the standard.

# **GC-MS** Analysis

About 100 g of the dried methanol leaf extract of *Terminalia ivorensis* was dissolved in 100 mL distilled water, further diluted with water 1:1 and filtered through a 0.22  $\mu$ m PVDF filter. GC-MS analysis of *Terminalia ivorensis* was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30 mm x 0.25 mm 1D x 1  $\mu$ Mdf, composed of 100%

Dimethylpolysiloxane) For GC-MS detection, an electron ionization system with ionizing energy in a single-phase ion mode of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min and an injection volume of 0.50 ml was employed (split ratio of 10:1); Injector temperature  $250^{\circ}$ C; Ion-source temperature  $280^{\circ}$ C. The oven temperature was programmed from  $110^{\circ}$ C (isothermal for 2 min.), with an increase of  $10^{\circ}$ C/min, to  $200^{\circ}$ C, then  $5^{\circ}$ C/min to  $280^{\circ}$ C, ending with a 9 min isothermal at  $280^{\circ}$ C. Mass spectra were taken at 70 eV; a scan-interval of 0.5 seconds and fragments from 45 to 450 Da. The total GC running time was 27 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was a Turbo mass.

#### Identification of components

Interpretation of mass spectrum from GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) and has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known and authentic samples stored in the NIST library. Computer searches in an HP Mass Spectral Library NIST98 were also applied. The name, molecular weight, and structure of the components of the test materials were ascertained.

# **RESULTS AND DISCUSSION**

The methanol extract of the leaves of *Terminalia ivorensis* was analyzed using proximate analysis for their nutritional contents, and the results obtained are presented in Table 1.0. Analysis of the samples showed that moisture content was high (41.02%), second to carbohydrates (36.70%), and the least was crude fibre (0.95%). Protein, crude fat, and ash content were also obtained.

## Table 1: Proximate analysis of methanol extract of Terminalia Ivorensis leaves

	Table 1. I toximate analysis of methanor extract of Terminana Tvorensis reaves					
ParametersMC (%)PROTEINCRUDE FATCRUDEAC (%)	NFE (%)					
(units) (%) (%) FIBRE (%)						
Plant extract 41.02±0.21 10.94±0.03 7.97±0.12 0.95±0.01 1.42±0.01	37.70±0.05					

Results are expressed as means  $\pm$  SEM of triplicate replication.

MC- Moisture Content, AC- Ash Content, NFE- Nitrogen Free Extract

*T. ivorensis* (coastal almonds) are rich in all primary nutrients. The percentage of proteins (10.3%) is high bearing in mind that this is a leaf extract (meat and fish possess between 15 and 20 g of proteins for each 100 g). crude fat of 7.9% with

less amount of carbohydrate chemically bound to other molecules in the form of NFE (37.70), MC gave the highest amount of 41 % which suggests a very poor shelf life of the leaf if not properly stored.

	Fable 2: Mineral anal	ysis of methanol leaf	f extract of <i>Terminalia</i>	ivorensis
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Parameters	Calcium (Ca)	Cupper (Cu)	Magnesium	Iron (Fe)	Manganese	Zinc (Zn)
			(Mg)		(Mn)	
Concentrations	641.2±1.20	6.28±0.02	186.16±0.29	17.13±0.06	3.47±0.01	3.82±0.01
Mg/100g						

Results are expressed as means  $\pm$  SEM of triplicate replication

Mineral analysis of T. *ivorensis* (coastal almonds) showed almonds are among the richest plant-based foods in calcium. They also contain significant amount of magnesium, potassium iron and zinc. The calcium content of T. *ivorensis* 

(641 mg/100) is much greater than milk, although the amounts of extract taken is usually less than that of milk or dairy products.



Each peak represents a distinct compound. The area covered by each peak correlates with the intensities of ions at specific mass to charge m/z values which corresponds to the relative abundance (concentration) of that compound. The higher the ion intensities the larger the peak area, which in turn

contributes to a higher area percentage. The identified compound corresponding to each peak with its area % value is presented in Table 4. The total analysis time per injection on the GC-MS system as shown in the chromatogram was 27 minutes.



Compound Name;	Retention time(min)	Area (%)	Chemical structure	Molecular weight(g/mol)	Compound type
Cyclohexanol	8.675	0.49	OH OH	154	Terpene
Citronellol	8.931	0.31		156	Monoterpene alcohol
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-;	9.197	0.33		154	Terpene Alcohol
1,2,3-Benzenetriol;	10.365	26.31	HO OH OH	126	Phenol
Propan-2-one;	11.245	0.68		190	Ketone
D-Allose	11.447	6.96		180	Aldose
Tetradec-11-en-1-ol acetate	11.642	0.72	OH "	268	Oxirane ring
3,3-Dimethyl-4- phenyl-4-penten-2- one	12.080	0.45		188	Ketone
Lilial	12.249	0.90	H <sub>3</sub> C CH <sub>3</sub> H <sub>2</sub> C CH <sub>3</sub> H <sub>3</sub> C CH <sub>3</sub> H CH <sub>3</sub> H	204	Aldehyde



7,9-Di-tert-butyl-1- oxaspiro	16.330	0.27		276	Oxaspiro compound
Dibutyl phthalate	16.659	0.20		278	Esters
n-Hexadecanoic acid	16.788	1.38	Y	256	Monocarboxylate
Phytol;	18.113	0.85	**	296	Monocarboxylate
9,12,15- Octadecatrienoic acid	18.178	0.79		278	Monocarboxylate
Octadecanoic acid;	18.386	0.37		284	Monocarboxylate
Phenol;	6.120	5.75	OH	94	Benzenol
Glycerin;	6.248	5.75	ОН ОН ОН	92	Trihydric alcohol

Table 4: Reported biological activity of compounds present in the methanol extracts of Terminalia ivorensis

Compound Name;	Biological Activity	Reference(s)
Cyclohexanol	Aromatic Odour;	(Salakhutdinov et al., 2017).
	Antimicrobial Properties;	
	Anti-Inflammatory Effects;	
	Analgesic (Pain-Relieving) Effects	
Citronellol;	Antimicrobial Properties;	(Salakhutdinov et al., 2017).
	Anti-Inflammatory Effects;	
	Analgesic (Pain-Relieving) Effects;	
	Antioxidant Activity;	
	Sedative and Anxiolytic Effects;	
2,6-Octadien-1-ol	Aromatic Odour;	
	Antioxidant Properties;	
	Anti-Inflammatory Effects;	
	Sedative and Anxiolytic Effects;	
	Anti-Infective Properties	
1,2,3-Benzenetriol	Cosmetic and Personal Care Products;	(Ozturk, 2015).
	Antioxidant Properties;	
	Anti-Inflammatory Effects;	
	Tyrosinase Inhibition;	
	Acetylcholinesterase inhibition.	
Propan-2-one;	Antimicrobial; anti-inflammatory activity.	(Zhang et al., 2015).

D-Allose	Antioxidant Activity;	(Shintani et al., 2020).
	Anti-Inflammatory Effects;	
	Anticancer Potential;	
	Diabetes Management	
Tetradec-11-en-1-ol	Antibacterial activity	
acetate		
3,3-Dimethyl-4-	Antioxidant Activity;	Gambhire, et al 2010).
phenyl-4-penten-2-	Anti-Inflammatory Effects	
one		
	Potential allergen	1.0010
Benzene	Antimicrobial Properties;	Alves et al 2012
	Antioxidant Activity;	
	Anti-Inflammatory Effects;	
	Analgesic (Pain-Relieving) Effects.	
Galactoluranose;	Antifungal Properties;	(Nurzynska-wierdak et al., 2014)
	Coll Surface Clycon	2014).
Cuelebevenene		
Disther Dist		$(1: \alpha = 1, 2021)$
Dietnyi Phinalate	Anumicrobial Activity	$(L1 \ et \ al., 2021).$
		(Auan <i>et al.</i> , 2006). (Yu at $al = 2010$ )
5 Isopropopul 2	Antimicrobial activity	$\frac{(1 \text{ u et al., 2010})}{(Chung et al., 2011)}$
5-1sopropenyi-2- mothyleyelopont 1	Anumerobiai activity	(Chung <i>et al.</i> , 2011).
anacarboyaldahyda		
1-Isobutyl-7	Antimicrobial activity	(Gupta $et al = 2017$ )
2-Cyclobeven-1-one	No activity	(Gupta et ul., 2017).
2-Cyclohexen-1-one	No activity	
2-Cyclonexen-1-one	No activity	
Cycloheyane	Anti-microhial activity	(Gunta et al. 2017)
propionic acid		(Gupta et al., 2017).
7.9-Di-tert-butyl-1-	Irritant	(Gupta <i>et al.</i> , 2017).
oxaspiro		(
Dibutyl phthalate;	Endocrine disruption	(Bhakta et al., 2009).
n-Hexadecanoic acid	n-Hexadecanoic acid serves as a major energy storage	(Biermann <i>et al.</i> , 2000).
	molecule, a crucial metabolite for the synthesis of	(Aparna <i>et al.</i> , 2012).
	complex lipids, and contributes to the structure and	
	stability of cell membranes. It also has anti-inflammatory	
	activity.	
Phytol;	Phytol shows anti-inflammatory properties, antioxidant	(Muhammad et al., 2018).
	activity, potential anti-cancer activity, and anti-microbial	
	activity against various microorganisms. It serves as a	
	metabolite in the human gut, produced through the action	
	of gut bacteria, with potential health impacts.	
9,12,15-	9,12,15-Octadecatrienoic has diverse biological activities	(Parvathi <i>et al.</i> , 2022).
Octadecatrienoic	that encompass anti-inflammatory effects, cardiovascular	(Abubakar and
acid	health benefits, support for brain health, promotion of	Majinda, 2016).
	skin health, potential anti-cancer effects, and a role in	(Akpuaka <i>et al.</i> , 2013)
	weight management.	
Octadecanoic acid	Energy Storage;	(Keawsa-ard <i>et al.</i> , 2012).
	Dreaurson for Linid Synthesis	
	Precursor for Lipid Synthesis; Pole in Cellular Signaling	
Phenol	Antiovidant:	$(\hat{A} _{varez} = S_{uarez} at al = 2012)$
I IICHUI	Anaesthetics:	(Anvaicz-Suaicz el ul., 2012). (Sakat Juvekar & Comphire
	Skin irritant	(Sakai, Juvekai, & Gambille, 2010)
Glycerin	Moisturizing agent:	(Szél et al. 2015)
Gijterin	Wound healing:	(Biörklund <i>et al</i> $2013$ )
	Laxatives.	(2) 51 (1010 Cr with 2010)

The proximate analysis technique helps in understanding the fundamental characteristics and properties of a substance, which can be valuable in nutrition and energy utilization (Ilouno, *et. al.*, 2018). Protein content and amino acid composition of the diet affect animal growth by regulating body protein synthesis and mean body mass that primarily

composes organs, red muscle, and white muscle. Proteins are needed for the build-up of body tissues and amino acids are the building blocks of proteins. In comparison to *Piper guineensis* (Benin pepper or uziza pepper), whose protein content was reported to be 8.75%, and *Morinda lucida* (Brimstone tree), whose protein content was reported to be 2.28%), Terminalia ivorensis leaves had a protein content of 10.94±0.03% (Table 3.1). Despite being somewhat higher than those mentioned above, the protein content of Terminalia ivorensis leaves is still much lower than that of Moringa oleifera leaves (22.99%) (Asaolu et al., 2012), Ageratum conyzoides leaves (18.62%), and Anthocleista djalonensis leaves (17.28%) (Nduche et al., 2015). This suggests that the plant Terminalia ivorensis is a convenient source of a protein that can be used as protein supplement. According to Khan et al. (2013), a plant's ash content is a measurement of the minerals it contains. The methanol leaf extract of Terminalia ivorensis has a 1.42±0.01% ash content. From this, it can be deduced that there are few or a few minerals present. According to Ahmed and Birnin-Yauri (2008), fibre in human diets is known to facilitate the flow of waste through the colon, acting as a potent anti-constipating agent. For the methanol leaf extract, the value for the crude fibre content from this investigation was 0.95±0.01%. The value obtained suggests that the extract is poor in fibre. Food moisture content provides important information on food preservation and storage. High moisture content aids in sustaining the protoplasmic contents of cells, however because of their short shelf lives, botanicals are perishable and more prone to microbial deterioration during storage. This explains why the majority of traditional users, including those who store Terminalia ivorensis in dried form (Gbadamosi et al., 2011; Ibukunoluwa et al., 2015), do so. The methanol leaf extract's moisture content was found to be 41.02±0.21% (Table 1.0). This result suggests that the moisture content is high. The methanol leaf extract's proximate analysis revealed a 7.97±0.21% lipid content. According to Ibukunoluwa et al. (2015), lipids are crucial for giving the body its maximum amount of energy, assisting in the movement of fat-soluble vitamins, insulating and protecting internal tissue, and supporting critical cellular functions. Plants contain carbohydrates in the form of nitrogen-free extract. Carbohydrates are one of the most important components in many foods. Carbohydrates may be present as isolated molecules or they may be physically associated or chemically bound to other molecules. Individual molecules can be classified according to the number of monomers that they monosaccharides, oligosaccharides contain as or polysaccharides which maybe covalently linked by glycosidic bonds. Molecules in which the carbohydrates are covalently attached to proteins are known as glycoproteins, whereas those in which the carbohydrates are covalently attached to lipids are known as glycolipids Terminalia ivorensis leaf extract in methanol has a high value of 37.70±0.05%. This number is considerably less than that reported by Nduche et al. (2015) for the leaves of Anthocleista djalonensis, which was 54.89%. However, Ipomoea batatas leaves were reported to contain 75% carbohydrates (Khan et al., 2013). In contrast, 94% of Talinum triangulare is made up of carbohydrate. The above result indicates the plant is a reliable source of energy. The mineral composition of extract as shown in Table 2.0 show the plant is rich in some minerals. Calcium plays a crucial role in the development and stability of cell walls as well as the preservation of membrane permeability and structure. It plays an important role in hormonal and nervous coordination (Felhi et al., 2016). Calcium as well as phosphorus and magnesium in the blood must be balanced, therefore the closer a food composition comes to that balance, the better for the body (Adlercreutz et. al., 1993). Reduced root growth, death of terminal buds, and distorted leaves are the effects of calcium deficiency in plants (Felhi et al., 2016). Calcium is directly involved in each heartbeat and in controlling arterial pressure. The extract richness in calcium

in addition to detected phytocompound (D- Allose) may have a very beneficial effect on cardiovascular health. Studies have shown that lack of calcium causes nervousness, the extract being very rich in calcium can also achieve the function of the nervous system. Magnesium, which is present in significant concentrations in the extract, functions as a cofactor for enzymes that catalyse several metabolic pathways e.g. hexokinase. Iron is a key component of heme, which functions as a prosthetic group for cytochrome and hemoglobin. Some enzymes required for metabolic activities are also activated by iron.

Forty-seven (47) phytochemical compounds were detected by GC-MS single phase ion mode including 1, 2, 3- Benzenetriol (26.31%), Diethyl Phthalate (14.93%), D-Allose (6.96%), Anhydro-.alpha-d-galactofuranose (5.09), and Glycerin (6.28%) as the most predominant. According to Li et al. (2002) diethyl phthalate exhibits antibacterial and antioxidant activity. According to Ozturk (2015), 1, 2, 3-Benzenetriol have anti-inflammatory and antioxidant properties as well as the ability to inhibit tyrosinase and acetylcholinesterase. D-Allose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) (6.96%) exhibits antioxidant, antiinflammatory and anti-cancer activities, with potential for managing diabetes (Shintani et al., 2020; Khadim et al., 2016). N-hexanedecanoic acid (1.38%), often referred to as palmitic acid, is a key energy storage molecule that is also essential for the creation of complex lipids and helps keep cell membranes stable. It also has anti-inflammatory properties (Aparna et al., 2012; Biermann et al., 2000). Phytol demonstrates anti-inflammatory, antioxidant, possible anticancer, and antimicrobial activities against a variety of bacteria. It functions as a metabolite in the human gut and is produced by gut bacteria, which may have its own impact in gut health (Muhammad et al., 2018). According to Nurzynska-Wierdak et al. (2014), 1, 6-Anhydro-. alpha-dgalactofuranose (C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>) (5.09%) functions as a cell surface glycan, has antifungal characteristics, and is employed as an antigenic determinant. Octadecanoic acid (0.37%) functions as an energy storage molecule, a molecule that enhances the structure of cell membranes, and a precursor for lipids synthesis, and antiviral activity and it has a role in cellular signalling (Keawsa-ard et al., 2012). Medicinal plants are known to play a very important role in diseased conditions by exhibiting anti-oxidative properties. The results obtained from this study is in agreement with the report by Enadeghe et al. 2022 that Terminalia catapppa possesses bioactives with antioxidant, antiviral, antimicrobial and antiinflammatory activities. The antioxidant activity could be related to the presence of hydroxyl groups, double bonds conjugation and resonance effect in the structure of the phenolic compounds detected in the extract. Terminalia ivorensis has also been shown to have antioxidant and antiinflammatory potential as reported by Aparna et al., 2012; Biermann et al., 2000). The antiviral activity of octadecanoic acid has been shown to inhibit the enzyme required for viral replication and cells invasion as reported by Keawsa-ard et al., (2012). These detected compounds have OH groups and oxygen atoms C=O with the ability to donate hydrogen atoms to free radicals, neutralize their scavenging effect, and chelate metals. The presence of alternating single and multiple bonds in detected structures as shown in (table 3 and figure 2-6) provides resonance stability to the molecule, thus allowing easy interaction with reactive oxygen species (ROS) with a powerful antioxidant potential. Phenolic compounds also have vasorelaxation and anti-allergenic activity (D'Archivio et al., 2010).

## CONCLUSION

The study provides scientific evidence that the methanol leaf extract of *Terminalia ivorensis* contains various volatile phytochemical compounds with reported medicinal potential. The structure, functional groups and resonance effect of these detected bioactive compounds may be responsible for the various reported antioxidants, biological and therapeutic potential of the plant extract. Thus may be considered as a potential drug, feed or fertilizer target.

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### REFERENCES

Abubakar, M.N. and Majinda, R.R. (2016). GC-MS analysis and preliminary antimicrobial activity of Albizia adianthifolia (Schumach) and Pterocarpus angolensis (DC). *Medicines*, *3*(1), p.3.

Adlercreutz, H., Markkanen, H., Watanabe, S. (1993). Plasma concentration of phytoestrogens in Japanese men. *Lancet*, **342**: 1209-1210

Ahmed, U. and Birnin-Yauri, U.A. (2008). Comparative analysis of the nutrient composition of cashew (Anacardium occidentale) apple and nut.

Akinyemi, K.O., Oluwa, O.K. and Omomigbehin, E.O. (2006). Antimicrobial activity of crude extracts of three medicinal plants used in South-West Nigerian folk medicine on some food borne bacterial pathogens. *African Journal of Traditional, Complementary and Alternative Medicines*, *3*(4), pp.13-22.

Akpuaka, A., Ekwenchi, M.M., Dashak, D.A. and Dildar, A. (2013). Biological activities of characterized isolates of n-hexane extract of Azadirachta indica A. Juss (Neem) leaves. *Nature and science*, *11*(5), pp.141-147.

Álvarez-Suarez, J. M., Giampieri, F., Gonzalez-Paramas, A. M., Elisabetta Damiani, E., Astolfi, P., Martinez-Sanchez, G., Battino, M. (2012). Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. *Food and Chemical Toxicology*, *50*, 1508– 1516.

Alves, M.J., Ferreira, I.C., Dias, J., Teixeira, V., Martins, A. and Pintado, M. (2012). A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta medica*, pp.1707-1718.

AOAC (Association of Official Analytical Chemists) (1990). *Official Methods of Analysis*. Washington D.C.

AOAC (Association of Official Analytical Chemists) (1997). *Official Methods of Analysis.* Washington D.C.

AOAC (Association of Official Analytical Chemists) (1973). *Official Methods of Analysis*. Washington D.C.

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design*, 80(3), pp.434-439.

Asaolu, T.O., Agorzie, C.J. and Unam, J.M. (2012). Materials management: an effective tool for optimizing profitability in the Nigerian food and beverage manufacturing industry. *Journal of Emerging Trends in Economics and Management Sciences*, 3(1), pp.25-31.

Ben-Azu, B., Aderibigbe, A.O., Adeoluwa, O.A. and Iwalewa, E.O. (2016). Ethanol Extracts of Terminalia ivorensis (Chev A.) Stem bark attenuates the positive, negative and cognitive symptoms of psychosis in experimental animal models. *Journal of* 

Bhakta, J., Majumdar, P. and Munekage, Y. (2009). Antimicrobial efficacies of methanol extract of Asteracantha longifolia, Ipomoea aquatica and Enhydra fluctuans against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus. *Int J Alter Med*, 7(2), pp.125-135.

Biermann, U., Friedt, W., Lang, S., Lühs, W., Machmüller, G., Metzger, J. O., Schneider, M. P. (2000). New Syntheses with Oils and Fats as Renewable Raw Materials for the Chemical Industry. *Angewandte Chemie International Edition*, 39, 2206–2224.

Burkill, H.M. (1985). The useful plants of west tropical Africa. ed. 2. Volume 1, Families AD. *Royal Botanic Gardens, Kew.* 

Chung IM, Ahmad A, Kim SJ, Naik PM, Nagella P. (2011). Composition of the essential oil constituents from leaves and stems of Korean Coriandrum sativum and their immunotoxicity activity on the Aedes aegypti L. *Immunopharmacol. Immunotoxicol*, pp1-5.

D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B. and Masella, R., (2010). Bioavailability of the polyphenols: status and controversies. *International journal of molecular sciences*, *11*(4), pp.1321-1342.

Doughari, J.H. (2012). *Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents* (pp. 1-33). Rijeka, Croatia: INTECH Open Access Publisher.

Enadeghe, R., Asoya, E.V., Iluma, J., Kolawole, O. and Omoregie, A.E., 2022. Phytochemistry and Acute Toxicity Study of Aqueous and Methanol Stem Bark Extract of Terminalia catappa. *Tropical Journal of Natural Product Research*, 6(6).

Felhi, S., Baccouch, N., Ben Salah, H., Smaoui, S., Allouche, N., Gharsallah, N. and Kadri, A. (2016). Nutritional constituents, phytochemical profiles, in vitro antioxidant and antimicrobial properties, and gas chromatography–mass spectrometry analysis of various solvent extracts from grape seeds (Vitis vinifera L.). *Food science and biotechnology*, *25*, pp.1537-1544.

Gupta, D. and Kumar, M. (2017). Evaluation of in vitro antimicrobial potential and GC–MS analysis of Camellia sinensis and Terminalia arjuna. *Biotechnology Reports*, *13*, pp.19-25.

Ibukunoluwa, M.R., Olusi, T.A. and Dada, E.O. (2015). Assessment of chemical compositions of three antimalarial

plants from Akure, Southwestern Nigeria: A preliminary study. *African Journal of Plant Science*, *9*(8), pp.313-319.

Ilouno, I.E., Gloria, O. Omaji and Anthony, E.O. (2018). Proximate, mineral and anti-viral composition of Jackal Berry (*Diospyros mespiliformis*) seeds. FUDMA Journal of Sciences. **2** (1) 56-65).

Keawsa-Ard, S., Liawruangrath, B., Liawruangrath, S., Teerawutgulrag, A. and Pyne, S.G. (2012). Chemical constituents and antioxidant and biological activities of the essential oil from leaves of Solanum spirale. *Natural product communications*, 7(7), p.1934578X1200700740.

Khan, M.S.M. and Kaneesamkandi, Z. (2013). Biodegradable waste to biogas: renewable energy option for the Kingdom of Saudi Arabia. *International Journal of Innovation and Applied Studies*, *4*(1), pp.101-113.

Li, X., Jing, T., Zhou, D., Zhang, M., Qi, D., Zang, X., Zhao, Y., Li, K., Tang, W., Chen, Y. and Qi, C. (2021). Biocontrol efficacy and possible mechanism of Streptomyces sp. H4 against postharvest anthracnose caused by Colletotrichum fragariae on strawberry fruit. *Postharvest Biology and Technology*, *175*, p.111401.

Nurzynska-Wierdak, R., Bogucka-Kocka, A., and Szymezak, G. (2014). "Volatile Constituents of Melissa officinalis Leaves Determined by Plant Ege." *Nat. Prod. Commun*, 9 (5): 152-60.

Mohd-Khan, M.S.A. and Ahmad, I. (2019). Herbal medicine: current trends and future prospects. In *New look to phytomedicine* (pp. 3-13). Academic Press.

Nduche, M.U., Omosun, G. and Okwulehie, I.C. (2015). Research Article Ethnobotanical Survey of Plants Used as Remedy for Fertility Conditions in Ebonyi State of Nigeria.

Parvathi, K., Kandeepan, C., Sabitha, M., Senthilkumar, N., Ramya, S., Boopathi, N., Ramanathan, L. and Jayakumararaj, R. (2022). "In-silico Absorption, Distribution, Metabolism, Elimination and Toxicity profile of 9, 12, 15-Octadecatrienoic acid (ODA) from Moringa oleifera", *Journal of Drug Delivery and Therapeutics*, 12(2-S), pp. 142-150. Sakat, S., Juvekar, A., & Gambhire, M. (2010). *In vitro* antioxidant and antiinflammatory activity of methanol extract of *oxalis corniculate* linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2, 146–155.

Salakhutdinov, N., Volcho, K. and Yarovaya, O. (2017). Monoterpenes as a renewable source of biologically active compounds. *Pure and Applied Chemistry*, Vol. 89 (Issue 8), pp. 1105-1117.

Shintani, H., Shintani, T. and Sato, M. (2020). D-Allose, a trace component in human serum, and its pharmaceutical applicability. *International Journal of Applied Biology and Pharmaceutical Technology*, 11(3), pp.200-213.

Sofowora, A., Ogunbodede, E. and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary and alternative medicines*, *10*(5), pp.210-229.

Sozturk Sarikaya, S.B. (2015). Acethylcholinesterase inhibitory potential and antioxidant properties of pyrogallol. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *30*(5), pp.761-766.

Suroowan, S., Pynee, K.B. and Mahomoodally, M.F. (2019). A comprehensive review of ethnopharmacologically important medicinal plant species from Mauritius. *South African Journal of Botany*, *122*, pp.189-213.

Szél, E., Polyánka, H., Szabó, K., Hartmann, P., Degovics, D., Balázs, B., Németh, I. B., Korponyai, C., Csányi, E., Kaszaki, J., Dikstein, S., Nagy, K., Kemény, L., & Erős, G. (2015). Anti-irritant and anti-inflammatory effects of glycerol and xylitol in sodium lauryl sulphate-induced acute irritation. *Journal of the European Academy of Dermatology and Venereology: JEADV*, 29(12), 2333–2341.

Zhang, J.H., Di, Y., Wu, L.Y., He, Y.L., Zhao, T., Huang, X., Ding, X.F., Wu, K.W., Fan, M. and Zhu, L.L. (2015). 5-HMF prevents against oxidative injury via APE/Ref-1. *Free Radical Research*, 49(1), pp.86-94.



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