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Original Research Article

Chemical profiles and proximate analysis of n-hexane extract of *Terminalia catappa* kernel from Nigeria

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ABSTRACT

Background: *Terminalia catappa* is a traditionally consumed edible plant used for various health conditions. This study aimed to characterise the phytochemical profile, nutritional composition, and acute oral toxicity of the n-hexane extract of *Terminalia catappa* nuts.
Methods: Gas chromatography-mass spectrometry (GC-MS) analysis was conducted to determine the phytochemical profile of the *n*-hexane extract of *Terminalia catappa* nuts. Proximate analysis was performed to evaluate the nutritional composition. Acute oral toxicity testing was carried out in Wistar rats as per OECD Guideline 425 to assess safety.
Results: The phytochemical analysis identified 19 bioactive compounds in the *Terminalia catappa* nut extract. The major components were cis-vaccenic acid (24.493%), propyleneglycol monoleate (23.783%), and mitotane (14.186%). Proximate analysis revealed the nuts to be rich sources of fat (56.71%) and protein (26.30%). The median lethal dose (LD50) of the n-hexane extract in rats was greater than 5000 mg/kg, indicating a high margin of safety.
Conclusions: This work provides valuable insights into the nutritional potential and safety profile of *Terminalia catappa* nuts. Phytochemical characterisation corroborates traditional uses, while the acute toxicity data establishes the relative safety of the n-hexane extract when consumed at high doses.

Keywords: GC-MS, n-hexane extract, Phytochemical profile, Proximate analysis, *Terminalia catappa*

INTRODUCTION

Plants have been indispensable sources of sustenance and remedies for humans and animals, a timeless partnership that still endures.[1] In contemporary times, plants remain pivotal in traditional and complementary medicine, constituting integral components of primary healthcare systems in many nations.[2] This enduring relevance of plants in health and wellness is attributable to their rich reservoir of metabolites, encompassing diverse chemical compounds endowed with valuable pharmacological properties.

However, it is essential to recognise that the vast realm of plant species remains uncharted, mainly regarding their potential nutritive and therapeutic contributions.[3] A striking paradox emerges where many plant species harbour latent benefits, yet only a fraction of these have been explored and substantiated. This under-exploration can be attributed, in part, to the absence or insufficiency of scientific evidence that confirms their significance and

potential value.[3] In plant science, the value of these botanical treasures is intrinsically tied to their chemical composition, the intricate interplay of chemical constituents, and their inherent biological potential. As such, the imperative arises to bridge this substantial gap in our knowledge, thereby unlocking the full potential of these plants as sources of nutrition, nutraceuticals, and future medicinal agents.

This present study focuses on one such plant, *Terminalia catappa* LINN, and its n-Hexane extract. *Terminalia catappa* LINN is a plant species classified under the family Combretaceae and the catappa genus. *Terminalia catappa* L is a tropical plant widely recognised by its common name, the Indian almond or tropical almond plant. It grows predominantly in the tropical regions of Asia, Africa, and Australia.[4] *Terminalia catappa* L. is locally known by different names in Nigeria, such as Mbansan Mbakara (groundnut of the Whiteman) in Efik/Ibibios, Ebelebo in Benin, Egboen-nebi in Edo, Afara dudu in Yoruba, Fasakorihi in Fulani and fruits by some Nigerians.[5] *Terminalia catappa* L. have significant cultural, culinary, and medicinal importance and are used for various purposes such as providing shade, wood for timbers, bark, leaves, fruit, and nuts for medicinal purposes.[6-8]

The leaves, bark, and roots have found application in Ayurvedic medicine for addressing various health issues, including hypertension, dysentery, and diarrhoea.[6-8] The leaves, bark, roots, and fruits of *Terminalia catappa* extract are believed to possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, making it a potential plant for exploration of its phytochemical constituent.[4,9] The fleshy ripe fruit of *Terminalia catappa* yields edible nuts known as 'tropical almonds' or 'Indian almonds.' The nut can be consumed directly or modified into different types of food for normal consumption.[4] The nuts of *Terminalia catappa* are recognised for their richness in fatty acids, contributing to their high nutritional value and cardiovascular benefits.[10,11]

The proximate analysis of *Terminalia catappa* nuts reveals an impressive nutritional profile, characterised by a substantial protein content ranging from 18.39% to 40.9%.[12-15] Additionally, these nuts exhibit a generous oil content ranging from 43.36% to 63.65% and the presence of essential amino acids in these seeds, which support growth and contribute to their high dietary protein quality.[16,17] Ezeokonkwo further identified tyrosine, lysine, and methionine as the limiting amino acids in *Terminalia catappa* seeds.[13] Several studies have demonstrated the presence of fatty acid in the seed oil of *Terminalia catappa*, consistently highlighting oleic acid as the predominant component.[15,18]

However, it is worth noting that findings from diverse studies have identified linoleic acid as the major fatty acid in *Terminalia catappa* nuts.[19,20] Furthermore, investigations into the sugar composition have revealed the presence of a small amount of sugar in these seeds.[17,21,22] This diverse range of nutritional components underscores the potential benefits of *Terminalia catappa* nuts as a valuable dietary resource. Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique widely used in the phytochemical analysis of plant extracts. It offers valuable insights into the chemical composition of plant extracts by separating and identifying minute volatile molecules, including hormones and steroids, relying on the NIST MS/MS Database and Library from the National Institute of Standards and Technology (NIST).[23-26]

In the quest to unlock the full potential of *Terminalia catappa* nuts, the objectives of this study are twofold. Firstly, we aim to investigate the phytochemical profile of the n-hexane extract of *Terminalia catappa* nuts using Gas Chromatography-Mass Spectrometry (GC-MS) and proximate analysis of the *n*-hexane extract of *Terminalia catappa* nut using spectrophotometry and (2) the determination of the lethal dose of the n-hexane extract of *Terminalia catappa* nut. Therefore, this study aimed to characterise the phytochemical profile, nutritional composition, and acute oral toxicity of the n-hexane extract of *Terminalia catappa* nuts.

METHODS

Study design

This research utilised an experimental study design to characterise the bioactive and nutritional composition and evaluate the acute oral toxicity of the *n*-hexane extract of *Terminalia catappa* nuts. The study was conducted from July 2023 to August 2023 at the Animal House of the Department of Human Physiology, University of Port Harcourt, Nigeria.

Collection and identification of plant materials

The ripe fruits of *Terminalia catappa* were collected from the campus grounds of the University of Port Harcourt (UPH) and the Rivers State University (RSU), Nigeria, between May and August 2023. The plant was identified and authenticated by Dr Chimezie Ekeke, a distinguished authority in the Department of Plant Science and Biotechnology at the University of Port-Harcourt, Nigeria. Subsequently, a unique reference number was assigned, UPH/PBS/2023/047. A voucher specimen of *Terminalia catappa* L., specifically UPH/P/398., was then conscientiously catalogued in the University of Port Harcourt herbarium.

Solvents and chemicals

Analytical grade methanol and n-hexane, both procured from Merck (Germany), were utilised for the study. Deionised water, meeting the stringent standards of the

Milli-Q purification system by Millipore (USA), was also employed.

Sample processing and extraction

The freshly harvested fruits of Terminalia catappa underwent a rigorous cleansing process under a steady stream of running water. Following this, they were allowed to drain and were gently pat-dried. The edible nut of *Terminalia catappa*, was subsequently extracted from its fibrous husk and rigid shell. These processes were then air-dried at ambient room temperature. Once sufficiently dried, they were finely pulverised into a uniform powder. Utilising a solid-to-liquid ratio of 1:30 (w/v), the powdered material underwent extraction with 85% aqueous n-hexane employing a Soxhlet apparatus, as outlined by Yahaya et al.[27] This extraction process was iterated four times to ensure a thorough and exhaustive extraction, with the solvent extract being carefully collected for each cycle. The consolidated extracts derived from *Terminalia catappa* were subsequently pooled, filtered, and subjected to concentration under reduced pressure, employing a rotary evaporator from Heidolph GmbH & Co. K.G. (Germany). The resulting concentrated extract was then freeze-dried and stored at -20°C before administration.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

One gram of the crude n-hexane extracts obtained from *Terminalia catappa* nuts was reconstituted in methanol. Subsequently, it underwent solvent partitioning into n-hexane, facilitating the separation of fatty acids and other non-polar metabolites from metabolites of higher polarity. The resulting hexane fractions were then subjected to freeze-drying and subsequently analysed via GCMS utilising the QP2010 Ultra GCMS system by Shimadzu, Japan. The system was equipped with an Rxi-5ms fused silica capillary column, boasting a length of 30 meters, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm, composed of 5% diphenyl and 95% dimethyl polysiloxane.

The analysis was conducted employing a gradient temperature program, commencing at an initial temperature of 50°C for 3 minutes, then gradually increasing to 300°C at a rate of 3°C per minute. Subsequently, the temperature was maintained at 300°C consistently for an additional 10 minutes. The sample elution occurred at a volume of 1 µL, with the mass conditions configured as follows: ionisation voltage of 70 eV, helium flowing at a rate of 11.8 mL/min, ion source temperature set at 250°C, and a scan range spanning m/z 40-700 amu. A comparative analysis of the mass data derived from the hexane fractions was conducted against the NIST 14 (National Institute of Standard Technologies, Mass Spectra) libraries for compound identification.

Proximate analysis

The proximate analysis of the n-hexane extract of *Terminalia catappa* nuts was conducted to determine the moisture, ash, crude fibre, crude protein, and crude fat content using standard AOAC methods. [28] Moisture content will be analysed by oven drying samples at 105°C until a constant weight is achieved. Ash content was measured by incineration in a muffle furnace at 600°C. Crude fibre will be determined by digesting samples with sulfuric acid and sodium hydroxide. Crude protein will be analysed using the Kjeldahl method, which involves digestion, distillation, and titration. Crude fat was determined by extracting samples with petroleum ether using a Soxhlet apparatus.[28] All analyses were performed in triplicate, and the results will be expressed as percentages.

Acute toxicity

An acute oral toxicity study was performed to determine the lethal dose (LD50) of the n-hexane extract of *Terminalia catappa* based on the Organization for Economic Co-operation and Development (OECD) Test Guideline 425.[29] Wistar rats aged 6-8 weeks were obtained and acclimatised for two weeks before dosing. The rats were randomly assigned to different treatment groups receiving ascending doses of the extract from 5 to 5000 mg/kg body weight via oral gavage. The rats were observed closely for signs of toxicity and mortality for the first 4 hours and daily for 14 days. The LD50 was estimated using probit analysis.

The animal experiments were performed per the Institute of Laboratory Animal Resources guidelines, a guide for the care and use of laboratory animals.[30] The study protocol was reviewed and approved by the Research Ethics Committee.

RESULTS

GC-MS metabolite profile

The GC-MS spectral data of the hexane fractions of *Terminalia catappa* nut is presented in Figure 1, together with the total ion chromatograms of the sample (Table 1).

The spectral data indicates various extract constituents' retention time and intensity counts. A minimum of 19 bioactive constituents were identified following GC-MS analysis (Table 1 and Figure 1). Gas chromatography-mass spectrometry (GC-MS) analysis of the n-hexane extract of *Terminalia catappa* nuts revealed the presence of several notable bioactive compounds. As shown in Table 1, the most abundant components identified were cis-vaccenic acid (24.493%), propyleneglycol monoleate (23.783%), mitotane (14.186%), and *n*-hexadecanoic acid (9.186%). Additionally, moderate percentages of 9-octadecenal (1.672%), tetradecanoic acid (1.247%), an unidentified compound (2.017%), 5-(methylthio) salicylic

acid derivative (3.888%), 1,4-benzenediamine derivative (1.518%), cholesta-3,5-diene (1.522%), and cholesterol (1.954%), each representing over 1% and dodecanoic acid (0.866%) below 1% of the total extract composition were detected. The profile indicates a diversity of fatty acids, aldehydes, steroids, and aromatic compounds that likely contribute to the nutritional and bioactive properties of *Terminalia catappa* nuts reported in folk medicine and scientific literature.

Table 1: Chemical formula, molecular weight, and percentage composition of identified compounds in n-hexane extract of *Terminalia catappa* nuts using GC-MS.

Peak	RT (mins)	Area (%)	Name of compound	Molecular weight (g/mol)	Molecular formula
1	7.928	0.866	Dodecanoic acid	200.32	$C_{12}H_{24}O_2$
2	9.038	1.247	Tetradecanoic acid	228.37	$C_{14}H_{28}O_2$
3	9.970	2.017	Unidentified		
4	10.091	9.186	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$
5	10.171	1.309	Unidentified		
6	10.783	1.081	Unidentified		
7	10.943	24.493	cis-Vaccenic acid	282.48	$C_{18}H_{34}O_2$
8	11.092	1.037	Unidentified		
9	11.624	4.624	Mitotane	320.88	C14H10Cl4O3
10	11.973	14.186	Mitotane	320.88	C14H10Cl4O3
11	13.054	1.672	9-Octadecenal	266.46	C18H34O
12	14.016	1.522	Cholesta-3,5-diene	368.64	$C_{27}H_{44}$
13	14.840	1.954	Cholesterol	386.65	C27H46O
14	16.556	1.748	Dodecanoic acid, 1,2,3-propanetriy l ester	639.00	$C_{39}H_{74}O_6$
15	19.005	0.781	Unidentified		
16	19.995	3.888	5-(Methylthio) salicylic acid, 2TMS derivative	328.58	C12H18O3SSi2
17	20.487	1.518	1,4-benzenediamine, N4-[4-(decylamino) phenyl]-N1,N1-dimethyl-	406.64	C26H42N4
18	21.365	3.086	Unidentified		
19	22.599	23.783	Propyleneglycol monoleate	230.3	C12H24O4

Note. RT: Retention time; Area %: Percentage composition

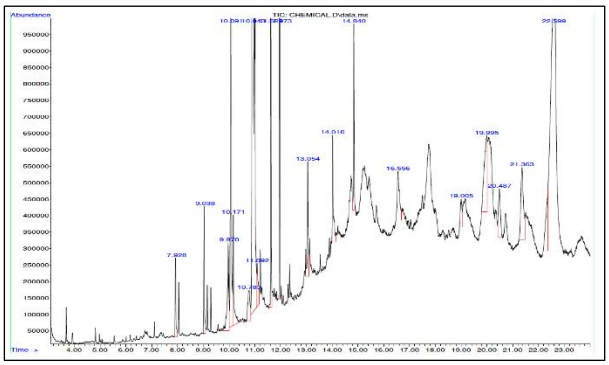

Figure 1: GCMS spectral data of metabolites identified in hexane fraction of *Terminalia catappa* nuts.

Proximate composition of Terminalia catappa nuts

The proximate composition of the *Terminalia catappa* nut is shown in Table 2. The proximate composition analysis of *Terminalia catappa* nut reveals significant nutritional components. The nut is notably rich in fat, constituting approximately 56.71% of its composition. Following fat, protein is the second most abundant component, accounting for approximately 26.30% of the nut's composition. Carbohydrates and fibre contribute to 6.50% and 4.40%, respectively, indicating moderate levels of these essential dietary constituents. The ash content, representing the inorganic mineral elements, is recorded at 4.55%, signifying the presence of minerals. Lastly, the moisture content is relatively low, accounting for approximately 1.54% of the nut's composition.

Acute toxicity study

The acute oral toxicity of the n-hexane extract of *Terminalia catappa* nuts was evaluated in female albino Wistar rats according to OECD Test Guideline 425. The extract was administered in ascending doses up to 5000 mg/kg body weight. Mortality and clinical signs of toxicity were monitored for 14 days post-dosing. No mortality or adverse effects were observed in rats treated at doses up to 5000 mg/kg body weight. The LD50 of the extract after single-dose oral administration is, therefore, greater than 5000 mg/kg body weight (Table 3). According to the OECD test guideline classifications, the nut extract can be considered Category 5 or "relatively harmless" based on the LD50 greater than 5000 mg/kg. Overall, the acute toxicity test suggests the n-hexane extract of *Terminalia catappa* nuts has a high safety

margin at acute oral doses, with no mortality or toxicity signs up to 5000 mg/kg in Wistar rats.

Table 2: Proximate composition of *n*-hexane extract of *Terminalia catappa* nuts.

Constituents	Percentage composition + SEM
Fat	56.71+ 1.66
Protein	26.30+ 0.14
Carbohydrate	6.50 + 0.38
Fibre	4.40 + 0.01
Ash	4.55 + 0.45
Moisture	1.54+ 0.29

Table 3: Acute toxicity study of the *n*-hexane extract of *Terminalia catappa* nuts.

Media	Dose (mg/kg)	*n*	Remark	Mortality	Clinical signs of toxicity	Lethal dose*	Safe dose
Distilled water	5	3	Normal, active	0/3	None	> 5000 mg/kg	0.1-5000 mg/kg
	50	3	Normal, active	0/3	None		
	300	3	Normal, active	0/3	None		
	2000	3	Normal, active	0/3	None		
	5000	3	Normal, active	0/3	None		

n: Number of Wistar rats

*According to the OECD test guideline classifications, the nuts extract can be considered Category 5 or "relatively harmless" based on the LD50 greater than 5000 mg/kg

DISCUSSION

The present study utilised Gas Chromatography-Mass Spectrometry (GC-MS) to analyse the phytochemical profile of the n-hexane extract of *Terminalia catappa* nuts. GC-MS is an invaluable analytical technique for separating, identifying, and quantifying volatile chemical compounds in complex mixtures.[31] The mass spectra generated were interpreted, and matching compounds were identified using the NIST mass spectral database.[26] The current study provides novel insights into the phytochemical profile and nutritional composition of the n-hexane extract of *Terminalia catappa* nuts. We pinpointed 19 bioactive components through GC-MS analysis, including fatty acids, aldehydes, steroids, and aromatic compounds. Notably, cis-vaccenic acid and propylene glycol monooleate hold significance, given their established anti-inflammatory, anticancer, antioxidant, and emollient properties.[32-35] The substantial presence of mitotane utilised as chemotherapy for adrenal cancers is also remarkable and indicates latent pharmacological potential.[36]

Propylene glycol monooleate is a common solvent used in medical preparations and has been found to produce significant apoptosis and anti-inflammatory properties.[37,38] Cis-vaccenic acid is an omega-7 monounsaturated fatty acid with antioxidant, anticancer, anti-inflammatory, and cardioprotective properties.[32-35] It has been found to lower the risk of incident type II diabetes.[39,40] The high percentage of cis-vaccenic acid likely contributes significantly to the nutritional and therapeutic benefits attributed to *Terminalia catappa* in traditional medicine systems.[40] Mitotane, known as Lysodren, was the second major constituent identified at 14.186%. This adrenolytic drug is utilised in chemotherapy for adrenal cortex cancers and Cushing's syndrome due to its cytotoxic effects on the adrenal cortex.[41] The substantial mitotane content of the nut extract suggests potential anticancer effects that merit further research, especially regarding adrenal tumours.[36]

Additionally, n-hexadecanoic acid possesses antimicrobial and hypocholesterolaemia activities, while 9-Octadecenal, commonly known as oleic acid, has demonstrated antimicrobial, antioxidant, and anti-inflammatory properties.[42-50] Their presence likely enhances the medicinal value of the extract. Furthermore, oxysterol cholesta-3,5-diene displays anti-inflammatory activities, whereas cholesterol plays indispensable structural and biochemical roles in the body.[51,52] Also, moderate levels of lauric acid, myristic acid, trilaurin, methylthio salicylic acid, and 1,4-benzenediamine derivatives were found. These compounds possess antimicrobial, antioxidant, anti-inflammatory, and anticancer effects based on documented research.[53]

With toxicity being a primary concern for therapeutic agents, this study established preliminary safety data regarding acute exposure to the nut extract. The high LD50 value (>5000 mg/kg) demonstrates an impressive safety profile and low toxicity risk following acute doses in animal models. However, future studies must also evaluate possible adverse effects associated with chronic exposures. Elucidating the pharmacological mechanisms and potential toxicological effects will be imperative as nut extracts are investigated for development into functional foods or phytopharmaceuticals.

Pharmacological and clinical relevance

The array of bioactive constituents identified in *Terminalia catappa* nut extracts suggests potential pharmacological and clinical applications that warrant further research. The high content of cis-vaccenic acid and other fatty acids indicates that anti-inflammatory effects may be clinically relevant, as inflammation underlies many chronic diseases.[32-35] Anti-cancer properties are also suggested based on the presence of mitotane and other anticarcinogenic compounds. In particular, the potential for development as an adjuvant therapy for adrenal cancers merits evaluation and further evaluation of mitotane in other forms of cancers.[36]

Antimicrobial effects against drug-resistant pathogens may also be pharmacologically relevant, given the combinations of lauric, palmitic, and oleic acids.[54] This could provide avenues for new anti-infective agents. The antioxidant capacity of the nuts could also confer clinical benefits in conditions involving excessive oxidative stress. Overall, the preliminary characterisation of the extract composition aligns with reported ethnomedical uses and suggests promise for managing an array of conditions.

Table 4: Possible biologic, pharmacologic effects, and industrial uses of identified compounds in the n-hexane extract of *Terminalia catappa* nuts.

Name of bioactive compound	Possible pharmacological, and biological effects
Dodecanoic acid	Antimicrobial[55,56], antioxidant[57], anti-inflammatory
Tetradecanoic acid	Antioxidant, anti-inflammatory, anticancer[53]
***n*-Hexadecanoic acid**	Antimicrobial, and hypocholesterolaemia properties[42-44]
cis-Vaccenic acid	Anticancer, antioxidant, anti-inflammatory properties[40]
Mitotane	Anticancer in the treatment of adrenocortical carcinoma[36]
9-Octadecenal	Antimicrobial[45,46], antioxidant[48,49], anti-inflammatory[50,58]
Cholesta-3,5-diene	Anti-inflammatory[51]
Cholesterol	Membrane stability, hormone precursor[52]
Dodecanoic acid, 1,2,3-propanetriyl ester	Exercise antioxidants, immunomodulators, and immunostimulants[58]
5-(Methylthio) salicylic acid, 2TMS derivative	Anti-inflammatory, antioxidant, anticancer
1,4-benzenediamine, N4-[4-(decylamino) phenyl]-N1,N1-dimethyl-	Anti-inflammatory, antioxidant, anticancer
Propyleneglycol monoleate	Used as emollient, emulsifier, penetration enhancer and food additive[37,38]

Key strengths of this study include the use of GC-MS for broad phytochemical profiling, which enabled the detection of diverse bioactive constituents. Thorough proximate analysis quantitatively validated the nutritional composition. The acute toxicity testing also provides initial data regarding the safety profile of the nut extracts. While the GC-MS furnished an initial metabolite profile, quantification of the identified compounds was not undertaken. Thus, the exact concentrations and proportions need to be clarified. Quantitative determination of the major constituent's merits investigation using validated spectrophotometric or chromatographic techniques.

CONCLUSION

The GC-MS results revealed the presence of several phytochemical compounds in the n-hexane extract of *Terminalia catappa* nuts, with a high amount of cis-vaccenic acid, mitotane and propylene glycol monooleate and are recommended as a nut of pharmaceutical importance. The proximate analysis shows that the extract has a richness of fats, protein, carbohydrates, and minerals, while the acute toxicity study in Wistar rats demonstrated an impressive safety profile.

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