



PHYTOCHEMICAL SCREENING, NUTRITIONAL COMPOSITION, FT-IR, AND GC-MS ANALYSIS OF THE ETHANOLIC EXTRACT OF *HUNTERIA UMBELLATA* SEEDS

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ABSTRACT

Hunteria umbellata is a medicinal plant widely used in traditional medicine for managing various ailments. This study presents the phytochemical screening, nutritional composition, FT-IR spectroscopy, and GC-MS profiling of *H. umbellata* seed extract. Phytochemical screening revealed high levels of alkaloids, coumarins, flavonoids, tannins, and phenols, while quantitative analysis showed alkaloids (2.31 mg/100 g), flavonoids (16.79 mg/100 g), phenols (5.36 mg/100 g), tannins (4.88 mg/100 g), and saponins (30.54 mg/100 g) as predominant compounds. Proximate analysis demonstrated the extract's richness in carbohydrates (33.44%), crude fiber (26.75%), protein (13.61%), fat (12.86%), moisture (9.51%), ash (3.83%), and a calorific value of 1277.34 kJ/100 g. The extract also contained essential minerals such as calcium (387.33 mg/100 g), magnesium (180.00 mg/100 g), sodium (170.10 mg/100 g), phosphorus (37.35 mg/100 g), potassium (19.17 mg/100 g), and manganese (2.16 mg/100 g). Vitamin analysis indicated the presence of vitamin A (5.89 µg RE/100 g), B1 (4.69 mg/100 g), B2 (4.87 mg/100 g), B3 (7.85 mg/100 g), B6 (2.57 mg/100 g), vitamin E (16.58 mg/100 g), and vitamin C (14.87 mg/100 g). FT-IR spectroscopy indicated the presence of hydroxyl, alkane, alkene, aromatic amine, and ether functional groups. GC-MS analysis identified key bioactive constituents, including cyclopropanoethanal derivatives and aminobenzene compounds. These findings validate the rich phytochemical and nutritional composition of *Hunteria umbellata* seeds and support their traditional and modern use in managing metabolic disorders.

KEYWORDS: *Hunteria umbellata*, Phytochemical screening, FT-IR spectroscopy, GC-MS analysis, Ethanolic extract, Medicinal plants, Bioactive compounds.

INTRODUCTION

Medicinal plants have long served as indispensable sources of bioactive compounds in traditional and modern medicine, providing therapeutic agents for the prevention and management of various ailments.^[1] Despite significant advancements in synthetic drug development, natural products continue to attract scientific interest due to their pharmacological efficacy, relative safety, and affordability. This interest is especially prominent in the search for novel agents to manage metabolic disorders, infectious diseases, and chronic conditions.^[2-3]

Hunteria umbellata (K. Schum.) Hallier f., a small glabrous tree of the *Apocynaceae* family, is widely distributed across the tropical forests of West African countries like Cameroon, Senegal, Ghana, Gabon, Congo, Ivory Coast, and Nigeria. It is locally known as “madaci” (Hausa), “mkpokiri” (Igbo), and “aberere” or “erin” (Yoruba). It is also referred to as “Asewa” (Ibibio),

“Osu” (Bini), and “Demouain” in Francophone West African countries.^[5-6]

Hunteria umbellata is rich in bioactive compounds such as alkaloids, flavonoids, phenolic acids, saponins, tannins, glycosides, terpenoids, and essential fatty acids. These constituents contribute to its wide-ranging biological activities, including antioxidant, anti-inflammatory, anti-obesity, hypoglycaemic, antimicrobial, analgesic, and hepatoprotective effects.^[7-8]

Various parts of the plant, including the seeds, bark, and roots, are traditionally utilized in African ethnomedicine for treating conditions such as menorrhagia, yaws, gastric ulcers, liver diseases, aphrodisiac, diabetes mellitus and obesity.^[9] The seeds are chewed or decocted to manage elevated blood glucose, body weight, and immune-related disorders, while the bark is used for fever and inflammation relief.^[10] In southwestern Nigeria, traditional birth attendants also use the leaves and fruit pulp to induce or augment labour in pregnant women.^[10]

This study aims to investigate the phytochemical profile of the ethanolic extract of *Hunteria umbellata* seeds through phytochemical screening, nutritional composition analysis, Fourier-transform infrared spectroscopy (FT-IR), and gas chromatography–mass spectrometry (GC-MS). These analyses are expected to provide insight into the metabolic and therapeutic potentials of the seeds extract in health.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Mature fruits of *Hunteria umbellata* were collected from the Pharmacy Plantation at the University of Uyo, Uyo,

Akwa Ibom State, Nigeria (Latitude: 5.04904° N, Longitude: 7.92695° E). Mrs. Emmanuella Udoma, a taxonomist in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy Herbarium, University of Uyo, Nigeria, identified and authenticated the plant samples. A voucher specimen with the reference number UUPH 6(I) was assigned to the plant and deposited in the herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria, for future reference.



Figure 1: (a) Fruit and (b) seeds of *Hunteria umbellata*.

PREPARATION OF EXTRACT AND CALCULATION OF PERCENTAGE YIELD

Fresh fruits of *Hunteria umbellata* were cut open, and the seeds were carefully collected. The seeds were manually dehusked, thoroughly washed with clean water, and air-dried at room temperature for three weeks to eliminate residual moisture. The dried seeds were then pulverized into a fine powder using a VTCL Solitaire mixer grinder (Model No.: Solitaire 4; VTCL, India).

About 3324 g of the powdered seed material was weighed using a Mettler analytical balance and soaked in 7.5 L of absolute ethanol in an air-tight container for 72 hours, with intermittent stirring to enhance extraction. The mixture was filtered using Whatman No. 1 filter paper, and the resulting filtrate was concentrated using a water bath (U-Clear Model: DK-420) maintained at 40 °C to evaporate the solvent completely and obtain the crude ethanolic extract.

The crude extract was weighed, and the percentage yield was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of crude extract obtained (g)}}{\text{Weight of pulverized dry seed extract (g)}} \times 100$$

The crude extract was then weighed, transferred to a sealed container, and stored at 4 °C prior to use.

QUALITATIVE ANALYSIS

Qualitative phytochemical screening of the ethanolic extract of *Hunteria umbellata* seeds was carried out to

detect the presence of major secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, phenols, and glycosides using standard protocols as described by.^[11]

QUANTITATIVE DETERMINATION OF HUS

Preliminary quantitative analysis of selected secondary metabolites, including alkaloids, flavonoids, phenols, and saponins, was carried out using established methods previously described by.^[12-13]

These assays provided approximate concentrations of these bioactive compounds in the seed extract, expressed as percentage (%) content relative to dry weight.

FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY ANALYSIS

FT-IR analysis was conducted using a Cary 630-IR spectrophotometer (Agilent Technologies, USA) at the Multi-User Science Research Laboratory, Ahmadu Bello University (ABU), Zaria, Nigeria, to identify the functional groups present in the HUS extract. Approximately 10 mg of dried powder of *Hunteria umbellata* seeds was mixed with 100 mg of potassium bromide (KBr) pellet to prepare translucent sample discs. The transmittance mode was employed, with 30 scans and a background scan of 16. A resolution of 8 cm⁻¹ was used, and spectra were recorded within the range of 4000 to 650 cm⁻¹, and the characteristic peaks were identified and interpreted based on standard FT-IR spectra data.

Each analysis was performed in triplicate for reproducibility, and functional groups were identified using the Happ-Genzel apodization function.

GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GC-MS) ANALYSIS OF BIOACTIVE COMPONENTS OF HUNTERIA UMBELLATA SEEDS

Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the ethanol extract of *Hunteria umbellata* seeds (HUS) was conducted at the Multi-User Science Research Laboratory, Ahmadu Bello University (ABU), Zaria, using an Agilent Technologies 7890A GC system coupled with a 5977B mass selective detector (MSD) and equipped with an automatic liquid sampler (ALS). A 2 µL aliquot of the extract was injected in splitless mode using a 10 µL syringe into the front injector maintained at 250 °C. High-purity helium (99.999%) served as the carrier gas at a constant flow rate of 1.2035 mL/min, with an inlet pressure of 11.089 psi and a total flow rate of 19.204 mL/min. The septum purge flow was set at 3 mL/min, and the purge flow to the split vent was activated at 15 mL/min after 0.75 minutes.

Chromatographic separation was achieved using an Agilent HP-5ms Ultra Inert capillary column (30 m × 250 µm × 0.25 µm). The oven temperature was initially held at 70 °C for 0 minutes, then ramped at 5 °C/min to 250 °C (held for 1 minute), followed by a second ramp at 30 °C/min to 300 °C with no final hold. The post-run oven temperature was set at 50 °C. The maximum allowable temperature was 325 °C. The average linear velocity was 40.367 cm/s with a holdup time of 1.2386 minutes.

The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. Full scan data were acquired over a mass range of m/z 50–650 with a solvent delay of 5 minutes. The ion source temperature was maintained at 230 °C and the quadrupole at 150 °C. The electron multiplier voltage (EMV) was set at 2182 V (actual value: 1907 V).

IDENTIFICATION OF BIOACTIVE COMPONENTS

Data acquisition and spectral analysis were performed using Agilent ChemStation software. Compound identification was based on comparisons with mass spectral data from the National Institute of Standards and Technology (NIST) Mass Spectral Library (version 2018), which contains over 62,000 reference spectra. The relative abundance of each compound was determined by calculating the area under the curve (AUC) of the respective chromatographic peak and expressing it as a percentage of the total ion chromatogram.

RESULTS AND DISCUSSION

The present study investigated the phytochemical screening, nutritional composition of *Hunteria umbellata* seeds (HUS) and explored their effects through

comprehensive spectroscopic and chromatographic analyses.

The ethanolic extract of *Hunteria umbellata* seeds resulted in an average yield of 7.22%, producing a dark brown, oily, sweet-smelling paste. This yield reflected the extractable bioactive components present in the seeds and provided a quantitative basis for further phytochemical and bioactivity analyses.

The qualitative analysis of *Hunteria umbellata* seeds extract revealed the abundant presence of alkaloids, saponins, phytosterols, coumarins, flavonoids, tannins, phenolic compounds, and carbohydrates. Moderate levels of triterpenoids, steroids, cardiac glycosides, anthraquinones, quinones, and diterpenes were detected. Additionally, phlobatannins and amino acids were present in trace amounts while proteins were not detected (Table 1). The abundance of saponins, flavonoids, phenolic compounds, and tannins is consistent with previous studies highlighting their roles in antioxidant defense and metabolic regulation.^[14-15] Moreover, the presence of alkaloids and triterpenoids further supports the ethnomedicinal relevance of the extract, considering their well-documented pharmacological activities, including anti-inflammatory, antimicrobial, and lipid-lowering effects.^[16-18]

Table 1: Preliminary Qualitative Phytochemical Analysis of HUS.

Phytochemical constituents	Inference
Alkaloids	+++
Saponin	++
Steroid	+
Phytosterol	+++
Triterpenoids	+
Cardiac Glycosides	+
Coumarins	+++
Anthraquinones	+
Quinones	+
Flavonoids	+++
Tannin	+++
Phenolic compounds	+++
Diterpenes	+
Phlobatannins	+
Carbohydrates	+++
Proteins	-
Amino acids	++

Legend

+ = Present in low concentration

++ = Present in moderate concentration

+++ = Present in high concentration

– = Not detected

The quantitative phytochemical analysis of *Hunteria umbellata* seeds (HUS) revealed a notable presence of secondary metabolites, expressed as milligrams per 100 grams (mg/100 g) of dry seed material. The results indicated the presence of alkaloids (2.31 mg/100 g),

flavonoids (16.79 mg/100 g), phenols (5.36 mg/100 g), tannins (4.88 mg/100 g), and saponins (30.54 mg/100 g). Among these, saponins and flavonoids were the most abundant, while phenols, tannins, and alkaloids were present in comparatively lower concentrations in the ethanolic extract. These findings are consistent with previous studies that identify saponins, flavonoids, and phenolics as dominant bioactive constituents in medicinal plants, contributing significantly to their pharmacological properties.^[19-20]

Studies have shown that saponin-rich extracts have demonstrated a wide range of therapeutic benefits, including energy enhancement, improvement of sexual function in men, and activity against central nervous system disorders, microbial infections, inflammation, diabetes, obesity, and cardiovascular diseases.^[21]

Flavonoids such as quercetin and catechins, along with phenolic acids like gallic and chlorogenic acid, have been shown to inhibit pancreatic lipase activity through competitive and non-competitive mechanisms, thereby reducing lipid digestion and absorption.^[22-24] These compounds are also recognized for their potent antioxidant, anti-inflammatory, and enzyme-modulating properties that are vital to the management of metabolic disorders.^[25]

Tannins, another group of polyphenolic compounds present in HUS, exhibit diverse biological activities. They have been associated with anti-inflammatory, immunoregulatory, hypoglycemic, and hypolipidemic effects, and contribute to metabolic regulation, DNA repair, and anticancer activity.^[26] It has also displayed antioxidant, antimutagenic, antiviral, antimicrobial, antinutrient, and free radical-scavenging activities.^[27] Their health-promoting effects extend to cardiovascular, metabolic, and neurological functions, including the modulation of gut microbiota and cognitive processes.^[28-30] These effects are further reinforced by studies showing that tannins influence various biochemical pathways associated with oxidative stress, inflammation, lipid metabolism, and gut-brain axis modulation, suggesting a complex and multi-targeted mechanism of action.^[31-32]

Alkaloids, though found in lower quantities, are bioactive molecules known for their roles in regulating appetite, lipid metabolism, and energy homeostasis.^[33] They possess a wide range of biological activities, including anti-inflammatory, analgesic, neuroprotective, anticancer, antimicrobial, anticholinergic, and antifungal activities.^[34]

These findings suggest that the phytochemical composition of *Hunteria umbellata* seeds supports their potential as a natural source of bioactive compounds with diverse pharmacological activities. The high levels of saponins and flavonoids, alongside phenols, tannins, and alkaloids, likely support the therapeutic benefits

observed in traditional and experimental studies, particularly regarding metabolic health and obesity management.^[7; 35]

The nutritional analysis of dried *Hunteria umbellata* seeds (HUS) confirmed their richness in essential dietary nutrients and bioactive compounds. The proximate composition revealed carbohydrates (33.44 ± 0.01 %), crude protein (13.61 ± 0.04 %), crude fiber (26.75 ± 0.06 %), fat (12.86 ± 0.03 %), ash (3.83 ± 0.00 %), and moisture (9.51 ± 0.02 %), with a calorific value of 1277.34 ± 3.18 kJ/100 g as shown in Table 2.

Among these macronutrients, carbohydrates were the most abundant, playing a crucial role as the primary energy source for vital organs, including the heart, brain, kidneys, and nervous system. This finding is consistent with the report of^[36], who documented similar high carbohydrate content in *Hunteria umbellata* seeds, indicating a consistent biochemical profile. The high carbohydrate content positions the seeds as a valuable energy-dense food source, especially in regions with limited dietary diversity. Additionally, consumption of carbohydrate-rich foods has been linked to reduced risks of chronic diseases such as cardiovascular disease, type 2 diabetes, obesity, and certain cancers.^[37-38] Therefore, incorporating *Hunteria umbellata* seeds, which are also rich in vitamins, minerals, and antioxidants, may confer nutritional benefits that support heart health, digestion, and overall wellness.^[39-41]

Moderate amounts of protein (13.61 ± 0.04 %) further enhance the nutritional quality of the seeds by providing essential amino acids required for tissue repair, immune function, fluid balance, hormonal synthesis, and enzymatic activity.^[42-43] Although not the dominant macronutrient, protein contributes substantially to the seeds' functional dietary value.

The lipid content (12.86 ± 0.03 %) contributes to the seeds' caloric density and is likely composed of essential fatty acids that play vital roles in cellular structure, immune regulation, inflammation control, neurodevelopment, cardiovascular protection, gene expression, aging, and metabolic health.^[44-46]

The ash content (3.83 ± 0.00 %) indicates the presence of essential minerals such as calcium, magnesium, and potassium, required for growth, bone development, neuromuscular function, and electrolyte balance.^[47] Dietary fiber (26.75 ± 0.06 %) in the seeds is associated with numerous health benefits, including cardiovascular protection, blood pressure regulation, improved gastrointestinal health, decreased risk of type 2 diabetes, and support for weight management.^[48]

Furthermore, the combination of nutritional and phytochemical richness, as evidenced by the concurrent presence of bioactive compounds such as saponins, flavonoids, alkaloids, and phenolics, points to the dual

functionality of the seeds. These compounds are known to exhibit antioxidant, anti-inflammatory, and lipid-lowering properties, thereby enhancing the seeds' therapeutic potential for integration into functional food systems or utilization in herbal formulations.^[49-50] In agreement with^[51], who emphasized the energy-dense and therapeutic value of *Hunteria umbellata*, the present findings reaffirm the seeds' dual role as a nutritionally beneficial food source and a plant of ethnomedicinal importance. This convergence of nutritional and medicinal properties aligns with the increasing global interest in nutraceuticals and supports the traditional use of *Hunteria umbellata* in African medicine.

Table 2: Proximate analysis of ethanol extract of *Hunteria umbellata* seeds.

Parameter	Value (%)
Moisture g/100	9.51 ± 0.02
Ash g/100	3.83 ± 0.00
Fat g/100	12.86 ± 0.03
Crude protein g/100	13.61 ± 0.04
Crude fibre g/100	26.75 ± 0.06
Carbohydrate g/100	33.44 ± 0.01
Calorific value (kJ/100 g)	1277.34 ± 3.18

Values in Mean ± SEM, (n= 3)

The mineral profiling of *Hunteria umbellata* seeds revealed substantial levels of essential minerals, including calcium (387.33 mg/100 g), magnesium (180.00 mg/100 g), sodium (170.10 mg/100 g), potassium (19.17 mg/100 g), phosphorus (37.35 mg/100 g), and manganese (2.16 mg/100 g) as summarized in Table 3. These minerals play critical roles in numerous metabolic and physiological functions such as acid-base balance, osmotic pressure regulation, nerve impulse conduction, muscle contraction (especially cardiac muscle), bone and teeth formation, and maintaining cell membrane integrity.^[52] The mineral content observed is consistent with previous findings reported by^[36], confirming the presence of these key elements in *Hunteria umbellata* seeds.

Table 3: Mineral contents of *Hunteria umbellata* seeds.

Parameter	Mineral Content (mg/100g)
Magnesium	180.00 ± 0.10
Calcium	387.33 ± 0.17
sodium	170.10 ± 0.03
Potassium	19.17 ± 0.27
Manganese	2.16 ± 0.00
Phosphorus	37.35 ± 0.04

Values in Mean ± SEM, (n= 3)

The high calcium content (387.33 mg/100 g) highlights its potential role in supporting bone health, muscle function, cardiovascular activity, blood clotting, prevention of preeclampsia, and cholesterol regulation.^[53] Moreover, calcium regulates lipid metabolism, reduces adipocyte lipid accumulation, and promotes energy

expenditure, contributing to weight reduction and the prevention of obesity-related metabolic disorders.^[54-55]

Magnesium, present at 180.00 mg/100 g, plays a central role in limiting adipose tissue accumulation, improving glucose and insulin metabolism, enhancing endothelium-dependent vasodilation, normalizing lipid profiles, and attenuating inflammatory processes.^[56] Its adequate presence in *Hunteria umbellata* seeds aligns with the plant's traditional use in managing metabolic syndromes, especially type 2 diabetes and obesity. Magnesium intake has been inversely associated with markers of systemic inflammation and metabolic risk.^[57]

The vitamin analysis of *Hunteria umbellata* seeds revealed the presence of several essential vitamins, including vitamin A (5.89 µg RE/100 g), vitamin B1 (4.69 mg/100 g), vitamin B2 (4.87 mg/100 g), vitamin B3 (7.85 mg/100 g), vitamin B6 (2.57 mg/100 g), vitamin C (14.87 mg/100 g), and vitamin E (16.58 mg/100 g), as presented in Table 4. These vitamins are integral to both antioxidant defense systems and metabolic processes, thereby underscoring the nutritional and potential therapeutic significance of the seeds.

Vitamin A, found at 5.89 µg RE/100 g, plays a vital role in maintaining healthy vision, enhancing immune function, and supporting cellular differentiation.^[58] It also exhibits potent antioxidant activity that helps combat oxidative damage, thereby lowering the risk of chronic diseases such as cardiovascular disorders and certain cancers.^[59]

Table 4: Vitamin contents of *Hunteria umbellata* seeds.

Parameter	Vitamin Content
Vitamin A (µg RE/100g)	5.89 ± 0.02
Vitamin B1 (mg/100g)	4.69 ± 0.00
Vitamin B2 (mg/100g)	4.87 ± 0.01
Vitamin B3 (mg/100g)	7.85 ± 0.03
Vitamin B6 (mg/100g)	2.57 ± 0.00
Vitamin C (mg/100g)	14.87 ± 0.04
Vitamin E (mg/100g)	16.58 ± 0.06

Values in Mean ± SEM, (n= 3)

The B-complex vitamins such as thiamine (B1), riboflavin (B2), niacin (B3), and pyridoxine (B6) were present in significant amounts. Specifically, thiamine (4.69 mg/100 g) is essential for carbohydrate metabolism and proper neural function^[60], while riboflavin (4.87 mg/100 g) and niacin (7.85 mg/100 g) are critical coenzymes in oxidative phosphorylation and energy metabolism.^[61] Pyridoxine (2.57 mg/100 g) is indispensable in amino acid metabolism and neurotransmitter biosynthesis, including serotonin and dopamine pathways.^[62] These B vitamins work synergistically to support energy production, neural integrity, and overall metabolic health, indicating that *Hunteria umbellata* seeds may help address B-vitamin deficiencies and support metabolic resilience.

Vitamins C (14.87 mg/100 g) and E (16.58 mg/100 g) were also present at appreciable levels. These antioxidants protect biological molecules from oxidative stress, which is implicated in ageing and chronic disease pathogenesis. Vitamin C serves as a water-soluble antioxidant, neutralizing free radicals in aqueous compartments of cells and regenerating vitamin E in lipid membranes.^[63] Meanwhile, vitamin E, a lipid-soluble antioxidant, prevents lipid peroxidation in cell membranes, preserving cellular function and integrity.^[64] Both vitamins have been shown to mitigate the risk of cardiovascular disease, cancer, and neurodegenerative disorders due to their ability to reduce oxidative damage.^[20]

The synergistic action of these vitamins enhances their bioavailability and efficacy, supporting the concept that consuming them in a whole food matrix, such as that provided by *Hunteria umbellata* offers more substantial health benefits than isolated supplementation.^[65] The combined presence of antioxidant and metabolic-supporting vitamins reinforces the seeds' dual functionality as both a nutritional and therapeutic agent.

These findings affirm the growing scientific consensus on the health-promoting properties of dietary antioxidants and B vitamins, while also supporting the traditional and ethnomedicinal use of *Hunteria umbellata*

in promoting vitality and preventing disease. The vitamin composition of the seeds not only supports their classification as a nutrient-dense food but also justifies their potential inclusion in functional food formulations or nutraceutical applications.

The Fourier-transform infrared (FTIR) spectrum of the ethanolic extract of *Hunteria umbellata* seeds revealed several distinct absorption bands corresponding to functional groups indicative of bioactive phytochemicals (Table 5; Figure 2). FTIR is a widely employed analytical method in phytochemical research, providing insights into the molecular structure and composition of plant-derived substances through the identification of characteristic vibrational frequencies.^[66]

A strong, broad absorption peak at 3276.3 cm^{-1} falls within the O–H stretching region (3200–3550 cm^{-1}), indicating the presence of alcohols and phenolic compounds, including polyphenols and flavonoids. These compounds contribute significantly to the antioxidant capacity of the extract. The detection of this band corresponds with GC-MS identification of fatty acids and alcohol derivatives such as oleic acid, linoleic acid, and 10-indecen-1-ol, which possess hydroxyl (–OH) groups and exhibit both antioxidant and antimicrobial properties.^[67-68]

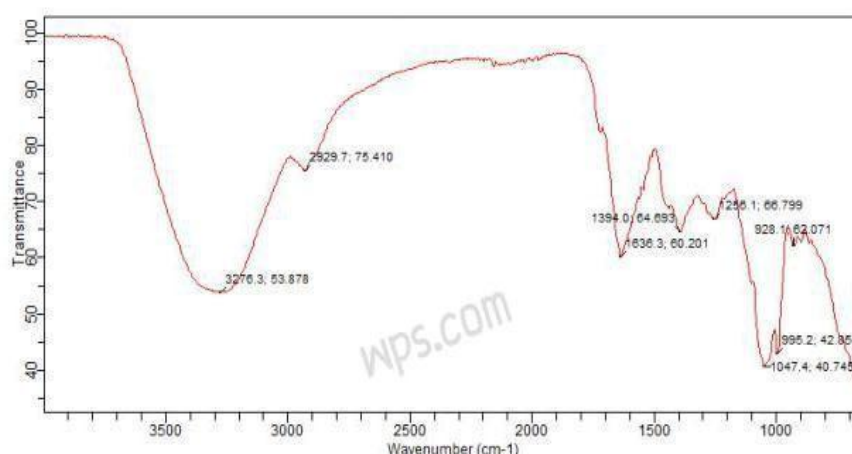


Figure 2: FTIR spectrum of *Hunteria umbellata* seed.

Table 5: FTIR Peak values and functional groups of ethanolic extract of *Hunteria umbellata* Seeds

S/No.	Standard Frequency Range (cm^{-1})	Absorption Frequency (cm^{-1})	Functional Group	Bond Type	Vibrations Mode	Intensity	Class of Compounds
1	3200-3550	3276.3	Alcohol	O-H	Stretching	Strong, broad	Polyphenols, Flavonoids
2	2840-3000	2929.7	Alkane	C-H	Stretching	Medium	Lipids, Fatty Acids
3	1395-1400	1394.0	Alkane	C-H	Bending (scissoring)	Medium	Lipids, Fatty Acids
4	1648-1638	1636.3	Alkene	C=C	Stretching	Strong	Unsaturated Fatty Acids, Terpenoids
5	1266-1342	1256.1	Aromatic amines	C-N	Stretching	Strong	Alkaloids
6	700-1000	928.1	Alkene	C-H	Bending	Strong	Unsaturated Fatty

							Acids, Terpenoids
7	700-1000	995.2	Alkene	C-H	Bending	Strong	Unsaturated Fatty Acids, Terpenoids
8	1040-1100	1047.4	Ethers	C-O	Stretching	Strong, broad	Glycosides, Flavonoids

Absorption bands at 2929.7 cm^{-1} (C–H stretching) and 1394.0 cm^{-1} (C–H bending) indicate the presence of aliphatic chains, corroborating the GC-MS identification of lipid-based esters such as methyl stearate, ethyl oleate, and palmitic acid esters. These compounds have been associated with lipid metabolism and energy homeostasis, with methyl stearate (derived from stearic acid) influencing mitochondrial function and energy regulation in humans^[69], and ethyl oleate shown to reduce food intake and body weight in animal models.^[70]

A strong peak at 1636.3 cm^{-1} , characteristic of C=C stretching, suggests the presence of unsaturated compounds such as flavonoids and sterols. This is supported by the detection of unsaturated fatty acids including linoleic and oleic acids, which have been shown to regulate adipogenesis by modulating transcription factors such as PPAR- γ and SREBP1c.^[71]

A strong absorption band at 1636.3 cm^{-1} is indicative of C=C stretching, pointing to the presence of unsaturated compounds such as flavonoids, terpenoids, and sterols. These bioactive molecules are known for modulating adipogenic transcription factors like PPAR- γ and SREBP1c, which are involved in lipid storage and adipocyte differentiation.^[71] The consistent detection of linoleic and oleic acids further supports the potential of the extract in regulating lipid metabolism.

The absorption band at 1256.1 cm^{-1} , attributed to C–N stretching vibrations, confirms the presence of aromatic amines, suggesting the occurrence of alkaloids. GC-MS analysis identified aminobenzene derivatives such as N-[5-chloro-2-hydroxybenzylidene]-2-methylthio-aminobenzene, which are associated with antimicrobial, anti-inflammatory, and metabolic-regulatory activities.^[72] This functional group supports the traditional use of *Hunteria umbellata* in ethnomedicine.

Additionally, a strong peak at 1047.4 cm^{-1} within the range for C–O stretching vibrations suggests the presence of ethers and glycosidic compounds. These structures, such as 1,3-propanediol and hydroxy[2-oxocyclohexylidene]acetic acid identified via GC-MS, are known for their antioxidant and anti-inflammatory properties.^[73] The occurrence of methyl and ethyl esters of fatty acids in this region further reinforces the lipid-modulating effects of the extract.^[74]

The combined FTIR and GC-MS data provide compelling evidence for the presence of multiple bioactive compound classes in *Hunteria umbellata* seeds, such as polyphenols (O–H), flavonoids and sterols (C=C),

alkaloids (C–N), esters (C–O), and glycosides. These compounds collectively underpin the extract's antioxidant, anti-inflammatory, antimicrobial, and lipid-lowering activities.^[75-76] These bioactivities are mechanistically linked to modulation of key obesity-related genes such as PPAR- γ , C/EBP- α , and SREBP1c, which regulate adipogenesis, lipogenesis, and energy metabolism.^[77]

In conclusion, the FTIR spectrum of the ethanolic extract of *Hunteria umbellata* seeds confirms the presence of diverse functional groups associated with health-promoting phytochemicals. These findings substantiate the ethnopharmacological use of *H. umbellata* in managing metabolic dysfunctions and provide a biochemical foundation for its therapeutic potential in obesity and related disorders.

The gas chromatography-mass spectrometry (GC-MS) analysis of the ethanolic extract of *Hunteria umbellata* seeds identified eleven major compounds based on their retention times, peak areas, molecular formulas, and molecular weights (Table 6; Figure 3). A total of eleven major peaks were identified based on their retention times and percentage composition. These compounds include a diverse array of phytochemicals, including fatty acid esters, aldehydes, and aromatic derivatives, reflecting the complex chemical composition and therapeutic potential of the extract as shown in Table 7. Table 8 outlines the proposed anti-obesity mechanisms of the identified compounds, supported by structural characteristics and relevant literature. Together with Table 7, they provide insight into the therapeutic potential of the extract.

The most prominent peak, accounting for 43.90% of the total extract composition, was identified as Cyclopropaneoctanal, 2-octyl- ($\text{C}_{19}\text{H}_{36}\text{O}$, 280.50 g/mol), eluting at 27.25 minutes. Although direct experimental data on this compound's anti-obesity activity are limited, its structural features suggest potential activation of peroxisome proliferator-activated receptors (PPARs), especially PPAR- α and PPAR- γ , key regulators of fatty acid oxidation, adipogenesis, and energy metabolism. These mechanisms align with physiological improvements such as reduced adiposity and enhanced lipid profiles observed in treated animal models.^[78-79]

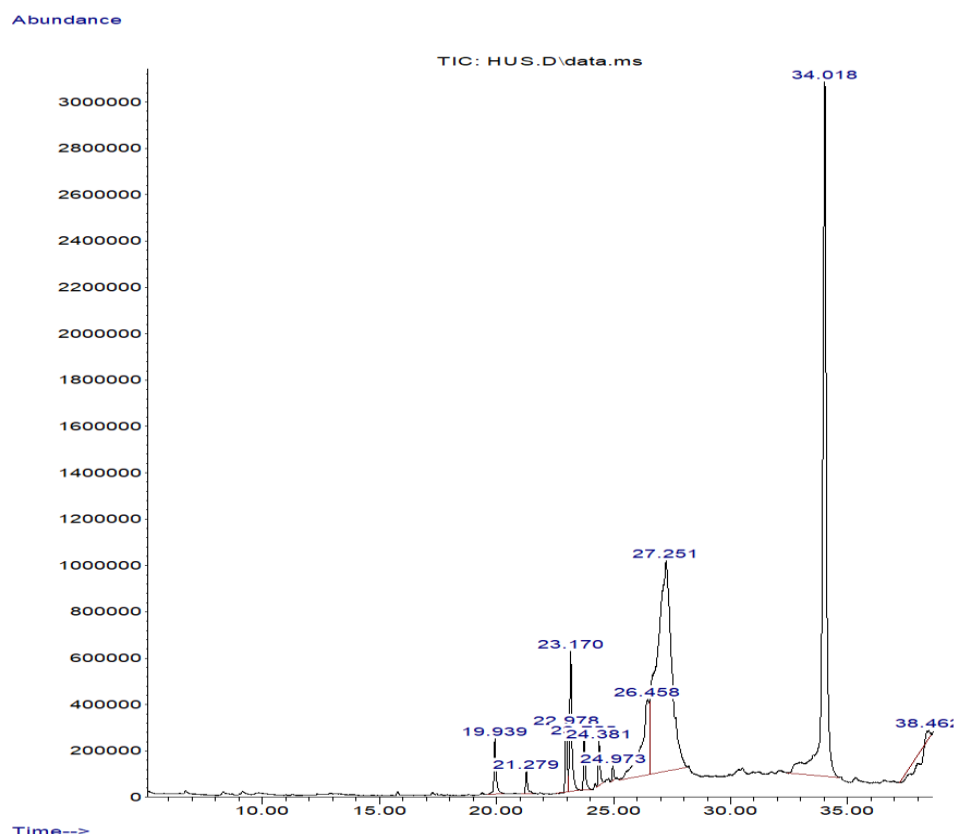


Figure 3: GC-MS chromatogram of *Hunteria umbellata* seed extract.

Another major component, Aminobenzene, N-[5-chloro-2-hydroxybenzylidene]-2-methylthio-, comprising 38.17% of the total GC-MS profile and detected at 34.02 minutes, shares structural features with chalcone derivatives. Chalcones have been shown to modulate adipocyte differentiation, lipid metabolism, and adipokine secretion (e.g., leptin and adiponectin) via PPAR- γ signaling pathways, further supporting its metabolic regulatory potential.^[80-81]

10-Undecenal, representing 8.42% of the GC-MS profile, is an aliphatic aldehyde with potential metabolic activity. It has been reported to enhance lipid metabolism by activating PPAR- α , thereby promoting fatty acid oxidation and improving lipid turnover. Its ability to modulate adipokines further supports its relevance in reducing adipose tissue mass and improving insulin responsiveness, in alignment with the outcomes observed in treated animals.^[82-83]

Among the significant fatty acid esters identified, 9-Octadecenoic acid (Z)-, methyl ester, also known as methyl oleate, was detected at 23.17 minutes with a peak area of 4.27%. This compound has been reported to enhance insulin sensitivity, suppress inflammation, and support lipid metabolism via PPAR- α activation.^[84-85] Similarly, 9,12-Octadecadienoic acid, methyl ester (linoleic acid methyl ester) appeared at 22.98 minutes with a peak area of 1.79% and is known to promote lipid oxidation and attenuate inflammatory responses.^[86]

Another compound, Pentadecanoic acid, 14-methyl-, methyl ester, was identified at 19.94 minutes (1.77%) and may stimulate mitochondrial β -oxidation and thermogenesis.^[87] Methyl stearate, detected at 23.76 minutes with a peak area of 1.48%, and (E)-9-Octadecenoic acid ethyl ester, observed at 24.38 minutes (1.17%), are both associated with anti-inflammatory and lipid-lowering effects that contribute to metabolic regulation.^[88]

Two additional minor constituents, Hexadecanoic acid, ethyl ester (21.28 minutes, 0.64%) and Octadecanoic acid, ethyl ester (24.97 minutes, 0.33%), have also demonstrated anti-inflammatory and metabolic benefits in previous studies.^[89-90]

At 38.46 minutes, a late-eluting peak revealed the presence of Cyclopropaneoctanal, 2-octyl- and 7,11-Hexadecadienal, though the peak area was recorded as negative suggesting potential integration artifacts. While the bioactivity of this compound could not be conclusively determined, its presence underscores the chemical complexity and structural diversity of the extract.

The bioactive constituents identified in the GC-MS analysis offer a mechanistic explanation for the extract's observed biological and anti-obesogenic activities. These compounds likely exert their actions through multiple pathways, including the activation of PPARs, enhancement of fatty acid oxidation, regulation of

adipokine secretion, and suppression of inflammation. The findings from this phytochemical investigation substantiate the traditional use of *Hunteria umbellata* in

managing metabolic disorders and support its potential development as a natural therapeutic agent for obesity and related complications.

Table 6: Identified Compounds in the GC-MS Analysis of *Hunteria umbellata* Seeds Extract

Peak No	Retention Time (min)	Peak Area	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Quality (%)
1	19.9390	1.7664	Pentadecanoic acid, 14-methyl, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	[98]
			Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	[97]
			Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	[97]
2	21.2795	0.6391	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.48	[96]
			Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.48	[93]
			Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.48	[91]
3	22.9783	1.7877	9,12-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.47	[99]
			9,12-Octadecadienoic acid (Z, Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.47	[99]
			9,12-Octadecadienoic acid, methyl ester, (E, E)-	C ₁₉ H ₃₄ O ₂	294.47	[99]
4	23.1698	4.2667	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.49	[99]
			9-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.49	[99]
			8-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296.49	[99]
5	23.7551	1.4833	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.50	[99]
			Methyl stearate	C ₁₉ H ₃₈ O ₂	298.50	[98]
			Methyl stearate	C ₁₉ H ₃₈ O ₂	298.50	[98]
6	24.3811	1.1705	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	310.51	[99]
			Ethyl 9-hexadecenoate	C ₁₈ H ₃₄ O ₂	282.46	[86]
			Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310.52	[81]
7	24.9729	0.3322	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312.53	[97]
			Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312.53	[93]
			Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312.53	[92]
8	26.4578	8.4159	10-Undecenal	C ₁₁ H ₂₀ O	168.28	[49]
			12-Methyl-E,E-2,13-octadecadien-1-ol	C ₁₉ H ₃₆ O	280.50	[46]
			Oleic Acid	C ₁₈ H ₃₄ O ₂	282.46	[43]
9	27.251	43.9044	Cyclopropanoethanal, 2-octyl-	C ₁₉ H ₃₆ O	280.50	[45]
			Oleic Acid	C ₁₈ H ₃₄ O ₂	282.46	[45]
			1,3-Propanediol, 2-dodecyl	C ₁₅ H ₃₂ O ₂	244.42	[44]
10	34.0181	38.1688	Aminobenzene, N-[5-chloro-2-hydroxybenzylidene]-2-methylthio-	C ₁₄ H ₁₂ ClNOS	277.80	[25]
			2-(2,3-Dimethoxy-benzylidene)-6,7-dimethylbenzo[4,5]imidazo[2,1-b]thiazol-3-one	C ₂₀ H ₁₈ N ₂ O ₃ S	382.43	[25]
			Hydroxy[2-oxocyclohexylidene]acetic acid	C ₈ H ₁₀ O ₄	170.17	[25]
11	38.4623	-1.935	Cyclopropanoethanal, 2-octyl-	C ₁₉ H ₃₆ O	280.51	[95]
			7,11-Hexadecadienal	C ₁₆ H ₂₈ O	236.39	[95]
			9,12-Octadecadien-1-ol, (Z,Z)-	C ₁₈ H ₃₄ O	266.46	[70]

Source: NIST Chemistry Webbook. <https://webbook.nist.gov/cgi/cbook.cgi?ID=019850-37-4&Units=SI>

Table 7: Biological Activities of Phytochemicals Identified in the GC-MS Analysis of *Hunteria umbellata* Seeds Extract.

Identified compounds	Compound Group	Biological Activities
Pentadecanoic acid, 14-methyl-, methyl ester	Fatty Acid Ester	Antifungal, Antimicrobial ^[91]
Hexadecanoic acid, ethyl ester	Fatty Acid Ester	Antioxidant, Hemolytic, Hypocholesterolemic ^[92]
9,12-Octadecadienoic acid, methyl ester	Fatty Acid Ester	Anti-inflammatory, Hepatoprotective ^[93]
9-Octadecenoic acid (Z)-, methyl ester	Fatty Acid Ester	Antioxidant ^[94] , Anti-inflammatory ^[95]
Methyl stearate	Fatty Acid Ester	Anti-inflammatory, Antioxidant, Antimicrobial ^[96]
(E)-9-Octadecenoic acid ethyl ester	Fatty Acid Ester	Antioxidant, Anti-inflammatory ^[97]
Octadecanoic acid, ethyl ester	Fatty Acid Ester	Emollient, Lubricant ^[98] , Biodegradable ^[99]
10-Undecenal	Aldehyde	Antimicrobial ^[100]
Cyclopropanoethanal, 2-octyl-	Cycloalkane	Antimicrobial, Antifungal, Cytotoxic ^[101]
Aminobenzene, N-[5-chloro-2-	Aromatic	Antioxidant ^[102] , Antimicrobial ^[103] , Anticancer ^[104]

hydroxybenzylidene]-2-methylthio- 7,11-Hexadecadienal	Compound Aldehyde	Antimicrobial ^[105] , Antioxidant ^[106]
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Table 8: Mechanistic roles of the major constituents in *Hunteria umbellata* seeds and their anti-obesity activity, based on their structure-function relationships and reported metabolic effects.

Compound	Relative abundance %	Mechanistic Role
Cyclopropaneoctanal, 2-octyl-	43.90	Potential PPAR- α/γ agonist; regulates lipid oxidation, anti-inflammatory, adipogenesis ^[78]
Aminobenzene, N-[5-chloro-2-hydroxybenzylidene]-2-methylthio-	38.17	Antioxidant, anti-inflammatory, and anti-adipogenic effects through ROS scavenging, cytokine suppression, inhibition of adipogenic transcription factors, and enzyme modulation ^[107]
10-Undecenal	8.42	Potential PPAR- α activator; promotes fatty acid oxidation, antimicrobial and anti-inflammatory effects ^[82-83]
9-Octadecenoic acid (Z)-, methyl ester (methyl oleate)	4.27	Improves insulin sensitivity, reduces inflammation, enhances cellular antioxidant capacity, supports lipid metabolism via PPAR- α ^[84-85]
9,12-Octadecadienoic acid, methyl ester	1.79	Promotes lipid oxidation and β -oxidation; modulates anti-inflammatory, antioxidant defense, and lipid metabolism via PPAR activation ^[86]
Pentadecanoic acid, 14-methyl-, methyl ester	1.77	Enhances mitochondrial β -oxidation and thermogenesis, intestinal barrier integrity, anti-inflammatory, and reduces oxidative stress ^[108]
Methyl stearate	1.48	Reduces fat accumulation and inflammation, improves insulin sensitivity, and exhibits antioxidant and antimicrobial properties ^[88]
(E)-9-Octadecenoic acid ethyl ester	1.17	Anti-diarrheal activity ^[109]
Hexadecanoic acid, ethyl ester	0.64	Exhibits anti-inflammatory and antioxidant activities, exerts lipid-lowering effects, improves cell membrane fluidity, and modulates lipid metabolism ^[89]
Octadecanoic acid, ethyl ester	0.33	Exhibits anti-inflammatory and antioxidant properties, modulates lipid metabolism, supports membrane integrity, and may aid in reducing fat accumulation. ^[90]

CONCLUSION

The findings highlight the rich composition of bioactive compounds and essential nutrients in *Hunteria umbellata* seed extract, reinforcing its antioxidant, anti-inflammatory, and metabolic benefits. These properties position it as a promising candidate for functional food applications and nutraceutical formulations, with potential for preventing and managing obesity and related metabolic disorders.

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