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Full Length Research Article

PHYTOCHEMICAL AND GC–MS CHARACTERIZATION OF THE HYDROETHANOLIC LEAF EXTRACT OF *CURCUMA LONGA* L. (ZINGIBERACEAE)

*[1]Okereke U.S., [1]Yayock H.C., [1]Nwoye Z.I., [1]Kasang N., [2]Idris Abdullahi

[1]Department of Biological Sciences, Faculty of Life Sciences, Kaduna State University, Kaduna
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna

*Corresponding Author Email Address: okerekeamaka8@gmail.com

ABSTRACT
Curcuma longa L. (Zingiberaceae) is a widely utilized medicinal plant, known for its anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective properties. While rhizome-based pharmacology has been extensively studied, leaf bioactivity remains underexplored. This study investigated the phytochemical composition of the leaf extract of *Curcuma longa* using standard qualitative tests and Gas Chromatography–Mass Spectrometry (GC–MS). Phytochemical screening revealed the presence of flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds. GC–MS analysis identified major constituents including ergosterol (18.03%), n-hexadecanoic acid (10.51%), oleic acid (∼10.22%), stearic acid (6.62%), phytol (0.70%), and squalene (0.57%). These bioactive compounds are reported to possess antioxidant, antimicrobial, and anti-inflammatory properties. This study characterises the chemical constituents identified in the leaves of Curcuma longa and highlights their potential as a source of bioactive compounds with possible pharmacological relevance.

Keywords: *Curcuma Longa* Leaves, Phytochemicals, GC–MS, Bioactive Compounds.

INTRODUCTION

Medicinal plants are important sources of natural bioactive compounds with therapeutic potential against various human diseases (Latif, 2025; Ansari *et al.,*2025). They play a critical role in primary health care, especially in developing countries where access to modern medicine is limited. The World Health Organization estimates that 60% of the World's population and about 80% of the population in developing countries rely on herbal medicines for their primary health needs. (Khan & Ahmad, 2019) This reliance is attributed to the rich diversity of bioactive phytochemicals present in these medicinal plants, many of which exhibit significant antimicrobial, anti-inflammatory, antiparasitic, and antioxidant properties. (Liang *et al.,* 2025). Consequently, the scientific characterization of plant metabolites has become essential for validating their applications and facilitating the discovery of novel compounds for drug development.

Curcuma longa L., commonly known as turmeric, belongs to the family Zingiberaceae. It is a perennial herb widely cultivated in tropical regions, including India, Thailand, Malaysia, Indonesia, and the Philippines. (Rajkumari & Sanatombi, 2017). It possesses pseudostems, and its leaves are large, lanceolate, or oblong. They are dark green from the upper surface and pale green from beneath. The petiole and sheath are about the same length as the blade. The spike appears before the leaves. Flowers are sterile, pale yellow with a reddish covering, while the rhizomes are yellowish-brown externally and dull orange internally, with tapered tubers. (Prasad & Aggarwal, 2021). The rhizomes emit a balmy odour and are bitter in taste, later ground into a yellow powder rich in curcumin (Puteri *et al.,* 2020; Sharma, 2020). The Plant is traditionally used to treat various ailments, such as inflammation, infections, respiratory conditions, and metabolic diseases. (Ayati *et al.,* 2019, Zhang *et al.,* 2013).

The pharmacological properties of *Curcuma longa* are attributed to its bioactive constituents, including curcuminoids, terpenoids, flavonoids, and essential oils. (Ammon & Wahl, 2021) . Bioactive compounds such as flavonoids, terpenoids, sterols, and fatty acids contribute to antioxidant, antimicrobial, anti-plasmodial, and antidepressant activities that can be exploited in drug discovery (Das *et al.,* 2015; Etemadi *et al.,* 2021). Its pharmacological effects validate its traditional uses. With minimal toxicity and proven efficacy, it remains a promising nutraceutical and pharmacological agent for modern healthcare applications (Fuloria *et al*., 2021). Although turmeric rhizomes have been extensively studied, the leaves remain underutilized despite evidence suggesting they contain significant bioactive constituents (Anekwe *et al.,* 2023; Rathore, 2012).

Phytochemical analysis provides a preliminary insight into plant metabolites while Gas Chromatography–Mass Spectrometry (GC–MS) enables the identification and quantification of chemical constituents with potential pharmacological properties (Maghsoudi *et al.,* 2017; Sun *et al.,* 2017).

This study aimed to characterize the phytochemical and GC-MS profiles of the hydroethanolic leaf extract of Curcuma longa, thereby generating foundational data for future pharmacological and nutraceutical research.

MATERIALS AND METHODS

Plant Collection, Identification, and Authentication

Leaves and stalks of Curcuma longa were collected from Zaria, Kaduna State, Nigeria. The plant was identified and authenticated at the Department of Biological Sciences, Faculty of Life Sciences, Kaduna State University, Kaduna. A voucher specimen (KASU/BSH/783) was deposited at the university herbarium for further reference.

Plant Preparation and Extraction

Leaves and stalks of Curcuma longa were washed, air-dried, and pulverized into a coarse powder. A total of two hundred and forty-one grams (241g) of powdered leaves and stalks were macerated at room temperature in one (1) litre of 70% ethanol for 72 hours

with occasional shaking. The mixture was filtered, and the filtrate was concentrated to dryness to obtain the crude extract. The dried extract was weighed, and the percentage yield calculated.

Qualitative Phytochemical Screening of Crude Plant Extract
The hydroethanolic leaf extract of *Curcuma longa* was screened for the presence or absence of flavonoids, alkaloids, tannins, saponins, and glycosides, using standard protocols (Trease & Evans, 1996; Tiwari *et al.,* 2021) as outlined below:

Test for Flavonoids:
A few drops of dilute sodium hydroxide (NaOH) were added to one millilitre (1ml) of the extract. An intense yellow colour appeared, which became colourless at the addition of dilute hydrochloric acid (HCL), indicating the presence of flavonoids.

Test for Alkaloids: Dragendoff's Test
To one milliliter (1ml) of the extract solution in a test tube, 5ml of 1% aqueous HCl was added with continuous stirring on a water bath for 5 minutes. The mixture was cooled and filtered, and a few drops of Dragendoff's reagent were added to the filtrate. A rose red precipitate was not formed, indicating the absence of alkaloids.

Test for Tannins: Lead Acetate Test
To one millilitre (1ml) of the extract, 3 drops of lead acetate solution were added. A light brown precipitate was formed, indicating the presence of tannins.

Test for Saponins: Frothing Test
Ten millilitres (10ml) of distilled water was added to the extract, and the mixture was shaken vigorously. The tube was left in a vertical position and observed for 30 minutes. A honeycomb froth that persists for ten (10) minutes indicates the presence of saponins.

Test for Glycosides
To two millilitres (2ml) of the extract, five milliliters (5ml) of dilute Sulphuric acid was added, and the mixture was boiled on a water bath for 10 minutes. It was then cooled and neutralized with 20% KOH. Five millilitres (5ml) of Fehling's solution were added and boiled. A brick red precipitate was observed as a result of the hydrolysis of glycosides, thus indicating the presence of glycosides.

Test for Phenolic Compounds: **Ferric Chloride Test**
A few drops of ferric chloride were added to one milliliter (1mL) of the crude extract.
The presence of green colouration indicates the presence of polyphenols.

Quantitative Phytochemical Analysis of Crude Plant Extract
Quantitative analysis of the crude plant extract was performed using the spectrophotometric method described by Ushie *et al.* (2018). The concentration of alkaloids, flavonoids, saponins, tannins, and polyphenols was determined.

Determination of Total Alkaloids Content
Five milligrams (5mg) of the crude extract was weighed, and two millilitres (2ml) of Hydrochloric acid (HCL) was added to the sample. The solution was completely dissolved. 1ml of the dissolved solution was pipetted into a separating funnel, 5ml of chloroform was introduced into the funnel, and the mixture was then shaken. 2.5ml of phosphate buffer was added. The solution formed two layers and was separated. The absorbance of the upper layer was measured at 725nm using a spectrophotometer.

Determination of Total Flavonoid Content
The total flavonoid content was determined using the aluminium chloride colourimetric assay method. Twenty microliters (20ul) of the sample were pipetted into a test tube, then fifteen microliters (15ul) of NaNO2 were added, and the mixture was left for five minutes. After, which fifteen microliters (15ul) of aluminium chloride and then eighty microliters (80ul) of 40% sodium hydroxide were added, and then allowed to stand for fifteen minutes at room temperature, to allow it to incubate. The absorbance was measured at 510nm using an ultraviolet-visible spectrophotometer.

Determination of Total Saponins Content
Twenty microliters (20ul) of the standard quercetin solution were pipetted into a clean test tube. Two hundred and fifty microliters (250ul) of vanillum reagent were added to the test tube, then 2.5ml of 72% sulphuric acid was also added. The sample was placed in a water bath for incubation at 60 °C for 10 minutes, after which it was allowed to cool. The absorbance of saponins was measured using a spectrophotometer at a wavelength of 544nm.

Determination of Total Polyphenols Content
Polyphenols were quantitatively analysed using the Folin-Ciocalteu assay. Twenty microliters (20ul) of the stock sample were pipetted into a test tube. One hundred and fifty microliters (150ul) of Folin-Ciocalteu reagent were added to each of the test tubes and then allowed to stay for 5-6 minutes, after which eighty microliters (80ul) of 20% solution were also added and then properly mixed. The sample was left to stand for about 60 minutes at room temperature. The absorbance was measured at 725nm using a spectrophotometer.

Gas Chromatography– Mass Spectrometry (GC–MS) Analysis
The phytochemical constituents of the ethanolic extract were analyzed using gas chromatography–mass spectrometry (GC–MS) on an Agilent system equipped with Mass-Hunter software for data acquisition and PBM Apex for library matching. Separation was achieved on a capillary column (HP-5MS, 30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed as follows: an initial temperature of 60 °C (held for 2 min), a ramp rate of 10 °C/min to 280 °C, and a 10 min hold. The injector and detector temperatures were maintained at 250 °C and 280 °C, respectively. A 1 µL aliquot of the extract (dissolved in ethanol) was injected in split-less mode. The mass spectrometer was operated in electron impact (EI) mode at 70 eV, with a mass scan range of 50–650 m/z. (Abate *et al*., 2012). These phytoconstituents were identified by comparing the spectra obtained with those in the NIST Mass Spectral Library, using PBM Apex and Agilent Mass Hunter software to confirm compound identity.

Liquid Partitioning of the Crude Plant Extract
Five milligrams (5mg) each of the crude extract of Curcuma longa was subjected to liquid partitioning using solvents of increasing polarity. The extract was dissolved in 50ml of distilled water. To isolate the fractions, 100 ml of n-Hexane was added to the mixture, which was vigorously shaken, then allowed to stand to allow phase

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separation. The upper n-hexane layer was carefully collected. The remaining aqueous phase was subsequently partitioned with ethyl acetate following the same procedure. The ethyl acetate fraction was collected while the last aqueous phase formed the ethanol fraction. All fractions were concentrated under reduced pressure to obtain the respective solvent fraction.

RESULTS

Percentage Yield of the Crude Extract

The Hydroethanolic leaf extract of *Curcuma longa* was dark brown in colour with a characteristic odour and a gummy texture.
The final percentage yield of the extract was calculated as follows:
The final percentage yield of the extract was calculated as follows:

$$\%\ Yield\ = \frac{weight\ of\ dried\ crude\ extract}{weight\ of\ crushed\ leaves\ extract} x\ \frac{100}{1}$$

$$\%\ Yield\ = \frac{31.6g}{241.0g} x\ \frac{100}{1}$$

% Yield = 13.11%
The hydro-ethanolic leaf extract of *Curcuma longa* yielded 13.11%, indicating a moderate extraction efficiency.

Qualitative Phytochemical Analysis

The qualitative phytochemical screening revealed the presence of flavonoids, polyphenols, tannins, saponins, and phenolic compounds, while alkaloids were absent.

Table 1: Qualitative phytochemical constituents of Hydro-ethanolic leaf extract of *Curcuma longa*

Phytochemicals	Results (+/−)
Flavonoids	+
Polyphenols	+
Tannins	+
Saponins	+
Alkaloids	−
Phenolic compounds	+

+ indicates present **– indicates absence**

Quantitative Phytochemical Analysis of *Curcuma longa* Hydro-ethanolic Leaf Extract

The quantitative phytochemical analysis of the crude ethanolic leaf extract of *Curcuma longa r*evealed the presence of tannins, saponins, alkaloids, flavonoids, and polyphenols, in varying concentrations as presented in Table 2. Flavonoids and Saponins were detected in higher concentrations compared to tannins and polyphenols. Alkaloids had the lowest concentrations.

Table 2: Results Showing the Quantitative Phytochemical Analysis of the Hydroethanolic Leaf Extract of *Curcuma longa*

Phytochemicals	Concentration (mg/g Extract)
Flavonoids	638.53 ± 8.16
Polyphenols	106.82 ± 0.08
Tannins	63.86 ± 0.81
Saponins	726.59 ± 3.88
Alkaloids	0.13 ± 0.00

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

A total of 120 compounds were detected, with several compounds occurring in significant proportions. The most abundant compounds were ergosterol (18.03%), palmitic acid (10.51%), octadecanoic (stearic) acid (6.62%), and oleic acid (10.22%). Other notable compounds detected include 1-octadecene (1.25%), phytol (0.70%), squalene (0.57%), various long-chain hydrocarbons, and fatty acid derivatives. These predominant compounds indicate that fatty acid and sterol derivatives constitute a major proportion of the extract. Table 3 shows 20 major compounds identified by relative abundance.

Table 3: The GC–MS profile of the Hydro-Ethanolic leaf extract of *Curcuma longa*, showing their retention time, relative abundance (Area %), and chemical class.

Rank	Retention Time (mins.)	Compound Name	Class of Compound	Relative Abundance			
1	26.0269	Ergosterol	Steroids	18.03	18.03	0.5380	83
2	13.0693	n-Hexadecanoic acid (Palmitic acid)	Saturated Fatty Acid	10.51	10.51	0.7555	35
3	15.7753	Octadecanoic acid (Stearic acid)	Saturated Fatty Acid	6.62	6.62	10.5135	99
4	15.4389	Oleic Acid	Monounsaturated Fatty Acid	5.43	5.43	1.2473	99
5	15.5117	Oleic Acid	Monounsaturated Fatty Acid	3.56	3.56	1.1769	99
6	27.4367	Hexadecanoic acid, 2-hydroxy-, methyl Ester	Fatty Acid Ester	2.44	2.44	1.2863	96

7	16.1910	1-Octadecene	Alkene	1.65	1.65	0.7045	97				
8	17.6121	9-Tricosene (Z)-	Alkene	1.57	1.57	6.6171	99				
9	14.8867	Heptadecane, 2,6,10,15-tetramethyl-	Branched Hydrocarbon	1.29	1.29	1.5692	99	9.7496	Z-2-Tridecen-1-ol	0.5380	83
10	13.4972	1-Octadecene	Alkene	1.25	1.25	0.7772	95				
11	14.8091	1-Nonadecene	Alkene	1.18	1.18	0.5670	90				
12	21.3089	Bis(2-ethylhexyl) Phthalate	Phthalate ester	0.46							
13	20.5055	Pentacos-1-ene	Long Chain Alkene	0.78	1.18	18.0326	94				
14	11.1918	Octadecane	Alkane (Hydrocarbon)	0.93	0.95	2.4426	55				
15	12.2660	Z-5-Nonadecene	Alkene	0.72	0.93						
16	15.0957	Phytol	Diterpene Alcohol	0.70	0.7						
17	9.4116	2,5-Dihydroxybenzoic Acid	Phenolic Acid Derivative	0.54	0.67						
18	9.7496	Z-2-Tridecen-1-ol	Fatty Alcohol	0.54	0.63						
19	10.5601	1-Hexacosene	Fatty Alcohol	0.59	0.58						
20	25.2269	Squalene	Triterpene	0.57	0.57						

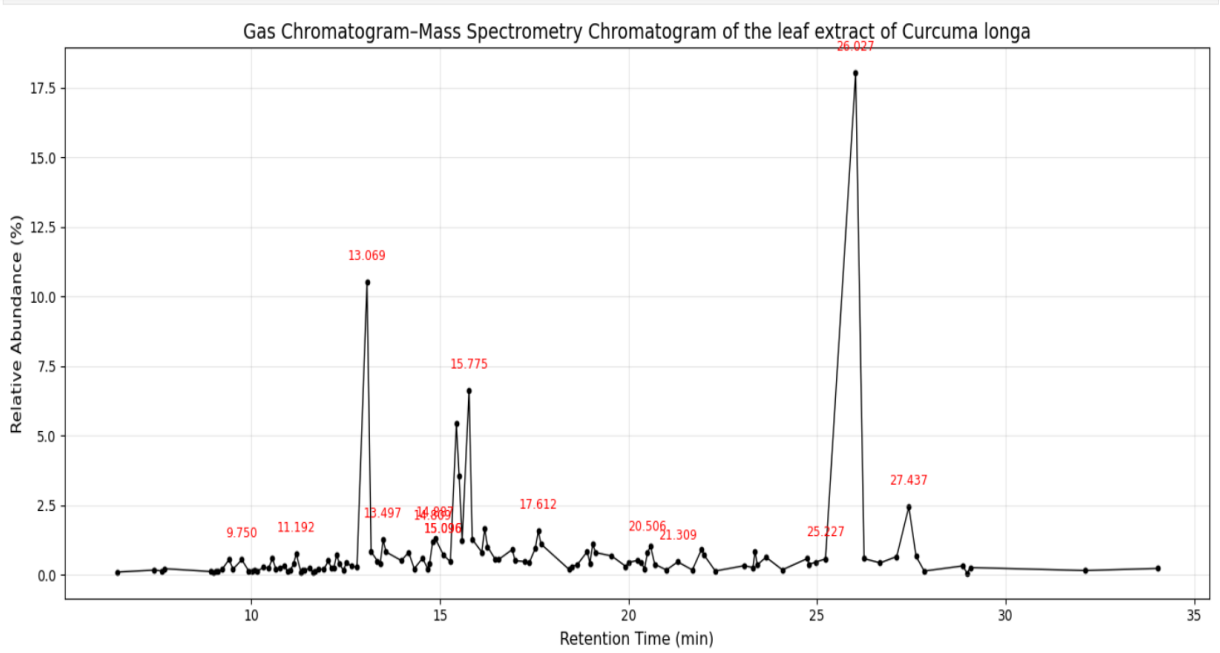

Figure 1: Total Ion Chromatogram (TIC) of GC-MS Analysis of the Leaf Extract of *Curcuma longa*

Liquid Partitioning of the Hydro-Ethanol Crude Extract of *Curcuma longa*

Liquid Partitioning of the crude extract yielded fractions of varying polarities as presented in Table 4. The following fractions: n-hexane, ethyl acetate, and ethanol were obtained. The ethanol fraction had the highest yield (43.83%), followed by the ethyl acetate fraction (25.28%), while n-hexane had the lowest yield (12.5%).

Table 4: Yield of fractions obtained from liquid- liquid partitioning of *Curcuma longa* ethanolic leaf extract

Fraction	Yield (g)	% Yield (W/W of Crude)
n- Hexane	0.75	12.5
Ethyl Acetate	1.55	25.8
Ethanol	2.63	43.83

DISCUSSION

The preliminary screening of the hydroethanolic leaf extract of *C. longa* revealed a rich phytochemical composition, with strong detection of major secondary metabolites such as flavonoids,

terpenoids, glycosides, phenolic compounds, and tannins, while alkaloids were absent. The presence of phytochemicals in the leaf extract of *Curcuma longa* agrees with a similar report by Rathore (2021), which shows that turmeric extracts have high flavonoid and saponin content, while the absence of alkaloids was also reported by Salma et al. (2022) and Zhran, Y.O. (2023). The qualitative absence of alkaloids despite trace quantitative detections suggests that the concentration was below the detection limit of the colourimetric assay. These secondary metabolites present in the crude extract of *Curcuma longa* are known to exhibit antioxidant, anti-inflammatory, antimicrobial, and anti-plasmodial properties (Das *et al.,* 2015; Etemadi *et al.,* 2021).

The GC–MS analysis of the leaf extract of Curcuma longa revealed the presence of diverse bioactive compounds, predominantly fatty acids and their derivatives, which are known for their antimicrobial, antioxidant, and anti-inflammatory properties. These findings are consistent with the report of Anekwe *et al*. (2023), who similarly identified fatty acid esters and related compounds in *Curcuma longa* leaf extracts. The presence of these bioactive constituents may account for the observed biological activities of the extract. Furthermore, the predominance of such compounds supports the earlier observation of higher yields in polar fractions, indicating that the extract's bioactivity is largely associated with polar-to-moderately polar phytochemicals. In addition, the detection of multiple bioactive constituents supports the earlier observation of higher extraction yield in polar fractions, as many of these compounds (especially oxygenated and phenolic-related compounds) exhibit moderate polarity. This further strengthens the link between solvent polarity, phytochemical composition, and biological activity. Therefore, the GC–MS profile obtained in this study corroborates existing literature and provides a chemical basis for the medicinal relevance of *Curcuma longa* leaf extract, particularly in the context of antimalarial and other therapeutic applications.

The higher fractionation yield observed in the polar solvent fractions of Curcuma longa compared to non-polar solvents such as n-hexane suggests a greater abundance of polar and moderately polar constituents in the plant. This is consistent with existing reports indicating that Curcuma longa is rich in phenolic compounds, including curcuminoids and flavonoids, which are more soluble in polar solvents such as ethanol and methanol (Matos *et al.,* 2021). The relatively low yield of the non-polar (n-hexane) fraction indicates a smaller proportion of non-polar constituents, such as lipids and terpenes. Given that phenolics and flavonoids are well-documented for their antioxidant, anti-inflammatory, and antimicrobial properties, their higher abundance in the polar fractions may account for the observed biological activities of the extract. Furthermore, these classes of compounds have been associated with antiplasmodial effects, suggesting that the enhanced recovery of polar constituents contributes significantly to Curcuma longa's therapeutic potential in malaria management (Konaté *et al.,* 2020). Therefore, the predominance of polar phytochemicals in the extract provides a plausible explanation for the bioactivity observed in this study.

Conclusion

The hydroethanolic leaf extract of *Curcuma longa* contains diverse bioactive compounds, including flavonoids, terpenoids, sterols, and fatty acids. These findings support its potential use in pharmacological and nutraceutical applications.

Recommendations

1. Evaluate antioxidant and antimicrobial activities of individual compounds.
2. Investigate synergistic effects of leaf constituents in pharmacological assays.
3. Explore in vivo toxicity studies and pharmacological studies for therapeutic application.

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