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Extraction and Identification of the Bioactive Compounds in Cola acuminata (STEM BARK AND LEAVES) Ethanol Extract Using Gas Chromatography-Mass Spectrometry (GC-MS)

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Abstract*—Bioactive compounds from plant extracts are increasingly recognized for their therapeutic relevance in both traditional and modern medicine, particularly in the context of rising drug resistance and adverse effects of synthetic drugs. This study presents a comparative GC–MS analysis of ethanol extracts of Cola acuminata stem bark and leaves to evaluate their phytochemical composition and medicinal potential. Both plant parts were harvested from vegetation located at Affa Community in Udi LGA of Enugu State, Nigeria. The extraction was carried out using soxhlet extractor and further concentrated with rotary evaporator and water bath at 90OC. From the result obtained the GC-MS analysis, both extracts contained common bioactive constituents such as acetic acid, octanoic acid, benzoic acid, phytol, conjugated linoleic acid, and α-linolenic acid, indicating a shared biochemical basis. However, the stem bark extract was quantitatively richer in long-chain fatty acids and esters, including n-hexadecanoic acid and linoleic acid derivatives, associated with antimicrobial and anti-inflammatory activities. In contrast, the leaf extract showed higher levels of phenolics, polyhydroxy compounds, and nitrogen-containing volatiles linked to antioxidant properties. Caffeine was detected only in the stem bark, suggesting tissue-specific metabolite distribution. The findings highlight distinct yet complementary bioactive profiles of C. acuminata plant parts, supporting their varied ethnomedicinal applications and potential for drug development.*

***Keywords*—** *Bioactive, Therapeutic, GC–MS, Phytochemical, Metabolite, Ethnomedicinal, Rotary Evaporator, Soxhlet Extractor.*

I. INTRODUCTION

Medicinal plants remain a cornerstone of traditional healthcare systems worldwide, especially in Africa where a large proportion of the population depends on herbal remedies for primary health needs. *Cola acuminata*, commonly known as kola nut, is one such plant of major cultural, economic, and medicinal importance in West Africa [1]. Traditionally, the plant is used as a stimulant, appetite suppressant, and in religious and social ceremonies. While research has largely focused on the seeds due to their high caffeine and theobromine content, increasing attention is being directed toward less-studied parts of the plant, particularly the stem bark, which is widely used in ethnomedicine [4].

Ethnobotanical evidence indicates that the stem bark of *C. acuminata* is employed in the treatment of ailments such as diarrhea, cough, wounds, and skin infections. These uses suggest the presence of bioactive phytochemicals with antimicrobial, anti-inflammatory, antioxidant, and possibly anticancer properties [7]. Such bioactive compounds, including alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic acids, are secondary metabolites that protect plants against pathogens and environmental stress. Their therapeutic relevance has gained renewed interest due to rising antibiotic resistance and the adverse effects associated with synthetic drugs [9,10].

Botanically, *Cola acuminata* belongs to the family Malvaceae and is an evergreen tropical tree indigenous to the rainforests of West and Central Africa, though it is now cultivated in other tropical regions [2,3]. The tree typically grows 12–20 meters tall, possesses a straight trunk with greyish-brown fissured bark, and exudes a reddish sap when cut. Its leaves are glossy, oblong to elliptical, and distinctly pointed, while its unisexual flowers are cream-colored with purple speckles and occur in clusters. The fruit is a star-shaped capsule containing several seeds known as kola nuts, which are bitter and astringent due to methylxanthines such as caffeine and theobromine [2,3,5,6].

C. acuminata thrives in humid tropical climates with high rainfall and well-drained loamy soils. The bark is thick and fibrous and has been reported to contain various phytochemicals responsible for its stimulant, aphrodisiac, and digestive benefits. Commonly known as “Oji,” “Obi,” or “Gworo” in Nigeria, the plant holds deep cultural significance across ethnic groups [15].

Ethanol is widely used in phytochemical extraction because it efficiently dissolves both polar and non-polar compounds while preserving thermolabile constituents. Its use enhances the likelihood of isolating pharmacologically active compounds from plant materials [1]. Despite widespread traditional use of *C. acuminata* stem bark, scientific validation of its bioactive composition remains limited. This study therefore seeks to bridge this gap through systematic

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phytochemical analysis of the ethanol extract, providing scientific support for its traditional applications and contributing to drug discovery and sustainable utilization efforts.

II. Methodology

2.1 Sample Collection and Preparation

The *C. acuminata* stem bark and leaves were collected from its whole *C. acuminata* tree located in vegetation of Affa Community, Udi LGA Enugu State, Nigeria. The samples were separated accordingly and packaged in a clean sterilized sample bags and further transported to the laboratory for the analysis.

The individual *C. acuminata* samples were washed thoroughly with tap water and further rinsed with distilled water. Prior to the extraction, the wet samples were spread over a clean sterile laboratory bench and allowed to dry under room temperature for about 21 days. The samples were crushed and milled into fine powder using grinding machine, packaged and kept in cool dry place for the extraction process.

2.2 Extraction Process

Soxhlet extraction method established by Joy (2015) was adopted in this study. 50 grams of each plant part (in powdered form) was added to the thimble and the extraction was carried out using ethanol as solvent. Further purification of the extract was performed using rotary evaporator until the entire solvent escaped through the condenser. The extract was concentrated using water bath at 90^{O}C until dryness. The extract was allowed to cool and then used for the analysis.

2.3 Preparation of Samples for GC Analysis

1ml of filtered extract was dissolved in 50ml of ethanol and transferred to a 100ml volumetric flask and diluted to the mark. The ethanol was evaporated at room temperature and 1ml of the reagent was added. It was sealed and heated at 40^{O}C water bath for 10 minutes. After heating, the organic sample was extracted with hexane and waters in the proportion of 1:1. The mixture was shaken vigorously by hand for 2min and was later transferred to the centrifuge. About half of the top hexane phase was transferred to a small test tube for injection.

2.4 The GC–MS Analysis

The bioactive compounds in the extracts were analyzed using GC–MS on an Agilent Technologies GC system with a GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) that included an HP-5MS column (30 m in length x 250 m in diameter x 0.25 m in film thickness). A GC–MS spectrometer detected the sample using an electron ionization system that employed high-energy electrons (70 eV). The carrier gas was 99.995% pure helium with a flow rate of 1 mL/min. The starting temperature range was 50 to 150 °C, with a rate of increase of 3 °C/min and a dwell period of around 10 minutes. Eventually, the temperature was raised at a rate of 10 °C/min until it reached 300 °C. In ansplitless mode, one microliter of the prepared 1% of the extracts, diluted with their respective solvents, was injected. Based on the peak area generated in the chromatogram, the relative amounts of the chemical components in each extract were expressed as percentages.

2.5 Identification of Components

The National Institute of Standards and Technology (NIST) database, which contains more than 82,000 patterns, was used to interpret the mass spectrum acquired via GC-MS. The NIST library's spectra of known compounds were compared to the spectrum of the unknown component. The test materials' components were identified by their name, molecular weight, molecular formula, and structure.

III. Results

GC–MS analysis of the ethanol extract of *Cola acuminata* stem bark revealed twenty bioactive compounds comprising alcohols, organic acids, fatty acids, esters, terpenoids, and alkaloids. Major constituents included n-hexadecanoic acid, methyl 2-octylcyclopropene-1-heptanoate, conjugated linoleic acid, and linoleic acid ethyl ester, indicating a dominance of long-chain fatty acids. Other notable compounds such as phytol, benzoic acid, caffeine, and α-linolenic acid suggest antioxidant, antimicrobial, anti-inflammatory, and stimulant potentials of the stem bark extract.

The results of the analysis are tabulated in the tables below (Table 1).

In table 2, GC–MS analysis of the ethanol extract of *Cola acuminata* leaves identified twenty-four compounds dominated by volatile nitrogenous compounds, alcohols, organic acids, fatty acids, phenolics, and terpenoids. Major constituents included (Z)-difluorodiazene, hydroxylamine O-methyl-, phytol, conjugated linoleic acid, and cis-13-octadecenoic acid. The presence of phenolics such as catechol and benzenetriol, alongside fatty acids and terpenoids, suggests strong antioxidant, antimicrobial, and anti-inflammatory potential of the leaf extract (Table 2).

IV. Discussion

Bioactive compounds derived from plant extracts play a vital role in modern and traditional medicine due to their diverse pharmacological properties. Plants have long served as primary sources of therapeutic agents, particularly in developing countries where herbal medicine remains integral to healthcare systems. The growing interest in plant-based bioactives is driven by increasing drug resistance and the adverse effects associated with synthetic drugs, highlighting their importance in drug discovery and development [10,14,15].

The comparative GC–MS analysis of the ethanol extracts of *Cola acuminata* stem bark and leaves reveals both shared and distinct phytochemical constituents, reflecting differences in metabolic roles and physiological functions of the two plant parts. Several low-molecular-weight volatile compounds, including acetic acid, octanoic acid, benzoic acid, phytol, conjugated linoleic acid and α-linolenic acid, were detected in both extracts, indicating a common biochemical foundation. The presence of these compounds supports earlier reports that *C. acuminata* contains diverse fatty acids, phenolics and

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terpenoids with documented antimicrobial, antioxidant and anti-inflammatory activities [2,3,14,15].

TABLE 1: GC–MS Identified Compounds in Ethanol Extract of *Cola acuminata* Stem Bark

Peak No.	Retention Time (min)	Molecular Weight (g/mol)	Molecular Formula	CAS Number	Identified Compound	Relative Height (%)	Relative Area (%)
1	2.54	66	F_2N_2	13812-43-6	(Z)-Difluorodiazene	1.60	2.42
2	2.77	60	C_3H_8O	71-23-8	1-Propanol	0.16	0.10
3	3.01	60	$C_2H_4O_2$	64-19-7	Acetic acid	0.30	0.86
4	3.64	102	$C_5H_{10}O_2$	503-74-2	3-Methylbutanoic acid	0.07	0.11
5	13.44	174	$C_8H_{16}O_2$	124-07-2	Octanoic acid	0.06	0.07
6	21.12	256	$C_{16}H_{32}O_2$	57-10-3	n-Hexadecanoic acid	5.67	6.53
7	25.00	270	$C_{15}H_{26}O$	0	Calarene epoxide	0.05	0.05
8	28.91	282	$C_{18}H_{34}O_2$	54546-22-4	Ethyl 9-hexadecenoate	0.05	0.05
9	29.18	222	$C_{15}H_{26}O$	38142-34-6	Cyclohexene-1-methanol derivative	1.55	1.81
10	30.23	294	$C_{19}H_{34}O_2$	18545-05-6	8-Octadecynoic acid, methyl ester	0.12	0.15
11	30.30	122	$C_7H_6O_2$	65-85-0	Benzoic acid	0.22	0.22
12	30.35	220	$C_{15}H_{24}O$	0	Longipinocarveol (trans)	0.60	0.59
13	30.51	294	$C_{19}H_{34}O_2$	5026-66-4	Methyl 2-octylcyclopropene-1-heptanoate	5.50	6.80
14	31.14	308	$C_{20}H_{36}O_2$	7619-08-1	Linoleic acid ethyl ester	2.36	2.67
15	32.51	296	$C_{20}H_{40}O$	150-86-7	Phytol	0.27	0.32
16	34.05	298	$C_{18}H_{34}O_3$	24560-98-3	Oxiraneoctanoic acid derivative	0.21	0.25
17	36.39	194	$C_8H_{10}N_4O_2$	58-08-2	Caffeine	0.18	0.18
18	36.52	280	$C_{18}H_{32}O_2$	544-71-8	Conjugated linoleic acid	3.08	4.64
19	37.48	236	$C_{15}H_{24}O_2$	0	Bicyclo[4.4.0]dec-2-ene-4-ol derivative	0.72	1.05
20	38.41	278	$C_{18}H_{30}O_2$	463-40-1	α-Linolenic acid	0.44	0.38

TABLE 2: GC–MS Identified Compounds in Ethanol Extract of *Cola acuminata* Leaves

Peak No.	Retention Time (min)	Molecular Weight (g/mol)	Molecular Formula	CAS Number	Identified Compound	Relative Height (%)	Relative Area (%)
1	2.49–2.75	66	F_2N_2	13812-43-6	(Z)-Difluorodiazene	7.14	10.46
2	2.58-2.72	47	CH_5NO	67-62-9	Hydroxylamine, O-methyl-	6.70	5.43
3	3.21-4.97	45	CH_3NO	865-40-7	Methane, nitroso-	0.14	0.15
4	3.31	60	C_3H_8O	67-63-0	Isopropyl alcohol	0.08	0.07
5	5.14	90	$C_4H_{10}O_2$	6982-25-8	DL-2,3-Butanediol	0.04	0.07
6	13.53	60	$C_2H_4O_2$	64-19-7	Acetic acid	0.07	0.12
7	26.72	144	$C_8H_{16}O_2$	124-07-2	Octanoic acid	0.03	0.07
8	28.03	90	$C_3H_6O_3$	79-33-4	L-Lactic acid	0.33	0.56
9	28.69	284	$C_{18}H_{36}O_2$	628-97-7	Hexadecanoic acid, ethyl ester	0.20	0.26
10	29.43	92	$C_3H_8O_3$	56-81-5	Glycerin	0.64	0.90
11	30.31	122	$C_7H_6O_2$	65-85-0	Benzoic acid	0.17	0.26
12	31.10	126	$C_6H_6O_3$	67-47-0	5-Hydroxymethylfurfural	0.04	0.06
13	31.24	290	$C_{20}H_{34}O$	24034-73-9	trans-Geranylgeraniol	0.08	0.08
14	31.69	180	$C_{10}H_{12}O_3$	28343-22-8	Phenol, 4-ethenyl-2,6-dimethoxy-	0.05	0.07
15	31.89-31.97	296	$C_{20}H_{40}O$	150-86-7	Phytol	6.59	8.34
16	32.52	228	$C_{14}H_{28}O_2$	544-63-8	Tetradecanoic acid	0.12	0.17
17	32.67	110	$C_6H_6O_2$	120-80-9	Catechol	0.11	0.13
18	35.74	284	$C_{18}H_{36}O_2$	57-11-4	Octadecanoic acid	1.38	2.19
19	36.02	282	$C_{18}H_{34}O_2$	13126-39-1	cis-13-Octadecenoic acid	3.02	4.67
20	36.53	280	$C_{18}H_{32}O_2$	544-71-8	Conjugated linoleic acid	3.42	5.87
21	37.27	278	$C_{18}H_{30}O_2$	463-40-1	α-Linolenic acid	0.39	0.53
22	37.84	312	$C_{20}H_{40}O_2$	506-30-9	Eicosanoic acid	0.49	0.79
23	38.23-38.26	310	$C_{20}H_{38}O_2$	17735-94-3	cis-13-Eicosenoic acid		
24	38.97-39.02	126	$C_6H_6O_3$	533-73-3	Benzenetriol isomers	0.48	0.54

Quantitatively, the stem bark extract showed a higher abundance of long-chain fatty acids and esters, notably n-hexadecanoic acid, methyl 2-octylcyclopropene-1-heptanoate, linoleic acid ethyl ester and conjugated linoleic acid. These compounds have been widely reported to possess antibacterial, antioxidant, hypocholesterolemic and anti-inflammatory properties [8,16]. The relatively high proportion of fatty acid derivatives in the stem bark may explain its traditional use in wound healing and treatment of infections, as fatty acids are known to disrupt microbial membranes and modulate inflammatory responses.

In contrast, the leaf extract exhibited higher levels of volatile nitrogen-containing compounds such as hydroxylamine, O-methyl- and nitroso derivatives, as well as polyhydroxy compounds like glycerin, catechol, benzenetriol isomers and 5-hydroxymethylfurfural. Phenolic compounds such as catechol and benzenetriols are potent antioxidants and free radical scavengers, contributing to cellular protection

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against oxidative stress [11,13]. The higher phytol content observed in the leaves further suggests strong antioxidant and anti-inflammatory potential, as phytol has been reported to exhibit anticancer, antimicrobial and immunomodulatory activities [12].

A notable distinction between the two extracts is the detection of caffeine exclusively in the stem bark, albeit at low concentration. This aligns with previous findings that methylxanthines, while abundant in kola nut seeds, can also be distributed in other tissues such as bark, contributing to stimulant and central nervous system effects [5,6]. The absence of caffeine in the leaves suggests differential biosynthesis or storage patterns within the plant. The comparative profile indicates that while both stem bark and leaves of *C. acuminata* are rich in bioactive compounds, the stem bark is more enriched in fatty acids and esters linked to antimicrobial and anti-inflammatory activities, whereas the leaves are richer in phenolics and oxygenated compounds associated with antioxidant potential. These findings are consistent with earlier phytochemical studies emphasizing tissue-specific variation in secondary metabolite composition and bioactivity in medicinal plants [9,10]. The results scientifically support the diverse ethnomedicinal applications of different parts of *C. acuminata*. More scientific validations are further required to elucidate and purify the various compound towards their application in modern medicine.

V. Conclusion and Further Considerations

The comparative GC–MS profiling of *Cola acuminata* stem bark and leaves demonstrates clear tissue-specific variations in bioactive compound composition, underpinning their diverse ethnomedicinal uses. While both extracts share common fatty acids, terpenoids, and phenolics, the stem bark is richer in long-chain fatty acids and esters associated with antimicrobial and anti-inflammatory activities, whereas the leaves contain higher levels of phenolic and oxygenated compounds linked to antioxidant potential. These findings scientifically validate traditional applications of different plant parts. Further studies are required to isolate, characterize, and purify the identified compounds, evaluate their toxicity and pharmacokinetics, and confirm their therapeutic efficacy through in vitro and in vivo models to support their integration into modern drug development.

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