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Pharmacognostic Characterization of *Cola millenii* K. Schum. (Malvaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MEB and ASU supervised the author IIJ who carried out the bench work and wrote the first draft of the manuscript. Authors RAU, UFU, ACI, MFA, NAA, IJU and OTU managed the literature search. Author MEB perfected the final manuscript which was approved by all authors for publication.

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ABSTRACT

Background: *Cola millenii* K. Schum. is a member of *Cola* which is lesser-known in the family Malvaceae. The leaf is used in the treatment of ringworm, scabies, gonorrhoea, diarrhoea, cough and ophthalmia by the locals.
Aim: The study determines various taxonomic, pharmacognostic and phytochemical standards helpful to ensure the identity, purity, safety and efficacy of the medicinal plant, *C. millenii*.
Methodology: The pharmacognostic characterization was determined following the guidelines given by the World Health Organization (WHO). Parameters determined included macroscopy,

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microscopy, powder microscopy, petiole anatomy, chemomicroscopy, micromeritic properties, ash values, extractive values, fluorescence analysis and preliminary phytochemical screening.
Results: Preliminary phytochemical screening for both the leaf and stem ethanol extracts showed the presence of phytochemicals. Epidermal cell shapes were polygonal with straight anticlinal walls. Stomatal distribution was hypostomatic with hexacytic, anisocytic and anomocytic stomata on the abaxial surface. Unicellular and glandular trichomes were observed on the abaxial surface. Areolation was quadrangular with calcium oxalate crystals observed along the veins. The chemomicroscopic study revealed the presence of lignin, starch, cellulose, oils, calcium oxalate crystals, mucilage and protein for both leaf and stem respectively. The fluorescence characteristics showed the presence of different colours supporting the presence of various phytoconstituents for both leaf and stem. The flow properties for both leaf and stem were fair and passable with the angle of repose of 40.6^{o} and 36.8^{o} respectively. The quantitative epidermal studies, chemomicroscopic and fluorescence characteristics revealed characteristic features for the drug. The physico-chemical results for leaf and stem gave total ash of 5.5 % and 12.07 %, water-soluble ash of 1.19 % and 3.39 %, acid-insoluble ash of 0.90 % and 2.29 % and moisture content of 4.49 % and 16.33 %, water-soluble extractive of 26.30 % and 22.50 %, ethanol-soluble extractive of 24.30 % and 16.80 %, methanol-soluble extractive of 20.90 % and 2.50 % and ethyl-acetate acetate-soluble extractive of 6.60 % and 1.10 % respectively. The GC-MS analysis revealed the presence of 31 and 45 phytochemical constituents for the leaf and stem respectively.
Conclusion: The data generated from the present study would help to authenticate *C*. *millenii* and also affirm its folklore use in traditional medicine.

Keywords: Cola millenii; flow rate; GC-MS; hexacytic; micromeritic; pharmacogostic.

1. INTRODUCTION

Plants in herbal medicine have become a basic interest for research as the major source of herbs for local people and the herbal drug industry is the wild source. Adulteration is often found in the raw materials when purchased from the market [1]. It is also reported that herbal industry and local residents face the problems of adulteration and substitution at a raw material stage [2].

Cola millenii is a tree 3-18 m high, with a low crown of arching branches and edible fruits found in deciduous, closed and transition forest of Southern Nigeria; it belongs to the family Malvaceae [3,4] with the common name Monkey Kola (English). The plant fruits between September – November.

Quality control of crude drugs and herbal formulation is of vital importance in justifying their acceptability in modern medicine. One of the main obstacles to the acceptance of traditional medicine in developed countries is lack of documentation and stringent quality control [5]. However, standardization of medicinal herbs includes proper identification, quality control and quality assurance. Therefore, the evaluation of standards can be done by assessing the organoleptic (colour, odour, taste) macroscopic, microscopic and physicochemical parameters [6]. It is known locally in Efik/Ibibio; Mba utong-ita, Mkpa mfet okpo ebot, Hausa, Goro mbiri, Yoruba; Obi edun, Igbo; Uto. In South-Western Nigeria, the Yoruba apply the leaves and fruits of *Cola millenii* in the treatment of ringworm, scabies, gonorrhoea, diarrhoea, cough, vomiting, dysentery and chest complaints [7]. They are eaten as edible fruits by the peasant farmers during the peak season. The pulp is licked as a dessert and it is used in the management of stomach aches [8].

Due to traditionally significant factors, acceptance and dependence on medicinal plants, they have become a topic of worldwide importance in both the developing and the developed climes. Due to limited data on safety and efficacy, poor regulation and control, medicinal plants have now become a key issue in industrialized and developing countries. Both the general consumer and health-care professionals need up-to-date authoritative information on the safety and efficacy of medicinal plants. World Health Organization encourages countries to provide safe and effective traditional remedies and practices in public and private health services. Preparations of monographs are intended primarily to promote harmonization in the use of herbal medicines with respect to levels of safety, efficacy, and quality control [9].

Despite these extensive uses of *Cola millenii* in folk medicine, there has been no report on the standardization of this drug. Therefore, this study is aimed at investigating the pharmacognostic and taxonomic parameters to aid in the identification and safe use of this drug.

1.1 Classification of *Cola millenii*:

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Malvales
Family:	Malvaceae
Genus:	*Cola*
Species:	*C. millenii* K. Schum.

Source: Angiosperm Phylogeny Group [10].

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The fresh samples of *Cola millenii* were collected in September, 2018 from Osomba Hills, Oban in Akamkpa Local Government Area of Cross River State with the GPS reading as ± 17m 5^{o} 27′59.28N, 008^{o} 37’39.6E and preserved in FAA (Formalin Acetic Acid). The plant was authenticated by Prof. M. E. Bassey a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo and voucher specimen have been deposited in the herbarium with the number “Johnny,UUH4082/Osomba”. The collected leaves and stems were washed under running tap water, rinsed with distilled water, chopped into pieces, dried under shade at room temperature. The dried leaves and stems were pulverized using Sonik SON/MPR/EE/1314 electric blender, sieved through 350 micron sieve size and stored in airtight bottles to avoid interference from some natural factors such as moisture, microorganisms, humidity etc. before use.

2.2 Macro-morphological Evaluation of Leaf and Stem

2.2.1 Organoleptic (sensory) parameters

Organoleptic (sensory) parameters of fresh leaf and stem as well as their powders such as colour, odour, and taste were evaluated by the sense organs and documented [11].

2.2.2 Morphological characteristics

Morphological and related taxonomic analysis were made on the stem, leaves (apex, base, margin, hairiness) petioles, stipules, fruits and the characters were described using standard methods [11].

2.3 Microscopic Evaluation of the Leaf

2.3.1 Qualitative microscopy

For anatomical studies, the standard median portion of the well expanded matured leaf was obtained. Epidermal peels of both adaxial and abaxial surfaces were made by placing the leaf on a clean glass slide with the surface to be studied facing down. The specimens were irrigated with water holding it downward from one end and then the epidermis above the desired surface was scrapped off carefully with sharp razor blade. The loose cells were then washed off with water and the epidermis was stained in 1 % aqueous solution of safranin-O for 2-3 minutes and washed again in water to remove excess stain and mounted in 10 % glycerol on a glass slide and covered with a glass cover slip before viewing with an Olympus CX21 binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 Amscope microscope eyepiece camera. Measurements were done at ×10 while ×40 for photomicrographs [12].

2.3.2 Quantitative microscopy

Quantitative microscopic parameters such as leaf constant studies viz. stomatal length and width, stomatal pore length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness, vein islet number, vein termination number, areole length and width and trichome length and width were carried out using standard procedures [10].

All measurements were made using a calibrated ocular micrometer and thirty (30) microscopic fields chosen at random were used and data presented as mean ± Standard Error of Mean (SEM).

The stomata index (S.I) was determined according to Killedar *et al* [12] using the formula:

Stomatal Index (SI) = S/E +S x 100
Where: S = number of stomata per unit area

E = number of epidermal cells in the same area.

2.4 Microscopic Evaluation of Petiole Section

Transverse section of the petioles was prepared by embedding the portion in pawpaw tissue after which free-hand sectioning was made with a sharp razor blade. The sections were cleared in 20 % sodium hypochlorite (NaOCl) for 3-5 minutes and thereafter thoroughly rinsed five times in distilled water. Sections were stained in safranin-O solution for a period of 2-3 minutes then rinsed carefully with distilled water to remove excess stain. Dehydration followed and then sections were mounted in 10 % glycerol solution and viewed with an Olympus CX21 binocular microscope. Photomicrographs were taken with an MD500 Amscope camera [12].

2.5 Evaluation of Powders

Chemomicroscopic studies of the coarse powders of the leaf and stem were carried out to study the microscopical characters as well as their chemomicroscopic properties viz. cellulose, mucilage, lignin, starch, protein, oils and calcium oxalate crystals [13,14].

Preliminary phytochemical screening was carried out on the powdered leaf and stem after extracting with 60 % ethanol and allowed to stand for 72 hours with occasional shaking. It was then filtered and concentrated to dryness. The extracts were then subjected to phytochemical screening using standard methods [15].

The fluorescent analysis of *C. millenii* dried leaf and stem powders was carried out using the standard method [16].

The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash), soluble extractive values viz. ethanol, ethyl acetate, methanol and water were performed according to the WHO prescribed guidelines on quality control methods for medicinal plant materials [17].

The micromeritic characteristics of leaf and stem powder viz. bulk density, tap density, angle of repose, Hausner's ratio, Carr's index and pH were determined according to standard methods [18].

Gas Chromatography-Mass Spectroscopy was carried out on the crude ethanol extracts according to standard methods [19].

All experiments were repeated at least three (3) times except for the quantitative microscopy where thirty (30) determinations were done. Results were reported as Mean±SEM (Standard Error of the Mean).

3. RESULTS

3.1 Macromorphological and Organoleptic Evaluation of Leaf and Stem of *C. millenii*

The results of the macro-morphological, micro-morphological and organoleptic evaluation of leaf and stem of *C. millenii* are summarized in Fig. 1 and Table 1.

The tree is about 12-18 m which occurs in the drier parts of the forest region, especially in secondary forest. The stem is erect and woody. The leaf is simple, petiolate, palmately tri-lobed but not dissected up to the petiole. The leaf is 7.5-22 cm long and 5.5-19 cm wide. The petiole is 12-22cm long; Leaf is hairy on the abaxial surface and scabrid on adaxial surface. Leaf apex is shortly acuminate to acute. Fruits occur in clusters of 5-11 orange-red velvety epicarp each containing 8-10 seeds. The seed is brown in colour with white fleshy mesocarp.

3.2 Microscopic Evaluation of Leaf

The microscopic evaluation of the leaf and petiole of *C. millenii* are summarized in Fig. 2.

The results for the qualitative and quantitative micro-morphological characters is summarized in Table 2.

C. *millenii* leaves were hypostomatic (stomata on the abaxial surface only). Unicellular trichomes were observed on the adaxial and abaxial surfaces. Lower values were obtained for areole length (211.99±11.33 µm) areole width (151.94±6.93 µm) and vein islets number (8.57±0.27) for the adaxial epidermis (Table 2). While higher values for areole length (255.91±12.88µm), areole width (175.06±6.69µm) and vein isles number (12.03±0.30) were obtained for the abaxial epidermis. On the other hand, the vein termination numbers were higher (4.20±0.81) for the adaxial epidermis than for the abaxial epidermis (2.87±0.15).

The preliminary phytochemical screening results are summarized in Table 3.

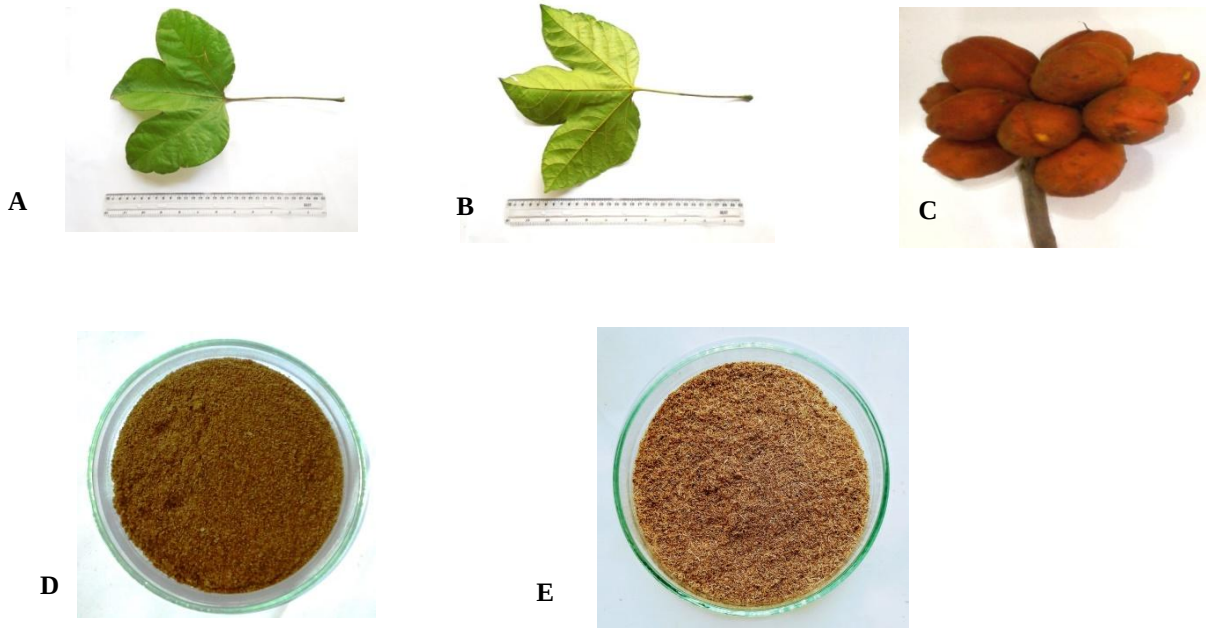


Fig. 1. A – Adaxial Surface, B – Abaxial Surface, C – whole fruit: D-deep brownish leaf powder, E- light brown stem powder

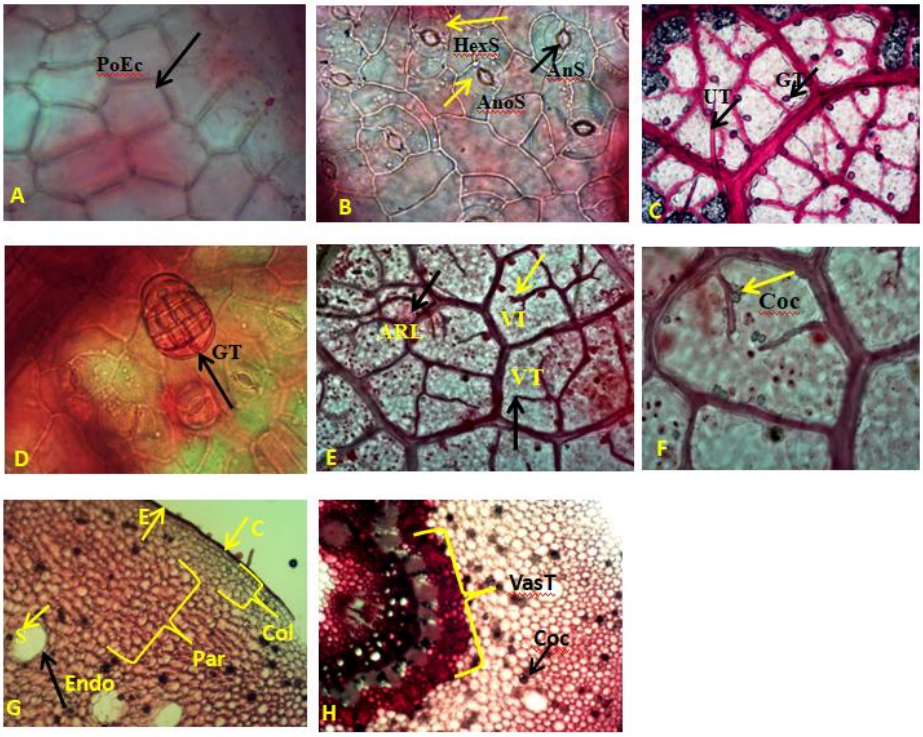


Fig. 2. (A): Polygonal epidermal cells and straight ACWP adaxial surface × 40: (B): Hexacytic (HexS), Anisocytic (AnS) and Anomocytic (AnoS) stomata abaxial × 40; (C): Unicellulat trichome (UT), Glandular trichome (GT) ×10 (D): Glandular trichome (GT) ×40; (E): Vein termination (VT), Veinislet (Vl) and areole (ARL) × 40, (F): Calcium oxalate crystals (Coc) druse; (G): Petiole showing- Cuticle (C); Epidermis (E); Collenchyma (Col); Parenchyma (Par); Endodermis (Endo); Stele (S); × 40 (H): Vascular tissues (VasT); Calcium oxalate crystals (Coc) petiole × 40.

Table 1. Macromorphological, micro-morphological and organoleptic evaluation of leaf, stem and fruit of *C. millenii*

Parameters	Macromorphological Characters	Micromorphological Characters		Organoleptic Characters
Leaf				
Type	Simple, petiolate, palmately tri-lobed			
Leaf length	7.5-22cm			
Leaf width	5.5-19cm			
Petiole length	12-22cm			
Leaf apex	Short acuminate to acute			
Margin	Entire to wavy			
Texture	Adaxial surface: Scabrid Abaxial surface: Pubescent			
Leaf shape	Palmately lobed			
Stem				
Type	Erect			
Shape	Cylindrical			
Size	7-11cm in diameter			
Indumentum	Pubescent			
Fruit				
Colour	Orange-red velvety epicarp Mesocarp-white			
Length	5-7cm			
Width	2-4cm			
Seeds	8-10 occurring in pod			
Epidermal cell		Adaxial	Abaxial	
Cell shape		Polygonal	Polygonal	
Anticlinal wall pattern		Straight	Straight	
Stomata type		-	Anisocytic, anomocytic and hexacytic	
Trichome		-	Unicellular and glandular	
Colour				Adaxial surface: green Abaxial surface: light green Leaf powder: deep brown Stem powder: light brown
Odour				No characteristic taste
Taste				No characteristic taste

Table 2. Qualitative and Quantitative micro-morphological characters of *C. millenii* (Mean ±SEM)

S/N	Parameters	Leaf surface	
1.		**Adaxial**	**Abaxial**
	Cell shape ×40	Polygonal	Polygonal
	pattern ×40	Straight	Straight
	Cell number ×10	398.10±8.40	135.87±2.81
	Cell length(µm) ±SEM ×10	87.75±2.49	59.40±2.70
	Cell width(µm)±SEM ×10	41.29±1.16	32.26±1.30
	Cell length/width ratio	2:1	1:1
	Cell wall thickness (µm)±SEM ×10	2.90±0.13	2.78±0.12
2.	**Vein architecture**		
	Areole length(µm)±SEM ×10	211.99±11.33	255.91±12.88
	Areole width(µm)±SEM ×10	151.94±6.93	175.06±6.69
	Termination number ×10	4.20±0.81	2.87±0.15
	Vein islet number ×10	8.57±0.27	12.03±0.30
3.	**Stomatal characters**		
	Length (µm) ±SEM ×10	-	42.07±0.59
	Width (µm) ±SEM ×10	-	32.52±1.35
	Guard cell length(µm)±SEM ×10	-	39.00±0.54
	Guard cell width (µm)±SEM ×10	-	18.79±0.82
	Stomatal number	-	67.43±1.26
	Stomatal type ×40	-	Hexacytic, anisocytic and anomocytic
	Pore length (µm)±SEM ×10	-	24.72±0.36
	Pore width(µm)±SEM ×10	-	14.98±0.49
	Stomatal index %	-	33.17
4.	**Trichome**		
	Length(µm)±SEM ×10	674.80±29.49	501.31±26.11
	Width(µm)±SEM ×10	19.97±0.59	20.54±0.48

Data was presented in Mean ±SEM (Mean±Standard Error of Mean of 30 determinations)

3.3 Physicochemical Evaluation

Dried powders of the leaf and stem were used for the quantitative evaluation of different physicochemical determinations. The moisture contents of the dried leaf and stem powders of 4.49 %w/w and 16.33 %w/w were determined by loss on drying method and presented in Table 4. These results showed that the moisture contents were not high for the leaf and could not encourage fungal and bacterial growth but high for the stem which care must be taken during preservation. The analytical results of the total ash were found to be 5.50%w/w and 12.07%w/w. The total ash for both leaf and stem were amorphous and greyish white in colour. The water-soluble ash of 1.19%w/w and 3.39%w/w, acid-insoluble ash of 0.90%w/w and 2.29%w/w were obtained respectively for the leaf and stem.

For the extractive values, the water-soluble extractive values for the leaf and stem were found to be higher in water-soluble fractions with values of 26.30±0.20 %w/w and 22.50±1.40%w/w compared to those of ethanol, ethyl acetate and methanol respectively as seen in Table 4.

3.4 Powdered Microscopic Analysis

The results of the powder analysis of *C. millenii* is presented in Fig. 3.

The results of the micromeritic evaluation is summarized in Table 5.

Table 3. Preliminary phytochemical screening result for leaf and stem of *C. millenii*

Test	*C. millenii*	
	Leaf	**Stem**
Alkaloids	+	+
Flavonoids	-	-
Saponins	+	+
Tannins	+	+
Cardiac glycosides	+	+
Anthraquinone	-	-

Key: + = Present, - = Absent

Table 4. Physicochemical constants of leaf and stem of *Cola millenii*

Parameter	Leaf (%w/w)	Stem (%w/w)
Moisture content	4.49	16.33
Total ash values	5.50	12.07
Water-soluble ash values	1.19	3.39
Acid-insoluble values	0.90	2.29
Extractive values (%w/w)		
Water-soluble	26.30 ± 0.20	22.50 ± 1.40
Ethanol	24.30 ± 0.10	16.80 ± 0.10
Methanol	20.90 ± 0.10	2.50 ± 0.40
Ethyl acetate	6.60 ± 0.40	1.10 ± 0.30

Results presented as Mean±SEM of Three (3) Replicate

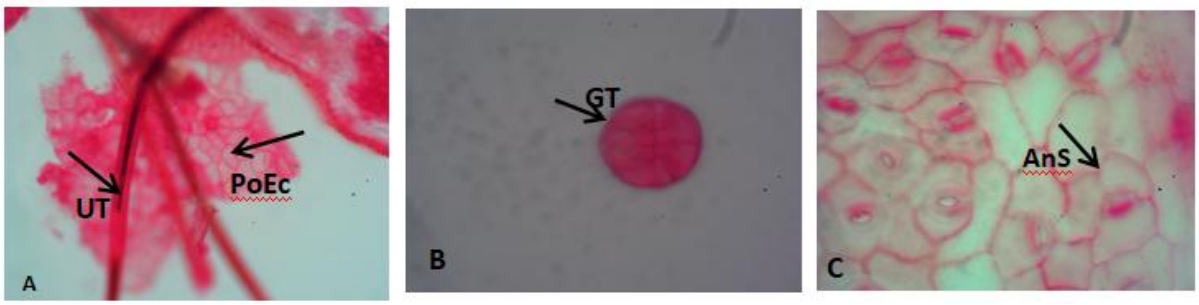


Fig. 3. (A): Polygonal epidermal cell with straight ACWP × 10; (B): Glandular trichome ×40; (C): Anisocytic stomata × 10

The chemomicroscopic examination of the leaf and stem revealed the presence of lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein as summarized in Table 6.

The colour behaviour of the dried leaf and stem powdered drug dissolved in organic solvents was observed both under visible and Ultra-violet (UV) light. The reactions of the drugs emitted fluorescence light as summarized in Table 7. The powdered drug solutions had exhibited a wide range of fluorescence colours under UV and visible light.

3.5 The Gas Chromatography-Mass Spectrometry

The Gas Chromatography-Mass Spectrometry (GC-MS) of the ethanol extracts for the leaf and stem of *C. millenii* revealed the presence of 31 and 45 suggested chemical constituents as presented in Tables 8 and Fig. 4 for the leaf and Table 9 and Fig. 5 for the stem respectively.

Table 5. Micromeritic evaluation of powdered leaf and stem of *C. millenii*

Micromeritic Parameters	*C. millenii* Leaf	*C. millenii* stem
Bulk Volume (mL)	52.17±0.17	46.00±0.50
Tapped Volume (mL)	37.17±0.44	32.83±0.17
Bulk Density (g/mL)	0.19±0.00	0.23±0.00
Tapped Density (g/mL)	0.27±0.00	0.31±0.00
Hausner's Ratio	1.42±0.02	1.36±0.03
Carr's Index (%)	29.63±0.90	26.00±1.73
Diameter of Heap (cm)	7.52±0.10	7.31±0.08
Height of Heap (cm)	3.20±0.03	2.75±0.06
Flow Time (sec)	27.34±0.33	23.67±0.88
Flow Rate (g/sec)	0.36	0.42
Angle of Repose (^{0})	40.60	36.8
pH Cold	4.24±0.01	3.96±0.00
pH Hot	6.23±0.00	6.77±0.00

Results presented as Mean±SEM of Three (3) Replicate

Table 6. Chemomicroscopic evaluation of the leaf and stem of *C. millenii*

Test	*C. millenii*	
	Leaf	Stem
Lignin	+	+
Starch	+	+
Cellulose	+	+
Oils	+	
Calcium oxalate crystals	+	+
Mucilage	+	+
Protein	+	+

Key: + = Present, - =Absent

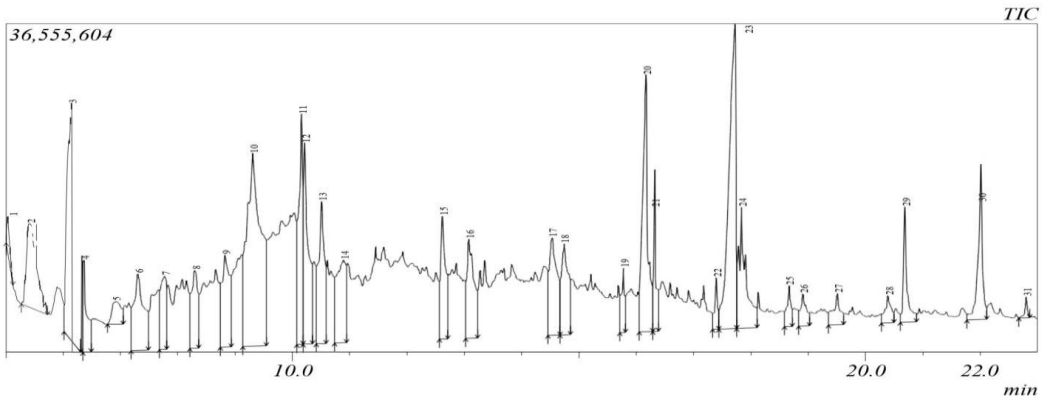


Fig. 4. GC-MS chromatogram of ethanolic leaf extract of *Cola millenii*

Table 7. Fluorescence analysis of *C. millenii* leaf and stem powder

Extract	Sample	Visible light	Under UV light Short Wave length (253.7nm)	Under UV light Long Wave length (365nm)
Picric acid(2,4,6-trinitrophenol)	Leaf	Brown	Green black	Black
	Stem	Light brown	Light green black	Black
Dichloromethane	Leaf	Green	Light brown	Brown
	Stem	Brown	Orange	Light brown
Ethyl acetate	Leaf	Green	Orange	Pink
	Stem	Light brown	Red	Grey
Methanol	Leaf	Light brown	Maroun	Brown
	Stem	Light brown	Blue	Grey
Water	Leaf	Green	Orange	Brown
	Stem	Dirty green	Red	Grey
Ferric chloride	Leaf	Green	Pink	Brown
	Stem	Light green	Maroon	Light brown
Acetic acid	Leaf	Yellowish green	Green	Light green
	Stem	Green	Light yellow	Deep green
Iodine in water (1%)	Leaf	Green	Black	Brown green
	Stem	Deep green	Ash	Green

Table 8. Phytochemical constituents identified from ethanol extract of leaf from *Cola millenii* by GC-MS analysis

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	5.030	Hydrazine, ethyl-	$C_2H_8N_2$	60	0.38
2	5.422	N,N-Dimethylaminoethanol	$C_4H_{11}NO$	89	4.16
3	6.150	2-Butenal, 2-methyl-, (E)-	C_5H_8O	84	5.09
4	6.367	2-Butenoic acid, 3-methyl-	$C_5H_8O_2$	100	1.57
5	6.921	Bicyclo[2.2.1]heptan-2-ol	$C_7H_{12}O$	112	1.02
6	7.307	Azacyclohexane, 3-methylamino-1-methyl-	$C_7H_{16}N_2$	128	3.68
7	7.774	Cyclopentanol, 2,4,4-trimethyl-	$C_8H_{16}O$	128	2.16
8	8.304	2-Dimethylaminomethyl-4-methoxy-cyclohexanone	$C_{10}H_{19}NO_2$	185	2.44
9	8.825	Pent-1-en-3-one, 1-(2-furyl)-5-dimethylamino-	$C_{11}H_{15}NO_2$	193	3.49
10	9.311	N-(4-Tolylsulfonylmethyl)formamide	$C_9H_{11}NO_3S$	213	12.99
11	10.165	4-Hydroxy-3-methylacetophenone	$C_9H_{10}O_2$	150	4.37
12	10.216	4-Hydroxy-3-methylacetophenone	$C_9H_{10}O_2$	150	4.66
13	10.512	2-Butenal, 2-methyl-, diethylhydrazone	$C_9H1_8N_2$	154	4.13
14	10.892	2-Dimethylaminomethyl-4-methoxy-cyclohexano	$C_{10}H_{19}NO_2$	185	3.77
15	12.621	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)-	$C_{10}H_{12}O_3$	180	2.99
16	13.082	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	$C_{10}H_{16}O_2$	168	3.45
17	14.533	9-Undecen-2-one, 6,10-dimethyl-	$C_{13}H_{24}O$	196	3.67
18	14.749	1-Heptadec-1-ynyl-cyclopentanol	$C_{22}H_{40}O$	320	3.04
19	15.777	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268	1.05
20	16.176	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.58
21	16.323	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.71
22	17.400	Phytol	$C_{20}H_{40}O$	296	0.68
23	17.722	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{13}H_{30}O_2$	278	10.05
24	17.837	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{13}H_{30}O_2$	278	4.14
25	18.671	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	0.80
26	18.911	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.88
27	19.509	Cyclopentadecanone	$C_{15}H_{28}O$	224	1.04
28	20.394	10-Undecen-1-al, 2-methyl-	$C_{12}H_{22}O$	182	0.78
29	20.690	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	1.91
30	22.015	Butyl 9,12,15-octadecatrienoate	$C_{22}H_{38}O_2$	334	3.07
31	22.806	3.beta.-Acetoxy-5-pregnen-7,20-dione	$C_{23}H_{32}O_4$	372	0.26

Table 9. Chemical components of ethanolic stem extract of *Cola millenii*

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	5.518	Cyclohexane, 1,4-dimethoxy-2-methyl-, stereoisomer	$C_9H_{18}O_2$	158	0.10
2	5.723	2(5H)-Furanone	$C_4H_4O_2$	84	5.12
3	5.983	5-Octen-2-ol, 5-methyl-	$C_9H_{18}O$	142	1.71
4	7.593	3-Cyclohexen-1-carboxaldehyde, 3-methyl-	$C_8H_{12}O$	124	6.31
5	8.485	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6 methyl-	$C_6H_8O_4$	144	18.21
6	9.244	2-Pentanone, 5-hydroxy-	$C_5H_{10}O_2$	102	12.40
7	9.942	10-Undecen-1-al, 2-methyl-	$C_{12}H_{22}O$	182	0.76
8	10.076	Cycloheptanol, 2-chloro-, trans-	$C_7H_{13}ClO$	148	0.99
9	10.153	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	2.67
10	10.481	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	2.32
11	10.617	5.alpha.-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	0.80
12	10.784	8-Methylenecyclooctene-3,4-diol	$C_9H_{14}O_2$	154	0.33
13	11.064	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236	2.09
14	11.426	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	1.65
15	11.580	.alpha.-D-Glucopyranoside,O-.alpha.-D-glucopyranosyl-	$C_{18}H_{32}O_{16}$	504	3.97
16	11.957	Methyl .beta.-d-ribofuranoside	$C_6H_{12}O_5$	164	7.59
17	12.168	2-Amino-8-[3-d-ribofuranosyl]imidazo[1,2-a]-s-triazin-4-one	$C_{10}H_{13}N_5O_5$	283	1.10
18	12.267	Silane, trimethyl(4-methyl-3-penten-1-ynyl)-	$C_9H_{16}Si$	152	1.78
19	12.509	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	$C_{16}H_{28}O_2$	252	0.98
20	12.601	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	$C_{16}H_{28}O_2$	252	1.18
21	13.101	Phenol, 3,4,5-trimethoxy-	$C_9H_{12}O_4$	184	1.10
22	13.821	.alpha.-D-Galactopyranoside, methyl	$C_7H_{14}O_6$	194	16.82
23	14.263	Lumazine, 8-ethyl-6,7-dimethyl-	$C_{10}H_{12}N_4O_2$	220	3.06
24	14.569	2-(2-Hydroxy-2-p-methoxyphenylethyl)-3-methylpyrazine	$C_{14}H_{16}N_2O_2$	244	2.91
25	15.201	8-Methyl-6-nonenoic acid	$C_{10}H_{18}O_2$	170	0.05
26	15.773	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)	$C_{25}H_{42}O_2$	374	0.08
27	16.063	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.81
28	16.199	Cyclopentanol, 3-methyl-2-(2-pentenyl)-	$C_{11}H_{20}O$	168	0.34

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
29	16.308	Ethyl cyclohexanepropionate	$C_{11}H_{20}O_2$	184	0.08
30	16.576	2-Propenoic acid, 3-[2-(aminocarbonyl)phenyl]-	$C_{10}H_9NO_3$	191	0.06
31	16.891	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	0.05
32	17.483	Cyclopropaneoctanoic acid,2-[[2-[(2 ethylcyclopropyl)methyl] cyclopropyl	$C_{22}H_{38}O_2$	334	0.26
33	17.549	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.35
34	17.764	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl	$C_{22}H_{38}O_2$	334	0.06
35	17.808	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.09
36	18.601	Cyclohexane, 1,4-dimethoxy-2-methyl-, stereoisomer	$C_9H_{18}O_2$	158	0.06
37	18.819	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl	$C_{30}H_{52}O_3Si$	488	0.07
38	18.905	i-Propyl 9,12,15-octadecatrienoate	$C_{21}H_{36}O_2$	320	0.06
39	19.067	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254	0.07
40	19.102	Isopropyl linoleate	$C_{21}H_{38}O_2$	322	0.06
41	19.464	8-Methyl-6-nonenamide	$C_{10}H_{19}NO$	169	0.13
42	20.102	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol,25-[(trimethylsilyl	$C_{30}H_{52}O_3Si$	488	0.13
43	21.034	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	$C_{31}H_{50}O_2$	454	0.43
44	21.663	Tetradecanoic acid, 3,3a,4,6a,7,8,9,10,10a,10b-decahydro-3a,10a	$C_{31}H_{50}O_6$	518	0.66
45	22.973	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl	$C_{15}H_{26}O$	222	0.14

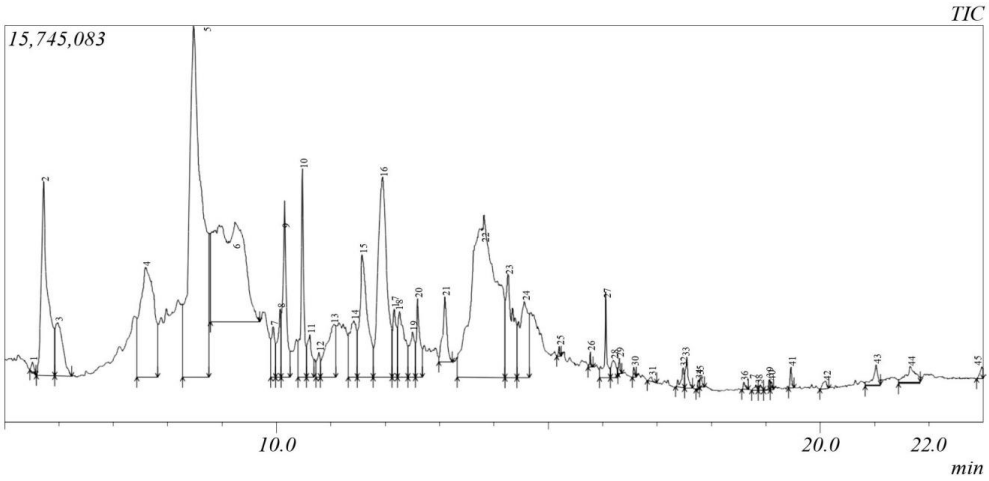
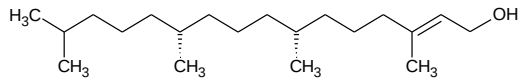
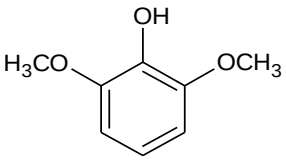


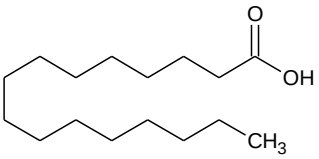
Fig. 5. GC-MS chromatogram of ethanolic stem extract of *Cola millenii*



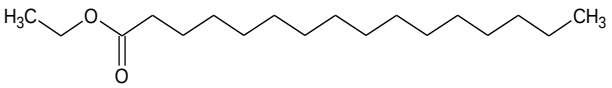
Phytol



Phenol, 2,6-dimethoxy



n-Hexadecanoic acid



Hexadecanoic ethyl ester

Fig. 6. Structures of some phytochemical constituents in the leaf and stem GC-MS ethanolic extract

4. DISCUSSION

The major problem of the commercial supply of crude drugs is the identification of the genuine drug. Taxonomic and pharmacognostic standardization including physicochemical evaluation is meant for identification, authentication and detection of adulteration and also a compilation of quality control measures of crude drugs. Many plants have not been evaluated for proper identification even though the first step towards standardization is ensuring the quality of its starting material is by proper authentication. With the application of modern techniques, identification of plant drugs by pharmacognostic studies is more reliable [20] and according to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such material.

The powdered drug of *C. millenii* was also evaluated organoleptically. The powder of the leaves is deep-brown color with no characteristic taste. The powder of stem is of light-brown color with no characteristic taste (Fig. 1 and Table 1).

The epidermal cells shape and anticlinal wall pattern of *C. millenii* were polygonal and straight on the adaxial surface but irregular and undulate anticlinal cell wall pattern on the abaxial surface as shown in Fig. 2A and 2B as also reported by Johnny and Bassey [21] for *C. pachycarpa*. Aworinde [22] reported on the irregular epidermal cell shape and also slightly curved anticlinal cell

wall pattern for some monkey *Cola*. These are distinctive delimiting features with regards to the epidermal cell shape and anticlinal cell wall pattern (ACWP). These findings were in agreement with Stace [23] who suggested that environmental conditions such as humidity play a significant role in determining the pattern of anticlinal cell walls.

C. millenii is hypostomatic (stomata on the abaxial surface only) with anomocytic, anisocytic and hexacytic stomatal complex type as Johnny and Bassey [21] reported same stomatal distribution for *C. pachycarpa* being a lesser-known member too but with anisocytic stomata only. Aworinde [22] reported anomocytic stomata for *C. millenii* in his study. Hexacytic has been documented by Pan and Jacobs as being scarce in occurrence in monkey *Cola* species but was detected in *C*. *millenii* in this study which is a distinctive and diagnostic feature. The position and number of subsidiary cells defines the different types of stomatal complexes. The occurrence of more stomata on the abaxial surface is an adaptation to water loss as this signifies a coping strategy to survive drought [24]. Therefore, *C. millenii* has the stomatal number of (67.43±1.26) with stomata index (33.17 %), stomatal length (42.07±0.59µm), stomatal width (32.52±1.35µm), stomatal pore length (24.72±0.36µm) and stomatal pore width (14.98±0.41µm) as recorded in this study (Table 2). Delimitation of some *Cola* species was done using same parameters [25]. *C*. *millenii* possesses both unicellular and glandular trichome but the glandular tichomes were found along the veins as this may serve as a diagnostic feature for its identity. Length of trichome was used to differentiate the forest and riverine variety of *Lasianthera africana* [Bassey and Sunday [26]. Studies on leaf epidermal characters in the Asteraceae family by Adedeji and Jewoola [27] differentiated *Chromolaena* as having amoeboid-shaped trichome as a diagnostic feature in the Asteraceae family.

The use of crystals as diagnostic tool has been extensively discussed by Amos [28], Terwelle [29], and Illoh and Inyang [30]. The presence of druse crystals was a common feature in the petiole (Fig. 2 H) and on the abaxial epidermal leaf surface (Fig. 2 F) along the veins which may be a distinctive feature for its identification as Dickson [31] was able to distinguish taxa in the family Connaraceae using similar observations of the calcium oxalate crystals.

Venation pattern is useful in the number of vein termination number, vein islet number, average areole length and width for delimiting the studied species of monkey *Cola*. For the adaxial and abaxial surfaces; vein termination number (16.20±0.81 µm and 1.87±0.15), vein islet number (8.57±0.27 µm and 12.03±0.03), areole length (211.99±11.33 µm and 255.91±12.88) and width (151.94±6.93 µm and 175.06±6.69) for *C*. *millenii* were recorded respectively. Therefore, these features can be used as diagnostic characters to aid in the identification of the *Cola* species studied.

Preliminary qualitative phytochemical analysis for the leaf and stem parts of *C. millenii* revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins and antraquinone was absent for the leaf and stem. These secondary metabolites are reported to have many biological and therapeutic properties [32-35]. Studies by Adewumi and Arije [36] and Ajayi and Ojelere [37] revealed the presence of alkaloids, saponins, tannins, glycosides, flavonoids, terpenoids but anthraqunone was found absent in all their researches. Aworinde *et al* [22]; Stace 1980 [23], Pan and Jacobs 2009 [38] used the presence of certain chemical constituents in solving taxonomical problems.

Mbah *et al*., [18] studied the pharmaceutical characterization of *Bridelia ferruginea* Benth (Euphorbiaceae) using the flow properties. Leaf and stem powder is deep brown and light brown respectively, with no specific odour or taste. They both have poor flow property with angle of repose 40.6^0 and 36.8^0 with a fine to coarse texture. Diagnostic powder microscopic features include polygonal epidermal cell shape and straight anticlinal cell wall pattern, unicellular trichome and anisocytic stomatal complex type (Fig. 3A, B and C) respectively.

For the micromeritic studies of the powders of *C. millenii*, the bulk and tapped densities of the leaf were (0.19 ± 0.00 and 0.27 ± 0.00), Hausner's ratio and Carr's index for the leaf were (1.42 ± 0.02 and 29.63 ± 0.90 %) and angle of repose 40.06^0 (Table 5). While the bulk and tapped densities of the stem were (0.23 ± 0.00 and 0.31 ± 0.00); Hausner ratio and Carr's index (1.36 ± 0.03 and 26.00 ± 1.73 %); angle of repose (36.8^0) (Table 5).

The Hausner's ratio and Carr's index are parameters that are used to determine the powder flow property and powder characteristics.

Hausner's ratio values less than 1.25 indicate good flow while those greater than 1.25 indicates poor flow. From the study, Hausner's ratio and Carr's index of both the leaf and stem were greater than 1.25 and 23 % respectively and these indicate that the powder has a poor flow property as shown in Table 5. This is as a result of some factors that affect a powder's flowability hence affecting the powder characteristics. The factors include: moisture contents, temperature, particle size, particle shape (texture) and time of storage at rest.

The angle of repose is considered to be the most classical technique used for characterizing the flow properties of powders. Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles [39,40,41]. For the monkey *Cola* studied, with angle of reprose put into consideration it exhibited poor flow due to the fiber-like nature of their powders as same poor flow was also recorded in *Cola pachycarpa* leaf and stem powders [21]. Umoh *et al* [42] used the micromeritic method to obtain a fair flow in *Solenostemon monostachyus*. This result may be used in the identification and authentication of *C. millenii* as seen to be an inherent characteristic feature.

The pH values for cold infusion for both leaf and stem ranges between 3.96 – 4.24 while hot infusion was between 6.23 – 6.77. Consumption of acidic beverages (as seen with herbal teas) can result in irritation of the oral mucosa. Phelan and Rees [43] have reported similar observation on their work adding that consumption of acidic infusion and decoction of herbal drugs has the potential of wearing off the teeth enamel leading to dental problems. From the results, the most acidic impacts on the dental enamel by causing demineralization of the tooth [44].

Quantitative study is an essential factor in setting standard of crude drugs and the physical constant parameters could be useful in detecting any adulterant in the drug. Low moisture content with less than 14% indicates less chances of microbial attack of crude drug during storage [45]. For the stem of *C. millenii* with 16.33% signifies that care must be taken during storage to avoid degradation of the drug as a lower value 4.49% was recorded for the leaf.

The moisture content of crude drug is directly related to its stability when there are chances of microbial growth. The lower the moisture content, the higher will be the stability of that drug as recorded in the leaf of *C. millenii* (4.49%), chances of microbial growth will be less and vice versa. The shelf life of the drug also increases with lower moisture contents [46,47]. Significant amount of moisture was found in air-dried material of stem (16.33%). Therefore, the powdered drug of *C. millenii* should be stored with care and in a dried form. The total ash for the leaf and stem as 5.5 and 12.02 %w/w respectively were within the accepted limit of 14 %w/w. Laitharani [48] reported that the total ash value is an indicative of the impurities present in a drug as this is constant for a givin plant sample. Various solvents like methanol, ethyl acetate, ethanol and water were used to determine the extractive value which is an important quality control parameter for herbal drugs. The extractive values for the leaf and stem were more with polar solvents, i.e water (26.30 and 22.50), ethanol (24.30 and 16.80), methanol (20.90 and 2.50) but decreased as the polarity decreased, e.g., ethyl acetate (6.60 and 1.10) respectively (Table 4). Fluorescence analysis is also an important tool for the determination of constituents in herbal drugs and it provides an idea about the chemical nature. The powder drug analysis was carried out by treating the samples with various chemical reagents, and observations were made in visible light and ultra violet light of short and long wavelengths (Table 7).

The Gas Chromatography-Mass Spectroscopy is a vital tool due to its potential to supply suggested qualitative and quantitative information on constituents based on their structural compositions. The GC-MS analysis showed the presence of thirty (31) phytochemical constituents (Table 8 and Fig. 4) for the leaf and forty-five (45) phytochemical constituents (Table 9 and Fig. 5) for the stem.

Hexadecanoic ethyl ester and and n-Hexadecanoic acid were found in both leaf and stem. The major components found in the leaf were: N-(4-Tolylsulfonylmethyl) formamide (12.99%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (10.05%), n-Hexadecanoic acid (6.58%) and 2-Butenal, 2-methyl-, (E)- (5.09%) while that of the stem were: 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6 methyl- (18.21%), .alpha.-D-Galactopyranoside, methyl (16.82%), 2-Pentanone, 5-hydroxy- (12.40%), methyl .beta.-d-ribofuranoside (7.59%) and 3-Cyclohexen-1-carboxaldehyde, 3-methyl- (6.31%).

The phytochemical, 9,12, 15-octadecatrienoic acid, methyl ester may act as anti-inflammatory, hypocholesterlemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic and antieczemic [49]. n-Hexadecanoic acid (fatty acid) may act as antioxidant and anti-inflammatory. Phytol being most dorminant may act as an antioxidant. Hexadecanoic acid (fatty acid) may act as antimicrobial and chemopreventive. Sonibare *et al*. [50] reported the antimicrobial effect of ethanol extracts of leaf of *Cola millenii* against human isolated strains of *Staphylococcus aureus, Staphylococcus albus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*. Ubon *et al* [51] reported the noncytotoxic, cardio-protective and hepato-protective properties of seeds of *C. millenii* While Giwa *et al*. [52] reported the antimicrobial activities of the pulp and seed. These biological properties are a function of the presence of these phytoconstituents.

5. CONCLUSION

The study sought to establish the diagnostic characteristic features of *C. millenii*. viz hypostomatic stomatal distribution, polygonal epidermal cell shape, straight anticlinal wall pattern, hexacytic stomata, presence of glandular and unicellular trichome as well as a the presence of druse calcium oxalate crystals along it vein serves as a diagnostic tool for its identification. The results of the moisture content and total ash value could be employed as suitable quality control measures to ensure quality, safety, and efficacy of this herbal drug material. The phytochemical profile from the GC-MS analysis may provide answers on problems related to phytochemical studies.

The presence of various bioactive constituents confirms the usage of *C*. *millenii* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may give birth to a novel drug.

NOTE

The study highlights the efficacy of "HERBAL" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adesina SK, Johnny, II. Plants in herbal medicine; their traditional uses, chemistry and biology. British Publishers International. 2021;1:1-785.
2. European Medicine Agency. Guideline on Quality of Herbal Medicine Products / Traditional Medicine Products. 2005; 3(1):31- 40.
3. Russell TA. The Kola of Nigeria and the Cameroon. Tropical Agriculture. 1955; 32(3):211- 277.
4. Keay RWJ, Onochie CFA, Stanfield DP. Nigerian trees. National Press Limited. 1964;(1):219.
5. Thomas S, Patil DA, Patil AG, Naresh C. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* fruit. Journal of Herbal Medicine and Toxicology. 2008:2(2):51-54.
6. Burkill HM. The Useful plants of West Tropical Africa. Vol. 5.2nd ed. Royal Botanic Gardens, Kew. 2000;686.
7. Odugbemi T. Medicinal Plants by Species Names: Outlines and Pictures of Medicinal Plants from Nigeria.10. University of Lagos Press. 2006;158.
8. Akinnibosun FI, Adewumi BL. Evaluation of phytochemical components of various parts of *Cola millenii* K. Schum. Ovidius University Annals of Chemistry. 2018; 29-35.
9. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere, 2003;52:1361-1371.
10. Angiosperm Phylogeny Group. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society. 2016;181(1):1-20.
11. Metcalfe CR, Chalk L. Anatomy of the dicotyledons. 2nd Ed. Clarendon Press, Oxford. 1979;1:279.
12. Killedar GS, Harianth N, Sameer J, Nadaf S, Karade R. Phytochemical potential of *Memecyclon umbellatum*. Burm. Leaf extracts. Journal of Drug Delivery and Therapeutics. 2014;4(2):30-35.
13. Kokate CK, Purohit AP, Gokhale SB. Analytical pharmacognosy, Nirali publication, 30th edition. 2005;199.

14. Evans WC. Trease and Evans Pharmacognosy. 16th edition. Elserviers Ltd. United Kingdom. 2009;560-562:568-570.
15. Sofowora A. Medicinal plants and traditional medicine in West Africa. John Wiley and Sons, New York. 1993;289.
16. Kumar D, Gupta J, Kumar S, Arya R, Kumar T, Gupta G. Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf. Asian Pacific Journal of Tropical Biomedicine. 2012;2(1):6-10
17. Khandelwal KR. Practical pharmacognosy techniques and experiments. New Delhi: Nirali Prakashan. 2002;15-163.
18. Mbah CC, Builders PF, Akuodor GC, Kunle OO. Pharmaceutical characterization of *Bridelia ferruginea* Benth (Euphorbiaceae). Tropical Journal of Pharmaceutical Research. 2012;11(4):637- 644.
19. Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC–MS. Pharmacognosy Research. 2009;1:152–156.
20. Dhale DA, Bhoi S. Pharmacognostic characterization and phytochemical screening of *Achyranthes aspera* Linn. Current Agriculture Research Journal. 2003;1(1):51 – 57.
21. Johnny II, Bassey, ME. Pharmacognostic and Taxonomic Studies of *Cola parchycarpa* K. Schum. (Malvaceae). Asian Plant Research Journal. 2020;6(3): 33-45.
22. Aworinde DO, Ogundairo BO, Osuntoyinbo KF, Olanloye OA. Foliar epidermal characters of some Sterculiaceae species in Nigeria. Bayero Journal of Pure and Applied Sciences. 2012;5(1):48-56.
23. Stace C. Plant taxonomy and biosystematics contemporary biology, The Pitman Press Great Britain. 1980;279.
24. Mbagwu FN, Nwachukwu CU, Okoro OO. Comparative leaf epidermal studies on *S. marcrocarpon* and *S. nigrum*. Research Journal of Botany. 2008;3(1):45-48.
25. Goji TC, Ayodele AE. Foliar epidermal and pollen characters in the genus *Cola* in Nigeria. Journal of life and Physical Sciences. 2005;2(2):57-63.
26. Bassey ME, Sunday RA. Epidermal morphology and stomatal ontogeny in two varieties of *Lasiathera africana* P. Beauv (Icacinaceae). Transactions of the Nigerian Society for Biological Conservation. 2000; 101 – 107.
27. Adedeji O, Jewoola OA. Importance of leaf epidermal characters in the Asteraceae family. Notulae Botanicae Horti Agrobotanici Chuj-Napoca. 2008;36(2):7 – 16.
28. Amos G. Some silicious timber of British Guyana. Carrib. For. 1951;9(1):33 – 37.
29. Terwelle BJH. Silica grains in woody plants in neotropic especially Surinam. Leid. Bot. Ser. 1976;3:107 – 142.
30. Illoh, HC, Inyang UE. Foliar epidermis and petiole anatomy in some Nigerian Solanum Linn. Species in the sub-genus Leptostemonum (BITT) DUN. Glimpses in Plant Research. 1998;12:73 – 86.
31. Dickson WC. Anatomy studies in the family Connaraceae II. Wood Anatomy. The Journal of the Mitchell Society. 1972;117 – 136.
32. Vishnu R, Nisha R, Jamuna S, Paulsamy S. Quantification of total phenolics and flavonoids and evaluation of in vitro antioxidant properties of methanolic leaf extract of *Tarenna asiatica* - an endemic medicinal plant species of Maruthamali hills, Western Ghats, Tami Nadu. Journal Research and Plant Science. 2013;2(2):196 – 204.
33. Benedec D, Vlase L, Oniga I, Mot AC, Damian G, Hanganu D. Polyphenolic composition, antioxidant and antibacterial activities for two Romanian subspecies of *Achillea distans* Waldst. et Kit. ex Wild. Molecules. 2013;18:8725-8739.
34. Charalampos P, Konstantina L, Olga KM, Panagiotis Z, Vassileia JS. Antioxidant capacity of selected plant extracts and their essential oils. Antioxidants. 2013;2: 11-22.
35. Narender PD, Ganga R, Sambasiva E, Mallikarjuna T, Praneeth VS. Quantification of phytochemical constituents and in vitro antioxidant activity of *Mesua ferrea* leaves. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(2):539-542.
36. Adewumi BL, Arije OC. An Assessment of Phytochemical Constituents and Acute Toxicity of *Cola millenii* Seed and Pulp used as bio-preservatives. International Journal of Interdisciplinary Innovative Research and Development. 2017;1(4):118-121.
37. Ajayi IA, Ojelere OO. Chemical Composition of ten medicinal plant seeds from Southwest Nigeria. Advances in Life Science and Technology, 2013;10:22-30.

38. Pan AD, Jacobs BF. The earliest record of the genus *Cola* (Malvaceae *sensu lato*: Sterculioideae) from the Late Oligocene (28-27Ma) of Ethiopia and leaf characteristics within the genus. Plant Systematic Evolution. 2009;283:247-262.
39. Rasanen E, Antikainen O, Yliruusi J. A new method to predict flowability using a microscale fluid bed. American Association of Pharmaceutical Scientists Pharmscitech. 2003;4:418–424.
40. Li Q, Rudolph V, Weigl B, Earl A. Interparticle van der Waals force in powder flowability and compactibility. International Journal of Pharmaceutics. 2004;280:77–93.
41. Dalla Valle JM. *Micromeritrics:* The Technology of Fine Particles. 1943;1906-1958.
42. Umoh RA, Johnny II, Udoh AE, Andy NA, Essien AC, Udoh IJ, Emeh WE, Umanah OE. Micromorphological and Pharmacognostic Studies of Leaf and Stem of *Solenostemon monostachyus* P. Beauv (Lamiaceae). Journal of Complementary and Alternative Medical Research. 2021;16(4): 230-240.
43. Phelan J, Rees J. The erosive potential of some herbal teas. Journal of Dentistry. 2003;31(4):241–246.
44. Zero DT. Etiology of dental erosion-extrinsic factors. European Journal of Oral Sciences. 1996;104(2):162–167.
45. Devi IA, Muthu AK. Gas Chromatography-Mass Spectrometry analysis of phytocomponents in the ethanolic extract from whole plant of *Lactuca runcinata* DC. Asian Journal of Pharmaceutical and Clinical Research. 2015;8(1):202 -206.
46. Musa KY, Katsayal AU, Ahmed A. Pharmacognostic investigation of the leaves of *Gisekia pharmacioides*. African Journal of Biotechnology, 2006;5:956–957.
47. Kunle OF, Eghareva HA, Ahmadu PU. Standardization of herbal medicines – A review. International Journal of Biodiversity and Conservation. 2012;4(3):101-112.
48. Lalitharani S, Mohan VR, Maruthupandian A. Pharmacognostic investigation of *Bulbophyllumalbidum* (Wight) Hook. f. International Journal of PharmTech Research. 2011;30(1):556 – 562.
49. Okhale SE, Amanabo MO, Jegede IA. Phytochemical and pharmacognostic investigation of antidiabetic *Scoparia dulcis* Linn Scrophulariaceae whole plant grown in Nigeria. Researcher. 2010;2:7–16.
50. Sonibare M, Micheal O, Oyedokun O, Oluwadayo O. Phytochemical and Antimicrobial Studies of Four Species of Cola Schott & Endl. (*Sterculiaceae*). African Journal of Traditional, Complementary and Alternative medicines. 2009;6(4):518-525.
51. Ubon JA, Akpanabiatu MI, Akpanyung EA, Ufot UF. Effects of ethanolic extracts of *Cola millenii* K. Schum seed on biochemical and toxicological indices of male wistar albino rats. Journal of Pharmacognosy and Phytochemistry. 2017; 6(1): 160-166.
52. Giwa OE, Onileke FO, Adesina IA, Adebote VT. Phytochemicals and Antimicrobial Properties of Seed and Pulp of Monkey Cola (*Cola millenii*) on some Selected Clinical and Food Borne Isolate. International Journal of Applied Biology and Pharmaceutical Technology. 2012; 3(3):390-400.

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