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Research Article

Assessment of the Phytochemicals, Antioxidant, and GC-MS Evaluation of Methanolic Leaf Extract of an Antidiabetic Plant, *Cola millenii*

Ogbe Emmanuel Ojonugwa[1], *Ogbe Kingsley Unekwu[2], Simon Sani Ocholi[3], Dangana Elizabeth Ojochebo[4], Wintola, H.U.[5], Dauda Jamiu Muhammed[6] and Obaka Emmanuel[7]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria
[2,5]Department of Animal and Environmental Biology, Faculty of Natural Science, Prince Abubakar Audu University Anyigba, Kogi State, Nigeria
[3]Department of Chemistry/Biochemistry, Federal Polytechnic Nasarawa, PMB 001, Nassarawa State, Nigeria[4]Department of Human physiology Prince Abubakar Audu University Anyigba, Kogi State, Nigeria
[6]Faculty of Agriculture, Confluence University of Science and Technology Osara, Kogi State, Nigeria
[7]Department of Plant Science and Technology, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria
***Corresponding Author's email*: ogbekingsley90@gmail.com**

ABSTRACT

The phytochemicals present in *Cola millenii* have been described in different reports as an effective anti-diabetic plant. This study evaluated the phytochemical contents and antioxidant potential of *Cola millenii* leaves. Using methanol, the phytochemicals in the leaves were extracted, and the methanol was distilled from the *Cola millenii* extract using a rotary evaporator. The total phenolic and flavonoid contents were determined spectrophotometrically. Using the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay, the *in-vitro* antioxidant potential of the extract was determined, respectively. The extract was also analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to determine the presence of volatile compounds. The results from this study showed a high total phenolic content (32.7±1.97mg/100g GAE), and the total flavonoid content in the extract had a concentration of (0.97±0.11 mg QE/g). The *in-vitro* antioxidant activity determined by DPPH showed an IC_{50} of 213.80 µg/mL compared to the standard Vitamin C; 1.87 µg/mL. The evaluation done by Ferric Reducing Antioxidant Power (FRAP) assay showed an increase in % of reducing power with an increase in extract concentration. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed that the leaf extract possesses 25 different compounds. However, five of the compounds, namely n-Hexadecanoic acid, 6-Octadecenoic acid, Octadecanoic acid, Squalene, and Vitamin E, had higher concentrations of 32.53%, 32.85%, 3.87%, 7.71%, and 7.86%, respectively. The antioxidant activity property and presence of the identified phytochemicals in *Cola milenii* leaf extract from this study may be responsible for its anti-diabetic activity in herbal medicine and other pharmacological benefits.

Keywords: Anti-diabetic; Antioxidant; *Cola millenii*; Methanolic leaf extract; Phytochemicals

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INTRODUCTION

Medicinal plants are described as those plants whose parts contain bioactive substances that can be used for therapeutic purposes or serve as precursor for the synthesis of useful drugs (Nwokocha and Williams, 2018). Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids, and other compounds which have preventive or curative properties (Nwankwo, 2017). These chemical substances generally occur as secondary plant metabolites in these plants and are of enormous use to humanity (Ganiyat *et al.,* 2010). Higher plants have traditionally been used in folk medicine as well as in the extension of the shelf life of foods in the case of those with antimicrobial activity (Akinnibosun and Adewumi, 2019) .

All over the world, hundreds of plants have been identified as good sources of medicinal agents and are used in traditional medicine for different purposes, including the treatment of bacterial and fungal infections (Obafemi *et al*., 206). Ethnopharmacological uses of plants feature strongly among Nigerians. It has been pointed out that plants continue to play a prominent role in primary health-care of about 80% of the world's population. *Cola millenii* commonly known as monkey Cola belongs to the genus Cola under the family *Sterculiaceae*. The genus Cola contains many species numbering up to 50 in West Africa. Of these, only a few are fruit-bearing, while majority are woody species of economic importance. The few fruit-bearing species are notably: *Cola nitida*, otherwise known as gbanja or goro (Yoruba), ojiawusa (Ibo), *Cola acuminate*, Obi gidi or Obi abata (Yoruba), ojiigbo (Ibo), *Cola verticillata*, Obi Olooyo or the slimy Cola (Yoruba), *Cola millenii* also known as Obi edun in Yoruba, Uto (Igbo), Ivyureanchere (Ebira) ekpaokuko, obukpehie

Diabetes, whichis characterized by elevated levels of blood glucose, is a chronic metabolic disease resulting in chronic hyperglycemia and hyperlipidemia that ultimately increases the risk of heart diseases, stroke and renal diseases. Despite the fact that there is an extensive use of *Cola millenii,* the pharmacological composition of *Cola millenii* leaves as an antidiabetic plant has been underexplored

MATERIALS AND METHODS

Plant material collection and authentication:

Fresh leaves of *Cola millenii* were collected in the month of August, 2022 from Ejegbo in Ankpa Local Government Kogi State; it was identified in the department of Botany Kogi State University Anyigba.

Processing of plant materials

The leaves of the *Cola millenii* plant were removed from the whole plant using a sharp laboratory knife The plant materials were washed with water and air dried under shade for 14 days on the laboratory bench to avoid destruction of active compounds to constant weight. The dried materials were ground with an electric grinder into powder. These were stored in an air-tight container ready for extraction.

Preparation of methanolic extracts of *Cola millenii* leaves

Pulverized leaves of *Cola millenii* (350 g) was weighed out into a container; using the electric weighing balance. Using measuring cylinder, 2100 ml of methanol was measured and added into the container until it reached the brim which was covered tightly with aluminium foil and was allowed to stand for 72 hours at ambient temperature. After 72 hours, the solution was filtered using vacuum filter pump into a conical flask (500ml×2) and the filtrate concentrated using a rotatory evaporator and a vacuum drier leaving the extract of *Cola millenii* leaves.

Determination of extract yield

From the weighed mass of the sample and the mass of solvents extracts, the percentage was calculated using the equation below:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of pulverized leaf}} \text{ x } 100$$

Quantitative phytochemical analysis of methanolic leaf extract of *Cola millenii*

Total phenolic content

Folin-Ciocalteu reagent was used to determine the total phenolic content (TPC). Gallic acid was used as a reference standard (20-100 µg/mL) for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 µg/ mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 3 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was kept in dark at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured by using double beam UV-Vis spectrophotometer (UV Analyst-CT 8200) at fixed wavelength of 765 nm. The TPCs were determined using linear regression equation obtained from the standard plot of gallic acid. The content of total phenolic compounds was calculated as "mean$\pm$SD" (n=3) and expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Total flavonoid content

Total flavonoid test was determined using sodium nitrate, aluminium chloride ($AlCl_3$) and sodium hydroxide (NaOH). 0.005 g of *Cola millenii* was mixed with 50 ml of ethanol in a beaker, solution treated to a set of standard solutions ascorbic acid (µg/ml) were prepared in the laboratory. To each volumetric flask, 1ml of 5% Sodium nitrite was added and then after 5minutes, 1ml of 10% Aluminium Chloride was added and left for 5minutes after which 2ml of Sodium Hydroxide was added and the absorbance was taken at 510nm.A blank solution which was prepared by the same procedure except replacing the plant extract with an equal volume of methanol, was zero on the spectrophotometer.

"2.2-diphenlypicrylhrdrazyldpph" radical scavenging activity

The free radical scavenging activity of different concentrations of methanolic extracts of *Cola millenii* leaves and of standard ascorbic acid was evaluated by using DPPH radical scavenging method as per reported method. The extracts or standard ascorbic acid solution of 1 mL at different concentrations (25, 50, 100, 200, 400, 800, 1600, and 3200 µg/mL) were taken in separate test tubes. Two milliliter of 1.0 mmol/L DPPH radical solution, prepared in methanol, was added to each test tube. The solution was rapidly mixed and allowed to stand in dark at 37 °C for 30 min. The blank was prepared in a similar way without extract or ascorbic acid. The decrease in absorbance of each solution was measure at 517 nm using UV-Vis spectrophotometer. The percentage of radical scavenging activity of tested extracts and positive control ascorbic acid was calculated by using the following formula. Ac is the absorbance of the control reacted with ethanol and the DPPH working solution (1ml). Ac is the absorbance of the sample.

$$\% \text{ Inhibition} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \text{ x } 100$$

Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed according to previous method.[6] 0.005g of the sample was weighed and poured into the beaker, then 3ml of ethanol was used to dissolve the sample, the stock solutions prepared were 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16.8ml $C_2H_4O_2$; pH 3.6), TPTZ solution (10mM TPTZ in 40mM HCl) and 20mM $FeCl_3.6H_2O$ solution. Working FRAP solution was prepared by mixing 25ml of acetate buffer, 2.5 ml TPTZ solution and 2.5 ml of FeCl3.6H2O solution. The mixture is then warmed at 37 °C. 50 µl of each sample (containing 25, 50, 100, 200, 400,800, 1600 and 3200 µg respectively) were mixed with 3 ml of FRAP solution and incubated in dark for 30 min. Absorbance was read at 593 nm

$$\% \text{ FRAP VALUE} = \frac{\text{Change in sample absorbance}}{\text{Change in absorbance standard}} \text{ x } 2$$

Gas chromatography-mass spectrometry (GC-MS) procedure

2µLof the sample extract was injected into the GC column for analysis. The GC (Agilent 6890N) and MS (5973 MSD) is equipped with DB-5ms capillary column (30 m×0.25 mm; film thickness 0.25 µm). The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C/min. The temperature was again increased to 230°C at the rate of 5°C/min. The process continued till the temperature reached 310°C at the rate of 20°C/min which was held for 8 minutes. The injector port temperature remained constant at 280°C and detector temperature was 250°C then. Helium was used as the carrier gas with a flow rate of 1 mL/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively.

To identify the unknown components, present in the samples, their individual mass spectral peak value was compared with the database of National Institute of Science and Technology 2014. Then the components were identified after comparing the unknown peak value and chromatogram from GC-MS against the known chromatogram, peak value from the NIST library database. Subsequently, the details about their molecular formula, molecular weight, retention time and percentage content were also obtained.

Statistical analysis

The obtained data was expressed as mean ±standard error of the mean (Mean ± SEM).Data was subjected to one analysis of variance (ANOVA) followed by Turkey post-hoc test using statistical package for social sciences (SPSS) software [version 23]. $P<0.05$ was consideredstatistically significant.

RESULTS AND DISCUSSION

Plants are widely used in many parts of the world, especially in Africa and Asia for treatment of various ailments, Phytochemicals are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth/defense against competitors, pathogens/predators. Major function of phytochemical is their role as antioxidant. Antioxidant is crucial in preventing degenerative diseases that result from oxidative stress of which diabetes is a good example. In living organism, complex natural substances (Vit. C, E

and glutathione) and enzymes (superoxide dismutase, glutathione, peroxidase, catalase) and foods of plant origin provides total antioxidant capacity for the system (Arogba, 2014). In vitro antioxidant activities of *Cola millenii* methanolic leaf extract was accessed by DPPH and FRAP assays.

From figure1 the phytochemical screening of the methanolic extract revealed the presence of high concentration of total phenol (32.70 ± 1.97 mg of GAE/g) and a total flavanoid concentration of (0.97 ± 0.11 QE/g). This result suggests that a high concentration of phenolics and flavonoids in *Cola millenii* leaves shows it has antioxidant properties. In accordance to Soobratee *et al*. (2005) the presence of phenolics and flavonoid in the leaves of *Cola millenii* indicates the leaves have high antioxidant properties. The presence of flavonoids in the methanol extract of *Cola millenii* shows that the plant possess antioxidant properties and high beneficial effects on disease prevention such as protection against allergies, inflammation, platelet aggregation, microbes, ulcers, viruses and tumor (Okwu, 2005). Oxidative stress and an increase in reactive oxygen species have many effects and it is mainly associated with diabetes. The ability to maintain β- cells performance and decrease glucose level in the blood of a diabetic patient is due to the presence of natural antioxidants like flavonoids, tannins, Vitamin C and E (Kooti *et al*., 2016). This result thus suggests that the presence of phenolics and flavonoids in *Cola millenii* plant make it effective in the management and treatment of diabetes, by increasing insulin secretion and reducing the intestinal absorption of glucose by pancreatic tissue. The improvement of the performance of pancreatic tissue by increasing insulin secretion or reducing the intestinal absorption of glucose is often as a result of the antihyperglycemic effect that results from plants that contains flavonoids, terpenoids, carotenoids and alkaloids. Hence, these plants can have antidiabetic effect. Numerous studies on phenolic compounds have shown their value in demonstrating possible biological effects such antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antibacterial, and anticancer properties (Kumar *et al.,* 2013).

The *invitro* antioxidants scavenging activity of the extract against free radical was also determined using ferricreducing antioxidant power (FRAP) and 2,2-diphenyl 1- 2- picryl hydrazyl (DPPH) as shown in figure2. This result suggests that it can produce a very strong reducing activity against free radical. However at a concentration of 25.00 µg/ml the reducing power was lowest. Increased absorbance of the reaction mixture indicates lower reducing power of the plant that is reducing power of the extract reduced with concentration as shown in figure 2.

Result showed that percentage FRAP increased with increase in concentration, highest FRAP activity was observed at concentration 3200 µg/ml having % FRAP value of 2.32 while the reducing antioxidant activity was low at a concentration of 25.00 µg/ml. This result suggests that the *Cola millenii* leaves can produce a very strong reducing activity against free radical at a high concentration as the pathogenesis of oxidative stress plays a role in the complications of diabetes. Hence, the result suggests that *Cola millenii* leaves are important in the management of diabetes. This result is in correlation with a study carried out by Nwaehujor *et al.* (2012) where the FRAP value of the extract increased concetration dependently from 1.1 to 1.7µM (100-400 µg ml) while ascorbic acid has a FRAP value of 2 at 1000 µg mL^{-1}. Thus suggesting that the extract could be a potential source of a novel anti- diabetic and antioxidant agent.

In this study the in vitro anti-oxidant activity of *Cola millenii* leaf extract was determined by the DPPH radical scavenging method based on the principle that reduction of the DPPH radical is characterized by a change in colour from violet to yellow indicating the reduction by the extract used. The plant extract was observed to have reduced free radical DPPH remarkably. IC_{50} values were calculated for the extract as well as the standard Vit C. Since a lower IC_{50} value indicates a higher antioxidant potential, vitamin C which was the standard used has the lower IC_{50} 1.87 µg/mL. Thus has the highest antioxidant potential when compared with the extract used having an IC_{50} of 213.80 µg/ml as shown in figure 2 since the extract has a high IC_{50} it will suggest its low activity to scavenge free radicals compare to vitamin C having a low IC_{50}.

The result of GC-MS analysis of methanolic extract of *Cola millenii* leaf in table 3.2 shows the presence of different compounds, the constituents of *Cola millenii* leaf extract has been structurally identified and elucidated by GC-MS table. identification of compounds was based on comparison with matching NIST using their retention time (Rt), GC-MS analysis of *Colamillenii* leaf extract revealed that active component consist of a mixture of 25 compound present in methanolic extract of *cola millenii,* the compounds include n-Hexadecanoic% (32.53) acid, 6-Octadecenoic acid (32.83%), Octadecanoic acid (3.87%), which is used in cosmetic, flavor, lubricant and perfumery.[13] Squalene (7.71%) an important ingredient in some vaccine adjuvant, act as adjunctive therapy in a variety of cancers, plays a role in topical skin lubrication and protection, according to the study done by Mirasnpour

et al. (2022) squalene has anti–inflammatory and antioxidant effect that have been considered in many studies. In patients with uncontrolled diabetes there is an increase in total cholesterol and low density lipoprotein alongside a decrease in high density lipoprotein, hence squalene has been proven to be useful in lowering total cholesterol and triglycerides, Vitamin E (7.86%) according to Baburao *et al.* (2021) supplementation has an important role in delaying the onset of the diabetic complications as well as slowing down the progression of the complications. prevents coronary heart disease, strengthen immune function, prevent inflammation, promote eye health and lower the risk of cancer, hence , L-Glucose, a compound also found in *Colamillenii* helps to keep the blood sugar level in its range to help prevent serious health problems such as diabetes, heart disease and kidney diseases.

These compounds play important roles in bioactivity of medicinal plants; thus medicinal value of this plant rely on the embedded phytochemicals and as such produce definite physiological actionon human body (Haralapidis *et al.,* 2002)

The study carried out to quantify the phytochemicals present in *Cola millenii* leaf using methanol extract revealed the presence of several bioactive compounds which account for the medicinal properties of the plant. The various compounds found in this have been proven to help in the management of diabetes and its complications.

Total phenolics and flavonoid contents of methanolic leaf extract of *Cola millenii*

Figure below shows total phenolics and total flavonoid contents of extract from *Cola millenii* leaf, values are expressed in mean standard deviation for triplicate determination (n=3).

DPPH inhibition by methanolic leaf extracts of *Cola millenii*

Figure 2 below shows the result of the free radical scavenging activity (DPPH), the chart indicate increasing radical inhibitory activity of the extract with increasing concentration. The result shows a high scavenging activity effect at the highest concentration of 3200 µg/mL.

Ferric reducing antioxidant power of methanolic extract of *Cola millenii*

Figure 3 presents the result of ferric acid reducing power of methanolic extract of *Cola millenii*. The result shows increasing FRAP value of the extract with increasing concentration.

GC-MS of methanolic leaf extract of *Cola millenii*

Chromatogram of gas chromatography analysis of methanolic extracts of *Cola millenii* leaf, the GC-MS analysis identified 25 compounds , with the five most abundant being (n-Hexadecanoic acid, 6-Octadecenoic acid, Octadecanoic acid, Squalene, Vitamin E). As shown in Table 2

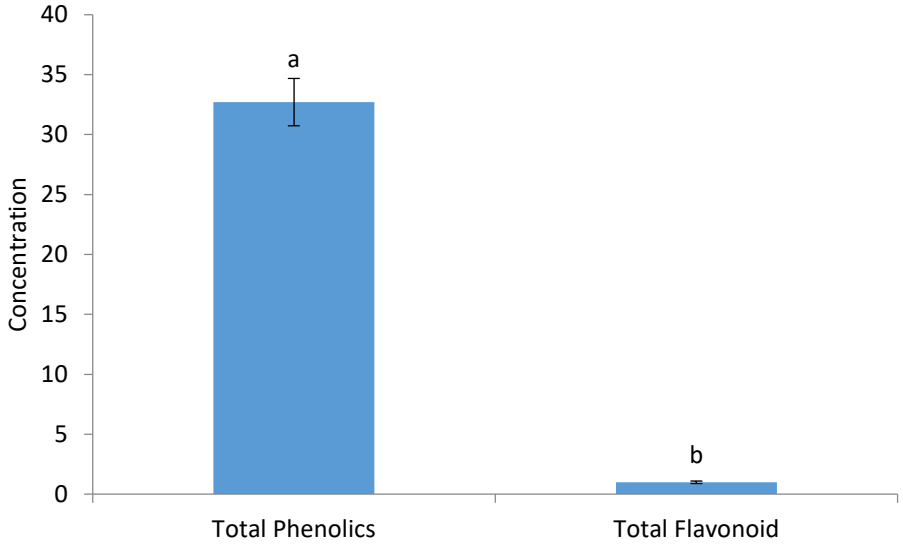


Figure 1 Total phenolic and total flavonoid content of methanolic extracts of *Cola millenii* leaf

Results are presented as mean ± S.E.M. Means. Bars having the same superscript are statistically the same while chart having different superscript are statistically different ($p<0.05$)

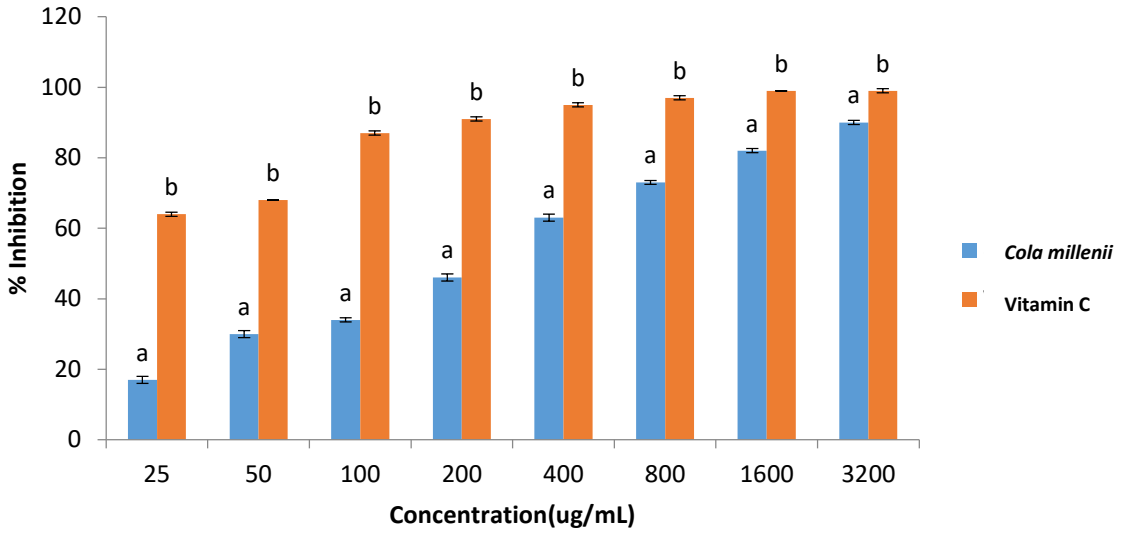


Figure 2: DPPH radical inhibition by varied concentrations of methanolic extract of *Cola millenii* leaf and standard antioxidant vitamin C

Bars for the same parameters with different superscripts are statistically significantly different ($p<0.05$).

Table 1: Inhibitory concentration of *Cola millenii* leaf extract and vitamin C against DPPH radical

Sample	IC_{50}(µg/mL)
Cola millenii leaf extract	213.80
Vitamin C	1.87

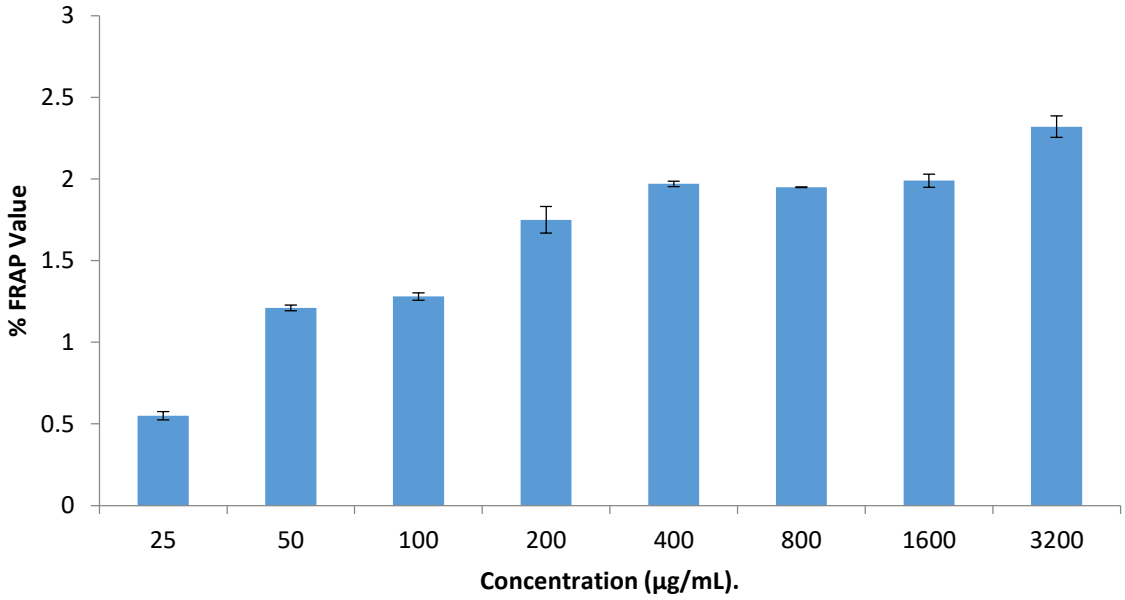


Figure 3: Ferric Reducing Antioxidant Power of varied concentration of methanolic extracts of *Cola millenii* leaf

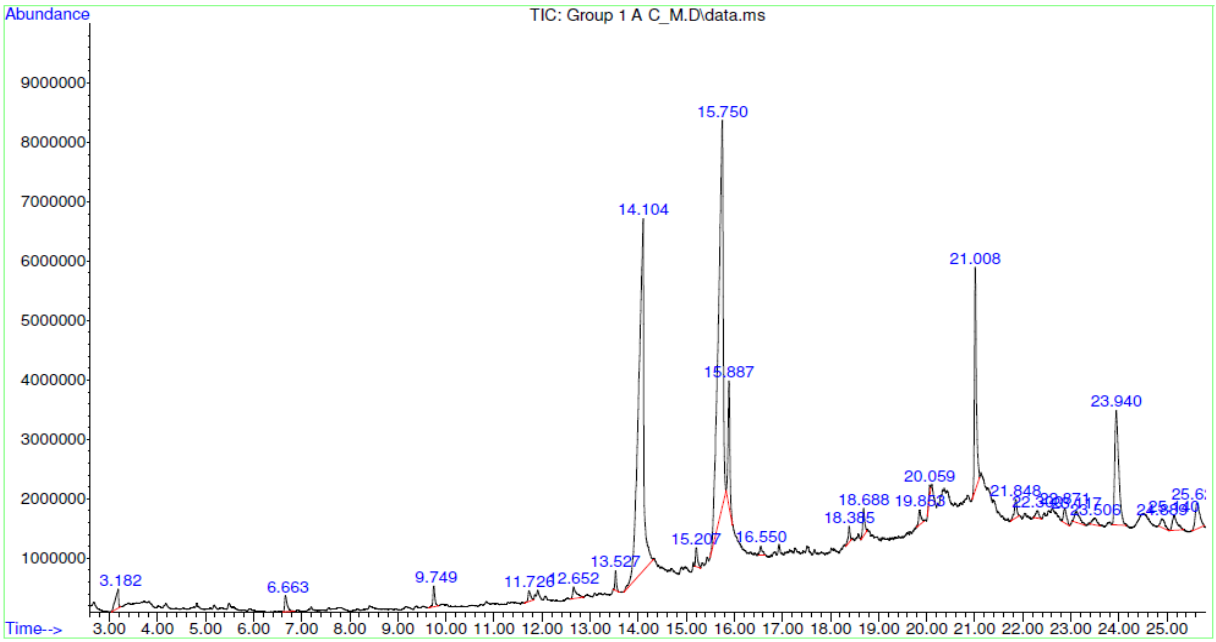
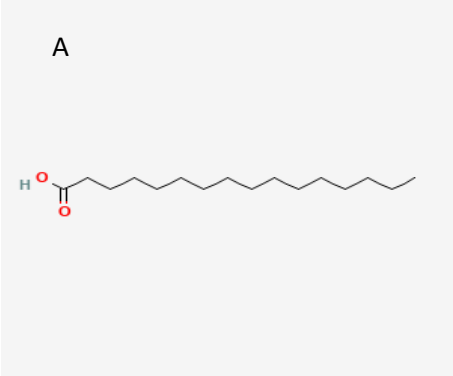


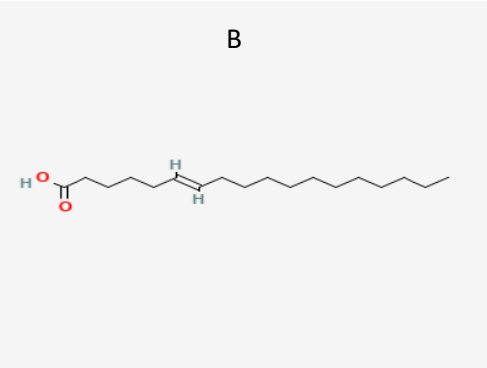
Figure 4. Gas Chromatography- Mass Spectrometry Chromatogram of methanolic extract of *Cola millenii* leaf

Table 2 Compounds Identified in Methanolic leaf extract of *Cola millenii* analysed by GC-MS

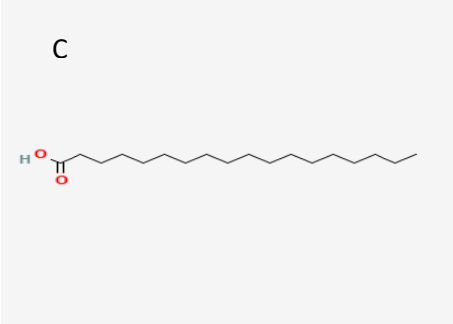
Peak Number	Retention Time(min)	Area%	Name of Compound	Molecular weight(g/mol)	Molecular formula
1	3.182	1.08	L-Glucose,	178	$C_7H_{14}O_5$
2	6.663	0.71	2-Methoxy-4-Vinylphenol	150	$C_9H_{10}O_2$
3	9.749	0.70	2,3,5,6-Tetrafluoroanisole	180	$C_7H_4F_4O$
4	11.726	0.48	4-((1E)-3-Hydroxy-1-propenyl)-2-me thoxyphenol	180	$C_{10}H_{12}O_3$
5	12.652	0.77	Cyclohexanone,	98	$C_6H_{10}O$
6	13.527	0.51	Hexadecanoic acid,	256	$C_{16}H_{32}O_2$
7	14.104	32.53	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
8	15.207	0.64	6-Octadecenoic acid,	296	$C_{19}H_{36}O_2$
9	15.750	32.85	6-Octadecenoic acid	282	$C_{18}H_{34}O_2$
10	15.887	3.87	Octadecanoic acid	284	$C_{18}H_{36}O_2$
11	16.550	0.39	Oleic Acid	282	$C_{18}H_{34}O_2$
12	18.385	0.52	i-Propyl 11-octadecenoate	324	$C_{21}H_{40}O_2$
13	18.688	0.99	9-Octadecenoic acid,	282	$C_{18}H_{34}O_2$
14	19.853	0.61	i-Propyl 11-octadecenoate	324	$C_{21}H_{40}O_2$
15	20.059	0.38	9-Octadecenoic acid (Z)-	282	$C_{18}H_{34}O_2$
16	21.008	7.71	Squalene	410	$C_{30}H_50$
17	21.848	0.82	2-Dodecen-1-yl(-)succinic anhydrid	266	$C_{16}H_{26}O_3$
18	22.300	0.39	Stigmasta-5,24(28)-dien-3-ol,	412	$C_{29}H_{48}O$
19	22.871	0.76	.beta.-Tocopherol	416	$C_{28}H_{48}O_2$
20	23.117	0.97	Methyl 2-hydroxy-pentadecanoate	272	$C_{16}H_{32}O_3$
21	23.506	0.54	1,3-Dioxolane, 4-ethyl-5-octyl-2,2 1,3-Dioxolane, 4-ethyl-5-octyl-2,2	322	$C_{13}H_{20}F_6O_2$
22	23.940	7.86	Vitamin E	430	$C_{29}H_{50}O_2$
23	24.889	0.66	2-Dodecen-1-yl(-)succinic anhydride	266	$C_{16}H_{26}O_3$
24	25.140	1.25	-Decyl icos-9-enoate	450	$C_{30}H_{58}O_2$
25	25.620	2.01	Stigmasterol 3-Pyrrolin-2-one	412	$C_{29}H_{48}O$



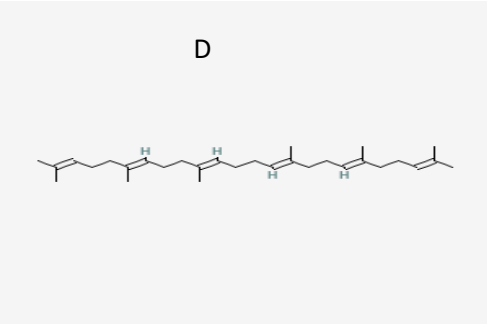
n-Hexadecanoic acid



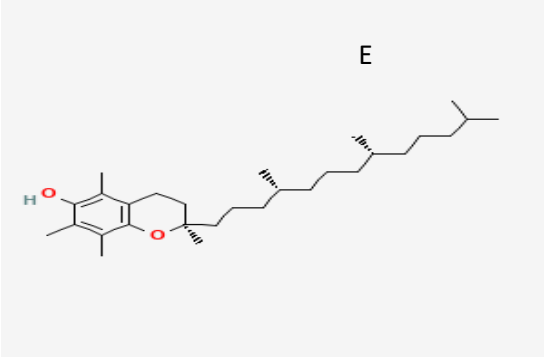
6-Octadecenoic acid



Octadecanoic acid



Squalene



Vitamin E

Figure 5: Structures of the most abundant compounds in methanolic leaf extract of *Cola millenii* identified by GC-MS

CONCLUSION

The results of the study reveal that methanol extract of *Cola millenii* leaves possess*invitro* antioxidant activity, which is an important and promising natural medicinal plant and could be utilized in several pharmaceuticals and medicinal medication because of its availability. The presence of the identified phytochemicals makes the leaves pharmacologically active for the treatment of diabetes.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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REFERENCES

Akinnibosun, F.I. and Adewumi, B.L (2019). Properties and Potentials of Cola milenii K. Schum: A Review.

Nigerian *Research Journal of Engineering and Environmental Sciences*, 4(1): 166-173.

Arogba, S.S (2014). Phenolics, antiradical assay and cytotoxicity of processed mango (Mangifera indica) and bush mango (Irvingia gabonensis) kernels. *Nigerian Food Journal*, 32(1): 62-72.

Baburao, D., Ali, S., Sadeghi, A., Saeed, M. and Moshavery, A (2021). Comparative histopathologic evaluation of the effects of Portulaca oleracea, Omega-3, and combination of sodium selenite and Vitamin E on hepatic enzymes of experimental diabetic rats. *Small Animal Advances*, 5: 4-9.

Benzie, I.F. and Strain, J.J (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem*. 239 (1), 70–76.

Ganiyat, K.O., Patricia, A.O., Sothka, J., Oguntokun, O. and Thoda, E. (2010). Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. *Annals of biological research,* 1(2): 114-120.

Haralampidis, K., Trojanowska, M. and Osbourn, A. E (2002). Biosynthesis of triterpenoid saponins in plants. *History and Trends in Bioprocessing and Biotransformation*, 31-49.

Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D. and Asadi-Samani, M (2016). The role of edicinal plants in the treatment of diabetes: a systematic review. *Electronic physician*, 8(1): 1832.

Kumar, S., Alhakmani, F. and Khan, S.A (2013). Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of Moringa oleifera. *Asian Pacific journal of tropical biomedicine*, 3(8): 623-627.

Mirmiranpour, H., Ashoori, M.R., Mikaeili, A.S., Pezeshki, S., Serani, A., Vassar,R., Martirosyan, D (2022). The effect of squalene on lipid profile and some oxidative biomarkers in patients with type 2 diabetes mellitus. *Functional Food Science*, 2(7): 144-156.

Nwaehujor, U., Obinna, K.N., Sunday, O.U., Chinaka, O.N. and Gregory, E.O (2012). Evaluation of anti-inflammatory activities of root extracts of Stephania dinklagei (Engl.) Diels. *African Journal of Pharmacy and Pharmacology*, 6**(**11): 834-839.

Nwankwo, C.E. (2017). Antimicrobial and Spectroscopic Analyses of Cola millenii K. Schum. An unpublished Dissertation in the Department of Biological Sciences, College of Natural Sciences, Redeemer's University, Ede, Nigeria, 2-31.

Nwokocha, L.M. and Williams, P.A. (2018). Characterization of the Polysaccharide from Cola millenii seeds. Conference Book of Proceeding, African Research Academy Conference, Ibis Styles, Accra, Ghana, 25-26.

Obafemi, C.A., Akinpelu, D.A., Taiwo, O.O. and Adeloye, A. (2006). Antimicrobial Activities of Solvent extracts of Terminalia catapa Linn leaves. *Ife Journal of Science*, **8**(1): 29-33.

Okwu, D (2005). Phytochemicals, Vitamins and Mineral Contents of Two Nigeria Medicinal Plants. *International Journal of Molecular Medicine and Advance Sciences*, **1**(4): 375-381.

Soobratee, M.A. and Neergheen, V.S (2005). Luximon-Ramma A, Arouma, AI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions .*Mutation research*, 579(1-2): 200-213.

Sudharsan, S.S., Kumar, A. and Singh, S.P. (2011). Antimicrobial activity of the major isolates of mentha oil and derivatives of menthol. *Analytical Chemistry Letters*, 1(1): 70-85.