

Cola millenii Leaf Ethyl Acetate Extract: Fourier Transform Infrared (FTIR) Spectroscopy; Modulatory Effect on Serum Indices and Redox Biomarkers

Original research article

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Abstract The ethyl acetate extract of the leaves of *Cola millenii* were investigated for its secondary metabolites as well as its effect on serum indices and antioxidant status in normal male albino rats. Fresh leaves of *C. millenii* were air dried, extracted with ethyl acetate and concentrated. The extract was analysed for its secondary metabolites using the Fourier transform infrared (FTIR) spectroscopy. Three concentrates consisting of 50, 100, and 200 mg/kg body weight (bw) of the extract were prepared. Groups of five rats were intraperitoneally injected with each of the doses, whilst a fourth group was not injected and served as the normal control. After 7 days, the rats were sacrificed by cervical dislocation. Blood serum was analysed for hepatic and renal biomarkers as well as cholesterol level. Hepatic tissue was analysed for malondialdehyde (MDA) level, superoxide dismutase (SOD) and catalase (CAT). FTIR spectroscopy revealed the presence of aliphatic, carboxylic acids, esters and alkenes functional groups. Administration of the extract doses led to the increased serum activities of hepatic and renal biomarkers. About 50 mg/kg bw of the extract had the least cholesterol level compared to the other doses. MDA level was significantly ($p < 0.05$) reduced in rats administered with 50 mg/kg bw of the extract. Except for the lowest dose, little or no significant effect was observed on SOD and CAT activities. These results indicate the medicinal potential of ethyl acetate extract of *C. millenii* leaves as portrayed by the low cholesterol and MDA levels and increased CAT activity with 50 mg/kg bw being the most active concentration.

Keywords Anti-oxidative stress – *Cola millenii* – Medicinal plants

INTRODUCTION

There have been increased interests in the use of medicinal plants in the management of various ailments (WHO, 2007). This has been attributed to the presence of phytochemicals. These phytochemicals have been documented for their medicinal properties with little or no side effects associated with synthetic drugs. Nigeria is endowed with several medicinal plants, most having little or no scientific evidence on their efficacy. Amongst such plants is the genus *Cola*. Commonly regarded as kola nuts, they belong to the family *Malvaceae*. The genus contains several species with only a few bearing fruits. Notably amongst the fruit bearing species are *Cola nitida*, *Cola acuminata*, *Cola verticillata* and *Cola millenii* (Orisakeye and Ojo, 2013; Ogbu and Umeokechukwu 2014). This present study focuses on the medicinal properties of the leaves of *C. millenii*. Commonly known as monkey kola, it belongs to the sub-family *Sterculioideae* (Ogbu

and Umeokechukwu 2014). It is a tree indigenous to Ivory Coast and Southern Nigeria that grows up to 12–20 m high (Orisakeye and Ojo, 2013). It is deciduous with low crown of arching branches (Burkil, 1985). The efficacy of the leaf extract against ring worm (Odugbemi and Akinsulire 2006), *Mycobacterium bovis* and strains of *Mycobacterium vaccae* (Adeniyi et al. 2004) has been reported. Its *in vitro* antioxidant and phytochemical properties have been documented in several studies (Orisakeye and Ojo 2013). They are commonly chewed ceremonially in many Nigerian cultures and are used in several traditional rites such as religious worship, wedding and naming ceremonies. They are important source of alkaloids in pharmaceutical preparations (Sonibare *et al.* 2009). Their use in the treatment of whooping cough and asthma is well documented and has been attributed to their high caffeine content (Jayeola 2001).

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Their use in the treatment of malaria and fever has also been reported (GRIN 2007)

This study aims at investigating the functional groups of the ethyl acetate extract of the *C. millenii* leaves using Fourier transform infrared (FTIR) spectroscopy as well as reporting its effect on serum indices and antioxidant status in normal male albino rats (Wistar strain).

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *C. millenii* were harvested from Ifako, Nigeria. They were rinsed with tap water before air drying. The dried leaves (100 g) were blended and extracted with ethyl acetate (1 L). The extract was concentrated and the residue stored in air tight container at -20°C until further analysis.

Fourier Transform Infrared (FTIR) Spectroscopy

The extract was mixed with KBr (potassium bromide) to ameliorate the scattering effect of large crystals. The mixture was then subjected to FTIR (PerkinElmer) analysis to determine the functional groups of compounds present in the sample.

Experimental Animals

Twenty male albino rats of Wistar strain with mean weights of 67–90 g were used for this study. They were acclimatised on normal pelleted diets for 7 days. They were provided water *ad libitum* and maintained under standard laboratory conditions of natural photo period of 12-h light-dark cycle. After acclimatisation, the rats were divided into four groups consisting of five animals each. Except for group 1 which served as control, the rats were injected intraperitoneally with different doses of the extract as shown below:

- Group 1: Control (no extract administered)
- Group 2: 50 mg/kg body weight (bw) of extract
- Group 3: 100 mg/kg bw of extract
- Group 4: 200 mg/kg bw of extract

The rats were monitored daily for food and water intake, bw and mortality. After 7 days, the rats were sacrificed by cervical dislocation.

The animals used in the present study were maintained in accordance with the approval of the Animal Ethical Committee, Federal Institute of Industrial Research, Lagos, Nigeria.

Collection of blood and preparation of serum

Blood was collected from each rat with a 5-mL syringe by cardiac puncture and transferred into clean plain centrifuge tube bottles as soon as it was collected. They were centrifuged at $6,440\times g$ for 10 min, and the serum (supernatant) was

transferred into labelled sample bottles. They were stored at 4°C to maintain enzyme activity.

Determination of Serum Indices

Blood serum was used for the evaluation of cholesterol and hepatic function biomarkers such as bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using commercial kits from Randox Laboratories, UK, according to the manufacturer's protocol. They were also analysed for renal function biomarkers such as urea and creatinine using commercial kits from Randox Laboratories, UK, according to the manufacturer's protocol.

Preparation of Tissue Homogenates

Hepatic organs were harvested, rinsed in ice-cold 1.15% KCl solution to wash off excess blood, blotted dry with filter paper and weighed. They were homogenised in phosphate buffer (0.01M; pH 7.4) and centrifuged at $6,440\times g$ for 15 min. The supernatant was decanted and stored at -20°C for subsequent analysis. Each time the supernatant was outside the freezer, it was kept in ice bags.

Determination of Oxidative Stress Parameters in Tissue Homogenates

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formed by thiobarbituric acid reaction (TBAR) as described by Chowdhury and Soulsby (2002). Catalase (CAT) activity was determined by monitoring the rate of decomposition of H_2O_2 (Aebi, 1983). The method of Misra and Fridovich (1972) was used in determining the superoxide dismutase (SOD) activity.

Statistical Analysis

To address the biological variability, each set of experiments was repeated at least three times. Differences between the groups were analysed by one-way analysis of variance (ANOVA) with the aid of SPSS software (SPSS Inc., Chicago, IL, USA) standard version 17. The p values of less than 0.05 were considered statistically significant for differences in mean using the least of significance difference, and data were reported as mean \pm standard deviation.

RESULTS

Figure 1 shows the chemical shifts from the FTIR spectroscopy of the extract. A weak alcoholic OH band stretch at $3,427\text{ cm}^{-1}$ and aliphatic CH band stretch at $2,922$ and $2,850\text{ cm}^{-1}$. The observed sharp shifts at $1,732$ and $1,464\text{ cm}^{-1}$, respectively, correspond to carbonyl and C=C stretching of carboxylic acids, esters and alkenes, whilst a CH band of alkene was observed at $1,377\text{ cm}^{-1}$. These observed chemical shifts

Table 1. Effects of ethyl acetate extract on plasma hepatic function markers

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)	Bilirubin (mg/dL)
Group 1	55.31±1.31	493.96±0.87	306.02±0.85	0.96±0.20
Group 2	113.69±2.08*	510.37±0.96	269.98±1.72	1.26±0.20
Group 3	89.11±1.67*	526.19±1.00*	223.11±1.27*	1.39±0.33
Group 4	122.9±1.89*	526.19±1.00*	318.97±1.19	1.30±0.13*

Values = Mean ± SEM; n = 5. *Statistically significantly ($p < 0.05$) compared to group 1

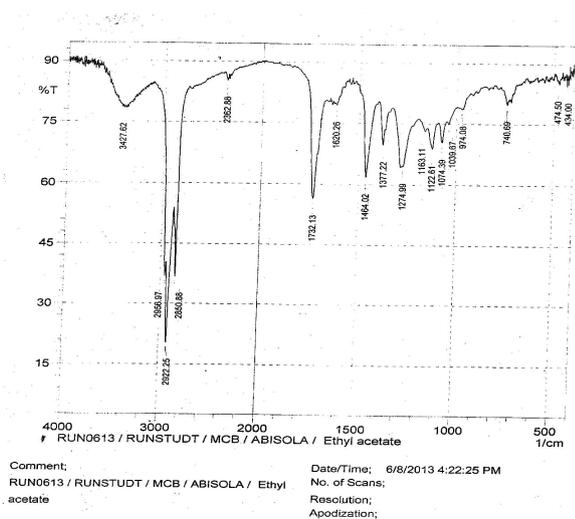


Figure 1. Fourier transform infrared (FTIR) spectroscopy of ethyl acetate extract of Cola millenii leaf.

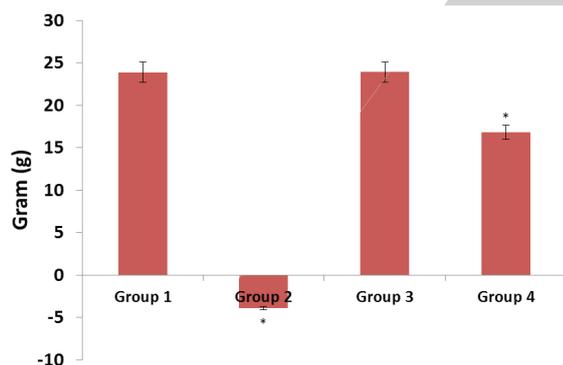


Figure 2. Body weight gain of experimental groups after 7 days of administration with different concentrations of ethyl acetate extract of Cola millenii.

indicate that the extract could be a mixture of compounds containing aliphatic, carboxylic acids, fatty acids, ester and alkenes.

No death was observed amongst the experimental groups. Significant ($p < 0.05$) weight lost was observed in rats administered 50 mg/kg bw of the extract (group 2) as depicted in Figure 2.

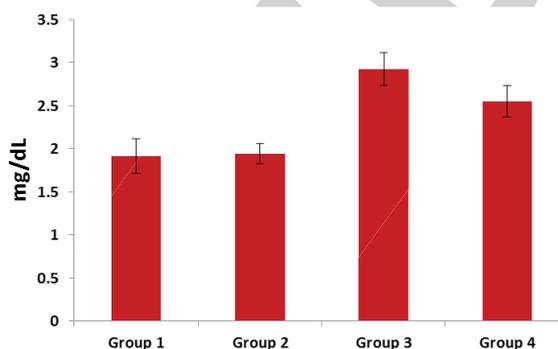


Figure 3. Serum cholesterol level of experimental groups after the administration of different concentrations of ethyl acetate extract of Cola millenii. Values = Mean ± SEM.

Administration of the extracts led to increased activities of the ALT, ALP and AST as well as increased bilirubin level indicating increased levels of serum hepatic markers (Table 1). Group 3 had the least increase in ALP and AST activities, whilst group 2 had the least in ALT.

Elevated level of urea and creatinine was observed in all groups administered with the extracts as shown in Table 2. These elevations indicate increased renal markers. Except for creatinine, group 2 had the least increase.

Significant increase in serum cholesterol level was observed as the extract concentration increased (Figure 3), thus indicating a dose – like dependent effect.

Administration of 50 mg/kg bw of the extract led to significant decrease in MDA level, signifying reduced lipid peroxidation as depicted in Figure 4. The other doses had little or no effect on the MDA level.

Administration of various doses of the extract had little or no effect on SOD activity as shown in Table 3. However, a dose-dependent effect was observed in the CAT activity with concomitant increase in the extract concentration.

DISCUSSION

The medicinal properties of plants have gained much research interests owing to the paradigm shift from synthetic drugs. This shift has been attributed to the lesser side effects of phytochemicals responsible for the medicinal properties

of these plants. In this study, the ethyl acetate extract of the leaves of *C. millenii* were investigated for its secondary metabolites functional groups as well as its effect on serum indices and antioxidant status in normal male albino rats (Wister strain).

The observed weight loss in the group administered 50 mg/kg bw of the extract is an indication of the weight control potential of the extract at that particular dose. Far-reaching health consequences such as type 2 diabetes, high blood pressure and stroke have been associated with overweight and/or obesity (ACS 2015). As many as one out of five of cancer deaths have been attributed to overweight (ACS 2015). Therefore, there is need for natural remedies coupled to physical exercise activities. Thus, the observed weight loss

by the extract at 50 mg/kg bw is of tremendous benefit in weight control.

The little or no effect on cholesterol level (Figure 3) observed in rats administered 50 mg/kg bw of the extract corroborates with the observed weight loss as compared to the other groups (figure 2). Hypercholesteremia has been associated with excess bw and/or obesity and linked with the aforementioned health consequences (ACS 2015). The reduced level further portrays the potential of the extract in the management and treatment of overweight and/or obesity-related ailments.

Elevated serum activities of hepatic and renal biomarkers have been associated with hepatocellular injury (Kaneko et al. 1997). These elevations have been attributed to the rupture of the plasma membrane and cellular damage, leading to release of the enzymes into the blood circulation (Abovwe et al. 2010). The increased activities (Tables 1 and 2) observed on the administration of the extract at various concentrations indicate that the extract may induce hepatic and renal injury in normal rats. The toxic effect of herbal medicine despite their efficacy is of major concern to health practitioners. Several studies have implicated herbal therapies in hepatic and renal toxicity (Efferth and Kaina, 2011; Asif, 2012), which can be attributed to their (hepatic and renal) increased metabolism of the extract. Thus, precaution should be taken when administering to healthy individuals.

Lipid peroxidation is a marker of oxidative stress and has been implicated in several diseases such as obesity, diabetes and cancer (Erukainure et al. 2014b). The observed reduced MDA level in rats administered 50 mg/kg bw of the extract (group 2) indicates the antioxidant potential of the extract at that concentration. This is further portrayed by the increased CAT activity by all the concentrations.

These observed biological activities can be attributed to the observed functional groups. Carboxylic acids such as nonanoic acid and octanoic acid are major constituents of most essential and fixed oils with reported antioxidant activities (Cunha et al. 2013). The double bonds present in alkenes especially unsaturated fatty acids have been recognised for their cardio-protective roles as well as their antiproliferative activities (Erukainure et al. 2014a; 2015). Therefore, there is need to isolate these active compounds and investigate their biological activities.

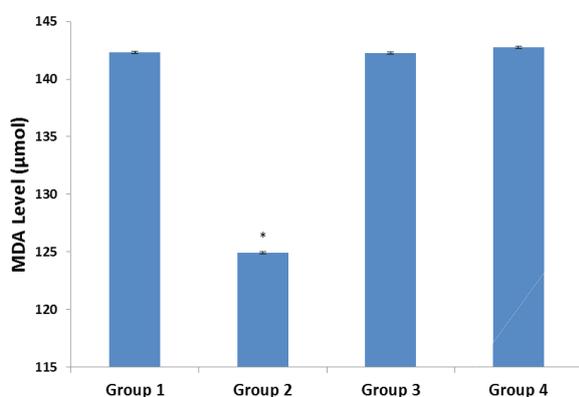


Figure 4. MDA levels of experimental groups after the administration of different concentrations of ethyl acetate extract of *Cola millenii*. Values = Mean \pm SEM; n = 5. *Statistically significant ($p < 0.05$) compared to group 1.

Table 2. Effects of ethyl acetate extract on plasma renal function markers

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Group 1	34.93 \pm 0.59	27.83 \pm 1.03
Group 2	36.56 \pm 0.57	33.39 \pm 1.36
Group 3	51.15 \pm 0.67*	20.87 \pm 0.72
Group 4	61.29 \pm 0.32*	53.43 \pm 1.09

Values = Mean \pm SEM; n = 5. *Statistically significantly ($p < 0.05$) compared to group 1

Table 3. Effects of ethyl acetate extract on antioxidant enzymes status in hepatic tissue of experimental groups

Parameters	Group 1	Group 2	Group 3	Group 3
Catalase activity (μ g/min/g)	26.53 \pm 0.26	28.25 \pm 0.34	26.78 \pm 0.39	28.28 \pm 0.30
Superoxide Dismutase activity (unit/g)	0.20 \pm 0.06	0.23 \pm 0.06	0.22 \pm 0.07	0.30 \pm 0.08

Values are expressed in Mean \pm SEM. *statistically significantly different compared to group 1

CONCLUSION

These results indicate the medicinal potential of ethyl acetate extract of *C. millenii* leaves as portrayed by the weight reduction, reduced MDA level and increased CAT activity with 50 mg/kg bw being the most active concentration. However, the increased serum activity of hepatic and renal biomarkers

poses a toxicity concern of the extract in normal individuals. Further isolation of the active compounds may reduce these toxic effects.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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