DE GRUYTER OPEN

DOI: 10.2478/auoc-2018-0004

Ovidius University Annals of Chemistry Volume 29, Number 1, pp. 29-35, 2018

Evaluation of phytochemical components of various parts of *Cola millenii* K. Schum

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Abstract. This study is aimed at evaluating the qualitative and quantitative phytochemical composition of various parts (leaf, stem bark, root, seed and pulp) of *Cola millenii* a medicinal plant of southwestern Nigeria. The bioactive ingredients were extracted using water, ethanol and *n*-hexane in a solvent-percolation protocol. The qualitative phytochemical screening result revealed the presence of alkaloids, saponins and tannins in all the parts of the plant analyzed. Glycosides was found in only the seed and pulp extracts while only the seed, leaf and stem bark contain terpenoids. Also, flavonoids were found in pulp extract only whereas, anthraquinones were not found in all the plant parts. In the quantitative analyses aqueous extracts of the pulp parts contained higher saponins (1.81%), tannins (0.77%) and flavonoids (1.12%) followed by seed aqueous extract which had 0.62%, 0.51%, 0.70% and 0.47% composition of alkaloids, saponins, glycosides and terpenoids respectively. In ethanol extract, pulp extract also had higher percentage of alkaloids (1.72%), saponins (2.24%), tannins (1.15%) and flavonoids (1.21%) compared to other parts of the plant however, glycosides was found in higher percentage in seed extracts (1.10 %) than in pulp (0.21%). Moreover, in *n*-hexane extracts of the plant parts, pulp extracts revealed higher percentage of alkaloids (1.71%), saponins (1.40%) and flavonoids (0.93%) followed by stem bark extract whereas glycosides was present in higher percentage in seed (0.82%) than pulp extracts (0.38%). In all, the pulp and seed extracts of the plant contained more phytochemicals than other parts screened. Moreover, pulp extracts contain higher percentage of these phytochemicals than the other parts except glycosides and terpenoids which were more abundant in seed extracts than the other parts. Among different solvents used for extraction in the series, ethanol had the highest extraction capacity in pulp, leaf and stem bark extracts while *n*-hexane had the best extraction capacity in the seed extract. Thus, *C. millenii* may possess medicinal properties which may be expeditiously utilized in the pharmaceutical industry.

Keywords: Cola millenii, phytochemicals, alkaloids, saponins, terpenoids, anthraquinones, glycosides.

1. Introduction

Generally, plants that contain any active substance which can be applied as remedy for the management of diseases or which may be used as starter for manufacturing of new drugs are regarded as medicinal plants [1, 2]. Furthermore, a plant becomes a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically established. Historically, plants have always played a major role in African traditional medicine. Nevertheless, among the vast array of these plant species, only few have been investigated for their chemical composition [3]. The folkloric knowledge of medically important plants passed down from generation to generation usually in oral form has made significant impact in the development of many useful compounds used in the modern medicine system.

Plants are invaluable source of medicines used in the management of several diseases especially in African traditional medical practices. According to the World Health Organization, 80% of the world population relies on medicine from plants in the management and treatment of various diseases; most of these people are located in African countries. Moreover, about 90% of people in the developing countries are estimated to be reliant on herbs and medicinal plants for their primary health remedies [4]. Furthermore, Food and Agriculture Organization reported that about a quarter of modern drugs are developed from plants while others are synthetic analogues of the species gotten from plants [5]. More than fifty percent of modern pharmaceuticals are developed from natural products especially from plants [6, 7].

The pharmaceutical properties of plants used in traditional medicine can be attributed to the presence of phytochemicals in their tissues [8]. Phytochemicals

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are the natural compounds present in plants and they interact with nutrients and fibers to form an integral part of human immunity system against various diseases. Phytochemicals are grouped into primary and secondary metabolites, the primary metabolites include sugars, amino acids, proteins, etc. whereas secondary metabolites include alkaloid, flavonoids, steroids, saponin, terpenoids, phenolics, etc. [9]. These substances are reportedly produced by the plants as defense against infectious agents and herbivores as well as for synthesis of new compounds.

Cola millenii K. Schum is a small tree with a low crown of spreading branches and edible fruits found in rain forests. It grows to a height of between 4.5 m and 18 m, developing either a robust or slender stem with either sparse or bushy foliage. It belongs to the family *Sterculiaceae,* it is commonly called Monkey kola (English) and "obi-edun" by the Yorubas of southwestern Nigeria. In African traditional medicine, the leaves, flowers, stem and fruit follicles and the bark are used in folk medicine to prepare a tonic used as a remedy for dysentery, coughs, diarrhea, vomiting and chest complaints. In Nigeria, *C. millenii* is used generally in ethnobotany for the treatment of diarrhea and dysentery [10]. Among the Yorubas of South Western Nigeria, the leaves and fruits of *C. millenii* is used in the treatment of ringworm, scabies, gonorrhea, dysentery and opthalmia [11]. There have been reports of the antimicrobial activities of this plant against human pathogens [12]. Despite several reports about the phytochemical properties and extensive uses of *C. millenii* K. Schum in folk medicine, there is a dearth of information on the quantities or proportion of these phytochemicals in major parts of the plant. Therefore, this research is undertaken to determine the phytochemical composition of different parts of *Cola millenii.*

2. Experimental

2.1. Materials

The high purity reagents were obtained from (Sigma-Aldrich Inc, Missouri, USA) being used as received without further purification.

2.2. Collection and preparation of plant samples

The leaf, root, stem bark and fruits of Monkey kola (*C. millenii*) were harvested fresh from the wild in a forest at Iyere, Owo local government, Ondo state. The plant materials were then authenticated at the Environmental Biology Unit of Science Laboratory Technology Department, Rufus Giwa Polytechnic, Owo and voucher specimens (CMP112IF - *C. millenii* fruit, CMP1122B - *C. millenii* stem bark, CMP1123R - *C. millenii* root and CMP1124L - *C. millenii* leaf) were deposited at the Department of Forestry Resources Technology, of the same institution. The seed and pulp were removed from the fruit manually using laboratory knife. Thereafter, the plant materials were washed thoroughly in distilled water and air dried for 21 days in the laboratory. The dried samples were then ground into powder with the aid of a mechanical grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use.

2.2. Extraction procedure

100 g portion of the powdered samples was soaked in 300 ml of different solvents (acetone and ethanol) for 48 h with intermittent stirring using sterile spatula. Thereafter, extracts were filtered through filter paper into sterile containers and then dried using rotary evaporator at 50 ^{o}C.

2.3. Qualitative phytochemical screening

All the tests described below were carried out on the extracts using the methods described by Harborne [13], Trease and Evans [7], and Sofowora [2].

2.3.1. Test for tannins. 1 ml of the extract was boiled in 10 ml of water and then filtered. An observation of green color on addition of drops of 0.1% ferric chloride ($FeCl_3$) confirms the presence of tannin.

2.3.2. Test for saponins. One portion of the extract was boiled in four part of water (1:4 v/v) followed by filtration. The filtrate was diluted with a little water and shaken for froth formation; olive oil was added to the mix and shaking continuously for 3 minutes. Appearance of emulsion confirmed the presence of saponins.

2.3.3. Test for flavonoids. 5 ml of the extract was mixed with 3 ml of 1% aluminum chloride ($AlCl_3$). A portion of 5 ml of dilute ammonia and concentrated H_2SO_4 solutions were added sequentially. The disappearance of yellow coloration indicated the presence of flavonoids.

2.3.4. Test for terpenoids (Salkowski test). 2 ml of chloroform were mixed with a 5 ml portion of the extract after which concentrated H_2SO_4 solution was gently added. Presence of terpenoids was confirmed with the appearance of a red-brown color at the interface.

2.3.5. Test for glycosides. A portion of 3 ml of the plant extract was treated with 1 ml of glacial acetic acid containing one drop of ferric chloride followed by addition of concentrated H_2SO_4 solution. The formation of violet-green layer underneath was taken as the positive test for the presence of glycoside.

2.3.6. Test for alkaloids. A 1:5 (v/v) mixture of the extract and 1% aqueous HCl was heated in water bath then filtered hot. The filtrate was diluted with water and 4 drops of Mayer's reagent was added. Conclusions were drawn based on color change.

2.3.7. Test for anthraquinone. The extract and benzene were mixed in ratio 1:2 (v/v), and then 10% NH_3 was added to the filtrate. Appearance of violet color after shaking of the mixture was taken as positive for the presence of anthraquinones.

2.4. Quantitative phytochemical determination

2.4.1. Tannins. A portion of the extract (2 g) was weighed into a beaker, 20 ml of methanol was added and homogenized by shaking. The beaker was sealed with Parafilm (Bemis Inc, USA) and heated in water bath at 80 ^{o}C for 1 h. The mixture was filtered using Whatman No. 1 filter paper into a volumetric flask then, 20 ml water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 were added and homogenized. This was made up to 100 ml with distilled water and left for 30 min to react. The absorbance of the tannic acid standard solution along with the sample was measured after color development using spectrophotometer (Buck Scientific 210VGP), at 760 nm [14]. The percentage of tannin was estimated by the formula:

$$\%Phytochemical = \frac{A \times GF \times DF}{m \times 10{,}000} \qquad (1)$$

where: A = absorbance of sample;
GF = gradient factor;
DF = dilution factor;
m = mass of sample [g].

2.4.2. Determination of alkaloids. A 2 g portion of finely ground sample and 1 g of magnesium oxide were mixed with absolute ethanol and then made up to 100 ml in a beaker. The mixture was digested in a boiling water bath for 2 h and then filtered immediately with the aid of a Buchner funnel. Further, the residue was re-digested for 45 min, thereafter, the alcohol was evaporated and water was added. After the removal of the alcohol, few drops of 10% hydrochloric acid was added. The solution was poured into a 250 ml volumetric flask then 5 ml each of zinc acetate solution and potassium ferrocyanide ($K_4[Fe(CN)_6]$) solution were added and homogenized to give a uniform solution. The mixture was left to react for some minutes, and then filtered with Whatman paper. Afterwards, 10 ml of the filtrate was transferred into a separator funnel and the alkaloids extracted by vigorous agitation with five successive portions of chloroform. The precipitate gotten was dissolved in hot water and transferred into a Kjeldahl bottle while 0.20 g sucrose, 0.02 g selenium and 5 ml concentrated sulfuric acid were added for digestion to determine percentage nitrogen [14].

The alkaloids percentage was estimated by the formula:

$$\%\ \text{Alkaloids} = \%\text{N} \times 3.26 \qquad (2)$$

where: %N = percentage nitrogen
3.26 = constant.

2.4.3. Determination of flavonoids. A portion of 1 g of finely ground sample was dissolved in 80 ml of absolute ethanol in a beaker with continuous stirring. After, the mixture was filtered with Whatman filter paper and then made up to 100 ml with ethanol in a volumetric flask. 1 ml of this solution was measured into 50 ml volumetric flask and 0.5 g of magnesium with few drops of concentrated hydrochloric acid were added (magenta red coloration developed). To a fresh standard flavonoids solution (5 ppm) was added 0.5 g of magnesium and few drops of hydrochloric acid. Absorbance of the sample as well as that of standard solutions were measured on a digital Jenway V6300 spectrophotometer at a wavelength of 520 nm [14]. The percentage of flavonoids was calculated using Eq. 1.

2.4.4. Determination of saponins. A portion of 2 g of finely ground sample was dissolved with 100 ml isobutyl alcohol in a beaker and homogenized by shaking for a few hours on an automated shaker. The mixture was later filtered with Whatman filter paper into a clean beaker, 20 ml of magnesium carbonate was added and the mixture was re-filtered. 1 ml of the filtrate was added to 2 ml of 5% $FeCl_3$ solution in volumetric flask and made up to 50 ml with distilled water. This mixture was left for 20 min for development of red color. A freshly prepared standard saponin solution (0-10 ppm) was also treated with $FeCl_3$. Thereafter, absorbance of the sample and standard saponins solutions were recorded in spectrophotometer at a wavelength of 380 nm.

The percentage of phytochemical was determined thus:

$$\%Phytochemical = \frac{A \times GF \times DF}{m \times 1000} \qquad (3)$$

where: A = absorbance of sample;
GF = gradient factor;
DF = dilution factor;
m = mass of sample [g].

2.4.5. Determination of glycosides. A 10 ml portion of each of the extract was mixed with 50 ml of chloroform in a conical flask, the mixture was then shaken for 1 hours using automated shaker then filtered into a conical flask. 10 ml of mixture and 2 ml of pyridine and 2% sodium nitroprusside ($Na_2[Fe(CN)_5NO]$) were added to the filtrate and vigorously shaken for a few minutes followed by addition of 3 ml of sodium hydroxide (NaOH) which leads to the development of brown-yellow coloration [14].

Glycoside standards which ranged from 0-5 mg/ml were prepared from 100 mg/ml stock glycoside standard. A series of fresh glycoside standards (0-5 mg/ml) were also treated with pyridine, sodium nitroprusside and NaOH like the test samples. Absorbance of the samples and the standards were read on a Spectronic 21D Digital spectrophotometer at a wavelength of 510 nm.

The percent of glucosides was estimated using Eq. 3.

2.4.6. Anthraquinones determination. A 1 g portion of the sample was dissolved in 60 ml benzene in a beaker with continuous stirring and then filtered. 10 ml of the filtrate was measured into a volumetric flask where 0.2 zinc dust and 50 ml hot 5% NaOH solution were added successively. This was heated in a water bath for about 7 min and then quickly filtered and

washed once in water. The filtrate was re-heated with 50 ml of 5% NaOH until a red color was developed [14].

Fresh standard anthraquinone solution (0-5 mg/ml) was also treated 0.2% zinc dust and NaOH like the sample. The absorbance of both sample and the standard anthraquinone solutions were recorded through spectrophotometer (Buck Scientific 210VGP) at a wavelength of 640 nm and the percent anthraquinone was estimated using Eq. 1.

2.4.7. Determination of terpenes. 20 ml of methanol and chloroform mixture (1:2 v/v) was used to dissolve 1g of sample in a conical flask, this was homogenized and allow to react for about 20 min after which it was centrifuged at 1,000 rpm for 15 min. The precipitate was recovered by decantation and later re-washed with another 20 ml methanol-chloroform mixture and centrifuged.

The precipitate was later dissolved in 40 ml of 10% sodium dodecyl sulfate solution with the addition of 1 ml of 0.01 M $FeCl_3$. The mixture was shaken and allowed to react for 30 min. The absorbance of the freshly prepared standard terpenes (0-5 mg/ml) solutions and that of the sample was read on spectrophotometer (Buck Scientific 210VGP) at a wavelength 510 nm [14]. The percent of terpenes was calculated using Eq. 1.

2.5. Data analysis

Data were recorded as mean ± standard error of mean (mean ± SEM). Significant difference between groups was tested using two-way analysis of variance and treatment means were compared with Duncan using SPSS version 17.0, while significance was taken at 95%.

3. Results and discussion

3.1. Results

Preliminary phytochemical screening results of various parts of *Cola millenii* K. Schum is presented in Table 1 where it is revealed that the extracts contain alkaloids, saponins, tannins, glycosides, flavonoids and terpenoids whereas anthraquinones were not found in any of the plant parts analyzed. The pulp and seed extracts of the plant had the highest number (5) of the phytochemicals screened while the root extracts (3) had the least number of the phytochemicals. Among different solvents used for extraction in the series, ethanol had the highest extraction capacity in pulp, leaf and stem bark extracts while *n*-hexane had the best extraction capacity in the seed extract.

Table 1. The qualitative phytochemical components of crude extracts of *Cola millenii.*

Phytochemical	Leaf			Stem bark			Root			Seed			Pulp		
	AE	EE	HE	AE	EE	HE	AE	EE	HE	AE	EE	HE	AE	EE	HE
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	-	+	+	+	+	+	-	-	+	+	+	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+
Flavonoids	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Terpenoids	-	+	+	-	+	+	-	-	-	+	+	+	-	-	-

Legend: ND = not detected; + = present; AE = aqueous extract; EE = ethanol extract; HE= *n*-hexane extract.

The results of quantitative evaluation of the phytochemicals in the different parts of the plant are presented in Figures 1 to 3. Fig. 1 shows that in aqueous extracts of the plant parts, saponins (1.81±0.01%), tannins (0.77±0.00%) and flavonoids (1.12±0.01%) were present at a higher percentage in aqueous pulp extract of *Cola millenii* followed by seed aqueous extract which had 0.62±0.00%, 0.51±0.01%, 0.70±0.01% and 0.47±0.01% composition of alkaloids, saponins, glycosides and terpenoids respectively.

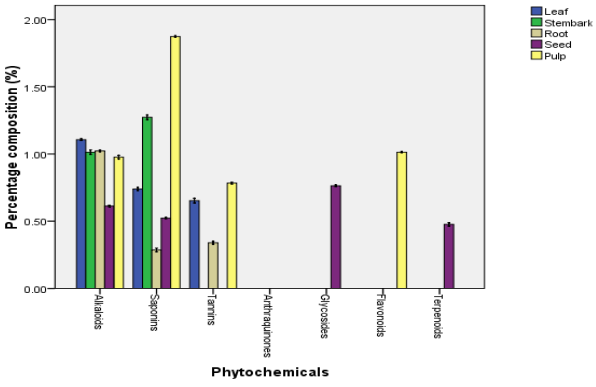

Figure 1. Phytochemical composition (%) of *Cola millenii* aqueous extract.

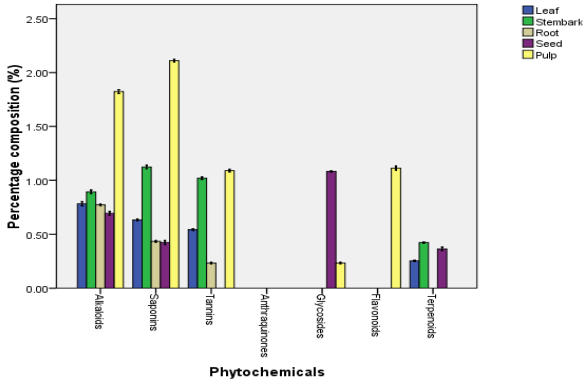

Figure 2. Phytochemical composition (%) of *Cola millenii* ethanol extract.

In crude ethanol extract of the *Cola millenii* parts, pulp extract contain higher percentage of alkaloids (1.72±0.01%), saponins (2.24±0.00%), tannins (1.15±0.02%) and flavonoids (1.21±0.01%) compared to other parts of the plant however, glycosides was found in higher percentage in seed extracts (1.10±0.00%) than in pulp (0.21±0.00%).

In *n*-hexane extracts of the plant parts, pulp extracts contained higher percentage of alkaloids (1.71±0.00%), saponins (1.40±0.01%) and flavonoids (0.93±0.00%) followed by stem bark extract whereas glycosides was present in higher percentage in seed (0.82±0.00%) than pulp extracts (0.38±0.01%).

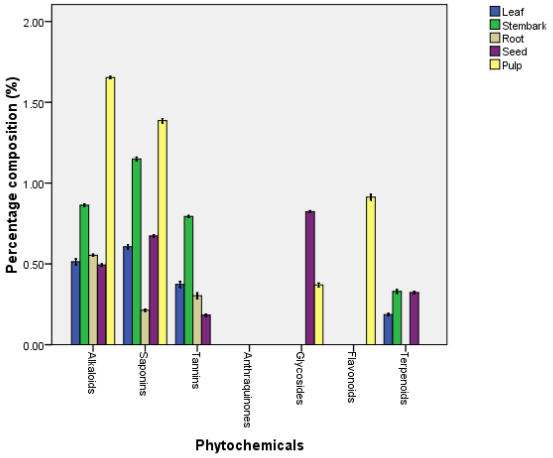

Figure 3. Percentage phytochemical composition of *Cola millenii n*-hexane extract.

3.2. Discussion

Phytochemicals are natural bioactive compounds produced by plants as secondary metabolites that work with nutrients to protect against pathogenic attacks. To survive in the harsh environment that plants live, they often synthesize various kinds of secondary metabolites that act to ameliorate such situations [15]. Nascimento *et al*. [16] reported that phytochemicals represents the most abundant and extensively distributed substances in the plant kingdom and that several plants and herb cells produce and father these range of medicinal phytochemicals. Cowan [17] and Cheeke [18] opined that some of the phytochemicals have great medicinal functions which play major roles in the new drug development process.

The presence of alkaloids, saponins, tannins, glycosides, flavonoids and terpenoids in seed and pulp of *C. millenii* in this present study is in consonance with the reports of earlier researchers [19, 20]. However, Ubon *et al*. [19] did not find flavonoids, tannins and anthraquinones in *C. millenii* seed they examined while Giwa *et al*. [20] did not find flavonoid and anthraquinones in the seed and pulp of *C. millenii* they screened. Ajayi and Ojelere [21] also reported the presence of terpenoids in the seed of *C. millenii* which is in conformity with the result of this research.

On the basis of distribution, the phytochemicals were found in varying quantities in the plant parts examined. Alkaloids and saponins were found to be higher in all the plant parts compared with the other phytochemicals. The presence of these secondary metabolites in high proportions suggests that the plant parts may possess pharmacological properties since alkaloids and saponins are two of the most revered phytochemicals in plants.

Alkaloids are believed to be one of the most effective and therapeutically significant plant substances [22], they are reported to be pharmacologically active and their actions are felt in different parts of the body system such as the nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases and malaria [7, 15]. This may explain the use of the plant parts especially the seed, pulp and leaf in treating several disease conditions [12, 23]. Alkaloids have analgesic, antiplasmodial and bactericidal effects [24] as well as marked physiological effects on animals [25]; this supports the folkloric use of the *C. millenii* pulp in treatment of various diseases in monkey.

Saponins have hypotensive and cardiac depressant properties according to Olaleye [26] and have been shown to possess beneficial properties by lowering the cholesterol level, have anti-diabetic and anticarcinogenic properties as well as being used as an expectorant and emulsifying agent [15, 27]. The high percentage of saponins in the leaf, stem bark, seed and pulp of *C. millenii* suggests that the plant may be useful in the treatment of diabetes and management of heart conditions.

The presence of tannins in high percentage in pulp and stem bark of the plant indicates that plant may be useful in the management of infectious diseases. Tannins are reportedly efficacious as antimicrobial agent [24] and to stop bleeding during circumcision. The skin regeneration actions of tannins could explain the usefulness of the plant wound healing [28].

Terpenoids were found in the leaf, stem bark and seed of *C. millenii*. They reportedly possess anti-hepatoxic properties, thus helping to prevent liver damage (cirrhosis); they equally have anti-microbial or anti-septic properties [15]. This suggests that the plant may be useful in the management of liver problems.

Interestingly, flavonoids was found only in the pulp extract of *C. millenii*, this is line with the reports of Ajayi and Ojelere [21], but at variance with the observation of Giwa *et al.* [19] who did not detect flavonoids in either seed or pulp extracts of *C. millenii* assayed. The presence of flavonoids in the pulp extracts of the plant is supported by earlier reports that flavonoids are found generally in plants. They are said to be produced by plants in response to microbial infection [15]. They are also known for their antioxidant, anticarcinogenic, antimicrobial and antitumor properties [29]. This may be an indication that *C. millenii* may possess the ability to boost the immune system.

Moreover, glycosides were found in seed and pulp of *C. millenii* albeit significantly high only in seed extract, this is in line with the reports of Giwa *et al*. [20], Ajayi and Ojelere [21] and Ubon *et al*. [19]. Glycosides are reported to possess clinical effects on congestive heart failure [15]. They are also said to be active on the heart muscles and increase renal flow (diuresis) [26]. All these are pointers to the potential ability of the plant part especially the seed to manage several degenerative diseases.

4. Conclusion

From the foregoing, various parts of *Cola millenii* K. Schum contain alkaloids, saponins, tannins, glycosides, flavonoids and terpenoids whereas anthraquinones was not detected in plant's parts analyzed. The pulp and seed extracts of the plant contained more phytochemicals than other parts screened. Moreover, pulp extracts contain higher percentage of these phytochemicals than the other parts except glycosides and terpenoids which were more abundant in seed extracts than the other parts. Among different solvents used for extraction in the series, ethanol had the highest extraction capacity in pulp, leaf and stem bark extracts while *n*-hexane had the best extraction capacity in the seed extract. Thus, *C. millenii* may possess medicinal properties which may be expeditiously utilized in the pharmaceutical industry. However, there is need for further research to elucidate the chemical species present in the various parts of *C. millenii*.

Conflicts of interest: The authors declare no conflict of interest.

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Received: 29.04.2018
Received in revised form: 03.06.2018
Accepted: 04.06.2018