IECAG 2021

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Proceedings

Influence of plant part and age on phytochemical constituents of Monkey Kola (*Cola millenii* K. Schum.); an important agro-forestry species †

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† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021

Abstract: The current available economic tree species in natural and urban forests are rapidly decreasing at a rate that needs urgent attention. This is mostly occurring because many natural plant potentials are yet to be discovered while food insecurity is heightening daily in many developing countries. *Cola millenii* K. Schum. is an important agroforestry plant utilized in traditional medicines for various sickness remedies in different parts of Nigeria. Often time, parts such as leaves, barks and fruit components are utilized as a conglomerate for the treatment of diseases without adequate knowledge of their phytochemical constituent, which is a measure of the medicinal efficacy. The plant growth stage has been reported to affect the phytochemical component in some medicinal plants, however, such information is scarce for *C. millenii*. This study, therefore, evaluated the phytochemical constituents of *Cola millenii* to provide the information required for its utilization and sustainability. Samples were collected from different parts of mother and young *Cola millenii* found in the University of Ibadan, Nigeria. Plant samples were analyzed for saponin, flavonoid, tannin, alkaloid, anthraquinone, terpenoid and steroid following a standard method. Data were subjected to descriptive statistics, t-test and analysis of variance (ANOVA). Results show that age and plant part significantly ($p<0.05$) influenced the presence of some analyzed phytochemicals. Flavonoid was the most concentrated phytochemical in the fruit of *C. millenii* with the highest value in epicarp (1.54 mg/100kg). None of the phytochemicals was found in the leaves of the plant. Plant parts and age play important role in the concentration of phytochemicals in *C. millenii*. Therefore, with the multipurpose benefits derived from *C. millenii*, incorporating it in agroforestry systems, where there will be limited deforestation by the farmers as tree component would be an alternative way to conserve it for posterity purpose.

Keywords: Phytochemicals; *Cola millenii*; tree species; Agroforestry; Drug development

Citation: Onefeli, A.O.; Jegede, O.C.; A. I. Tay Influence of plant part and age on phytochemical constituents of Monkey Kola (*Cola millenii* K. Schum.); an important agroforestry species. **2021**, *4*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

The tropical forest is a natural home for many important fruit trees. Unfortunately, most of these valuable trees are falling victims to drastic deforestation due to indiscriminate harvesting occasioned by a rapid increase in the population [1–5]. It has been reported that a good number of multipurpose tree species are domiciled in patches of forests or as an amenity and avenue trees in research institutes and other academic environments like Universities, Colleges and polytechnics [6-8]. But recently, some of these trees have also witnessed rapid destruction without replacement as a result of several threats posed to the environment. Hence, the population of these trees have now diminished both in the natural forest and urban environment. In a bid to make food available for the populace, an expanse of the residual forests is removed for agricultural purposes. Consequently,

available potential medicinal trees, shrubs and climber's component are now being threatened. To salvage the destruction of the relic trees as well as sustaining the benefits derived from them, there must be deliberate incorporation of the trees on the agricultural production by the farmers. The deliberate cultivation of crops and selective trees is popularly known as agroforestry. However, successful adaptability and subsequent inclusion of trees into agricultural production land are vastly dependent on the benefits derived from trees and the compatibility of the system [9-11]. One of such benefits is the medicinal applicability of the plant. *Cola millenii* K. Schum. is a small tree in the family of Sterculiaceae that can grow up to about 365cm in height. It produces edible fruits, born in clusters having pink to orange-red colour. It has been reported with multipurpose benefits; the fruit nuts are mostly consumed by monkeys, from which the name "Monkey Kola" was derived. Its fruits and leaves have been utilized in Southwestern Nigeria for the treatment of infections like gonorrhoea, scabies, ringworm and dysentery [12]. The antimicrobial effect of *C. millenii* was reported by [13]. However, there is limited information on its phytochemical properties. Phytochemicals refer to chemical compounds, which are particularly originated from plants. They are produced either through primary or secondary metabolism. They are purposely used by the plant as a defense mechanism against predators. They are very essential for the physiological functioning of the human system; however, some may be toxic and are therefore referred to as phytotoxins.

2. Materials and Methods

2.1. Collection of Samples and preparation for phytochemical extraction

Samples of *C. millenii* parts (leaf, fruit and barks) used for this study were obtained from the mother trees with the aid of a cutlass and pruning hook. The mother trees were located within the remaining patches of forests at different roads and places in the University of Ibadan. These include Abadina road, Amina way, Dairy farm, Ijoma road, Obong road, Registrar's office and sawmill (Figure 1). Samples were also collected from the young plants in their distributional range on the university campus. Collected samples were taken to Eltolad Scientific Ventures located at Ogbere-ti-o-ya Olorunsogo, Akanran Road, Ibadan, Oyo State, Nigeria for phytochemical assessment.

The University of Ibadan is the oldest in Nigeria. It is located at about eight kilometres away from the centre of Ibadan city on latitudes 7°46′20.27″ N and 7°43′28.33′′N longitudes 3°88′27.93″ E and 3°90′78′′E, located on the edge of the derived savannah ecosystem and it has a humid tropical wet and dry climate and relatively cool environment with an average rainfall of 1420.06mm and relative humidity of 74.55%. According to the reports by authors [6-8, 14], who had studied the plant diversity and benefits on the campus, the University is blessed with some important fruit tree species, which are deliberately retained due to the microclimatic amelioration of the university and other benefits derived from them by the university community dwellers.

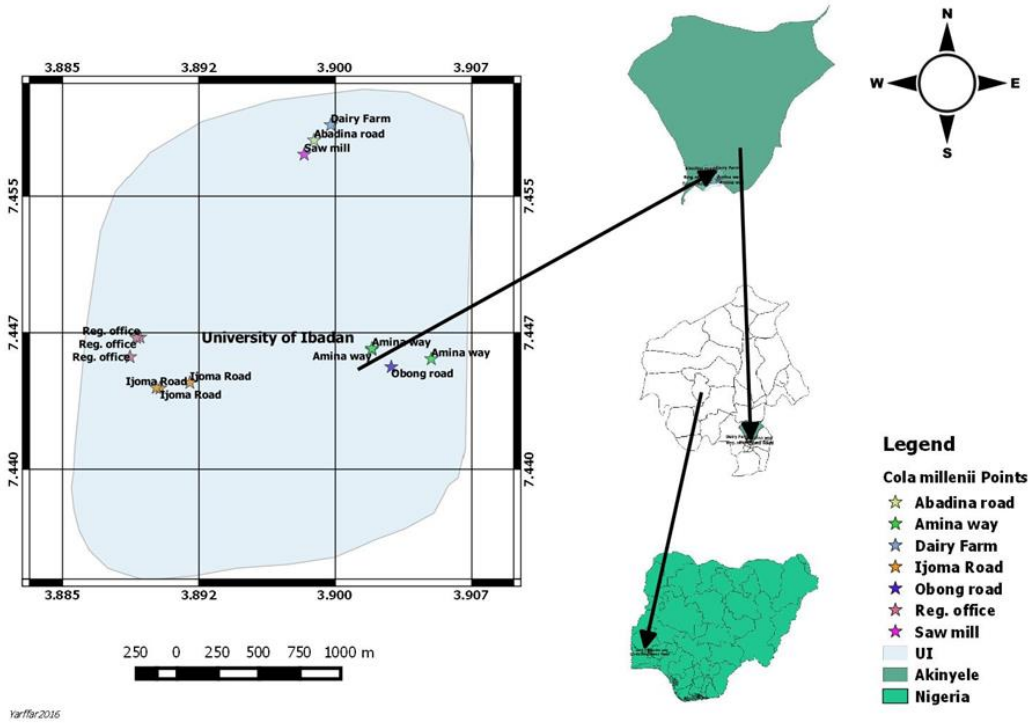

Figure 1. Collection points for the samples in the University of Ibadan.

All the collected samples were washed thoroughly while the fruit samples were separated into different parts (seeds, mesocarp, epicarp and endocarp). Samples were air-dried, chopped, crushed and powdered with the electrical grinder and then the dried powdered samples were stored in polyethylene bottles for further processes according to [15].

The phytochemical extraction adopted the standard method by [16]. Individual phytochemical was screened as follows:

2.2. Tannins

Approximately 1g of powdered sample was subjected to boiling in 20ml of water for five minutes in a water bath. This was subsequently filtered, distilled with the addition of drops of iron (III) chloride (FeCl3). The resulted solution was observed for colour changes and the formation of precipitates. The observation of colours such as blue-black or brownish-green indicated the existence of Tannins in the samples.

2.3. Saponins

One gram (1g) of the samples was boiled in 10ml of water for about 10mins using a water bath. After which filtering was carried out and then subjected to frothing. While demonstrating the frothing, about 2.5ml of the filtrate was diluted in 10ml of distilled water with vigorous shaking. The formation of a stable froth is an indication of Saponins.

2.4. Steroids

Samples of about 0.2grams for each plant part was dissolved in chloroform with the addition of concentrated H_2SO_4 of about 0.2ml. this was done for layers' formation. The appearance of a reddish-brown colour in between the layers was identified, which is a clear indication that steroid is present in the samples.

2.5. Alkaloids

Powdered samples of about 1gram each were boiled with water in a water bath with the addition of 5ml of 10% Hydrochloric acid. Potassium mercuric iodine was added to

the resulting solution in a test tube and observed. The creamy precipitate was formed, which indicates the presence of alkaloid in the samples.

2.6. Anthraquinones

About 0.5 grams powdered sample of each plant part was prepared and 5ml of chloroform was added. The solution was shaken for approximately 5 minutes and then filtered. This was followed by the addition of a 10% ammonium solution equivalent to the filtrate volume while the resulting solution was shaken together. An appearance of pink colour in the aqueous layer indicated the presence of anthraquinones

2.7. Flavonoids

One gram of the sample was boiled in about 20ml of water. The solution was filtered and 5ml of ammonium solution and concentrated H2S04 were added. The appearance of yellow colour was an indication of flavonoid.

2.8. Terpenoids

This was determined by mixing about 5 milligrams of the samples extract with 2mls of chloroform. This was followed by the addition of concentrated H2S04, which resulted in layer formation. The occurrence of a reddish-brown colour in between the layer formed was an indication of terpenoid.

Quantitative assessment of the phytochemicals in *Cola millenii* was achieved following a standard method by [16].

2.9. Data Analysis

Data collected were subjected to descriptive statistics, t-test and analysis of variance (ANOVA). T-test was used to determine the significant difference between the young and matured *Cola millenii*'s phytochemical properties as well as between the bark and leaf samples. ANOVA was used to analyze the statistical difference between the fruit parts. All these were achieved using Microsoft excel and Statistica version 7.

3. Results

Table 1 shows that *Cola millenii* leaves are deficient in all the phytochemical analyzed. Terpenoids and steroids were also absent in the bark, while saponins (0.02 mg/100kg) and flavonoids (0.01 mg/100kg) were in very little quantity. Tannins (0.97 mg/100kg) had the highest concentration, which was followed by alkaloid (0.85 mg/100kg) and anthraquinone (mg/100kg). T-test analysis revealed that the effect of plant parts is significant ($p<0.05$) on the concentration of saponin, tannin, alkaloid and anthraquinone in *C. millenii*.

Flavonoid is the most concentrated phytochemicals in the fruit parts of *Cola millenii* having 0.48 mg/100kg, 0.88 mg/100kg, 1.54 mg/100kg and 1.08 mg/100kg values in seeds, mesocarp, epicarp and endocarp respectively (Table 2). Saponin is absent in mesocarp, epicarp and endocarp but with an insignificant concentration in seeds (0,02 mg/100kg). Anthraquinone is completely absent in all the fruit parts. There was a very low concentration of terpenoid in all the fruit parts, while tannin was slightly available in seeds (0.01 mg/100kg), mesocarp (0.01 mg/100kg) and endocarp (0.02 mg/100kg). Alkaloid was also not found in seeds, mesocarp and epicarp but had very quantity in endocarp (0.01 mg/100kg). Small quantity of steroid was discovered in seed (0.01 mg/100kg), mesocarp (0.01 mg/100kg) and epicarp (0.03 mg/100kg) but absolutely absent in endocarp. Analysis of variance shows that the effect of fruit parts was significant on the available quantity of saponin in *C. millenii*.

Matured *C. millenii* had a higher concentration of the available phytochemicals in *C. millenii* (Table 3). The age of *C. millenii* significantly ($p<0.05$) influenced its available saponin, alkaloid and anthraquinone but insignificant ($p>0.05$) on the concentration of tannin (Table 3).

Table 1. Phytochemical constituents of leaves and barks of *Cola millenii* K. Schum.

Phytochemicals (mg/100kg)	Leaves	Bark	t-value	df	p-value
Saponin	0.00	0.02	5.00	10	0.000*
Flavonoid	0.00	0.01	2.24	10	0.490ns
Tannin	0.00	0.97	5.29	10	0.000*
Alkaloid	0.00	0.85	3.71	10	0.000*
Anthraquinone	0.00	0.82	3.60	10	0.010*
Terpenoid	0.00	0.00	-	-	-
Steroid	0.00	0.00	-	-	-

*= Significant at 5% probability level; ns= not significant at 5% probability level.

Table 2. Phytochemical constituents of seeds, fruits parts of *Cola millenii* K. Schum.

Phytochemicals (mg/100kg)	Seeds	Mesocarp	Epicarp	Endocarp	p-value
Saponin	0.02	0.00	0.00	0.00	0.000*
Flavonoid	0.48	0.88	1.54	1.08	0.000*
Tannin	0.01	0.01	0.00	0.02	-
Alkaloid	0.00	0.00	0.00	0.01	-
Anthraquinone	0.00	0.00	0.00	0.00	-
Terpenoid	0.03	0.02	0.01	0.04	0.61ns
Steroid	0.01	0.01	0.03	0.00	-

*= Significant at 5% probability level; ns= not significant at 5% probability level.

Table 3. Phytochemical constituents of barks of young and matured *Cola millenii* K. Schum.

Phytochemicals (mg/100kg)	Young	Matured	t-value	df	p-value
Saponin	0.10	0.23	4	4	0.020*
Flavonoid	0.00	0.01	-	-	-
Tannins	0.70	1.24	1.84	4	0.140ns
Alkaloid	0.34	1.37	36.81	4	0.000*
Anthraquinone	0.31	1.34	64.01	4	0.000*
Terpenoid	-	-	-	-	-
Steroid	-	-	-	-	-

*= Significant at 5% probability level; ns= not significant at 5% probability level.

4. Discussion

The most popularly utilized trees for agroforestry activities in many small and large farms in tropical areas have been the leguminous trees and shrubs. This is consequent from the fact that the leguminous plants are multi-beneficial coupled with the fact that they contribute significantly to the wellbeing of the farmers and nutrient composition of the soil for the growth of crops. Meanwhile, findings from the phytochemical analysis of *C. millenii* is an indication that the species could be categorized as a valuable tree. More importantly, the presence of alkaloid, tannin, anthraquinone and flavonoid in good quantity, which agrees with the findings of [17] makes it more advantageous for farmers to be incorporated into agroforestry practices. This is particularly important in an area where farmers do not have access to orthodox health facilities. According to [18], alkaloids act as narcotics and anaesthetics as well as effectively used for treatment against motion sickness, malaria, hypertension, rheumatism and neuralgia.

Previous researches [19-21] have reported the efficacy of flavonoids in eliminating carcinogens from the body. It was emphasized that Flavonoids such as quercetin, act as

chain-breaking antioxidants, and by preventing the oxidation of low-density lipoprotein by macrophages and metal ions like copper [21]. Aside from the direct application of *C. millenii* as an ethnomedicinal plant for the treatments of these diseases, which lead many local farmers to an early grave, the pharmaceutical industry can equally take advantage of its phytochemical properties for drug development such as anticancer and anti-malaria drugs.

The occurrence of phytochemical in seeds and fruits of *C. millenii* conforms to [22], who discovered cardiac glycosides in addition to the phytochemicals identified in this study. It was also stated by the authors that alkaloids, saponins and cardiac glycosides were equally found in the leaves of *C. millenii* samples analyzed. The identification of the three phytochemicals in the leaves of the same plant which were absent in the current study may be due to the effects of the environmental variations, which would have influenced the chemical compositions of the plants.

Another important finding worthy of note in this study is the age of the plant which contributed significantly to the concentration of the available phytochemicals in *C. millenii*. A similar finding was reported by [23] and later re-emphasized by [24] that age of the plant when harvested and the season of the plant determines the amount of the active constituents and since the active ingredients of plants can vary in quality and quantity from season to season

4. Conclusions

This study confirmed the presence of phytochemicals in *C. millenii*. It was discovered that both young and matured plants are endowed with these chemicals but more concentrated in the matured ones. In as much that *C. millenii* has been proven to some extent to be a valuable tree regarding its phytochemical properties. It is expedient that farmers both on large and small scales take advantage of this to incorporate the plant into farming systems. With the rate at which economic trees, shrubs and climbers are being indiscriminately destroyed without considering its uses for future purpose, conservation of *C. millenii* may be tantamount to vague if a concerted effort is not taken to harness its present potential.

Author Contributions: conceptualization, A.O. Onefeli; methodology, A.O Onefeli, O.C. Jegede and A.I. Tayo software, , A.O Onefeli.; validation, , A.O Onefeli and A.I. Tayo; formal analysis, A.I. Tayo; investigation, A.I. Tayo; resources, O.C. Jegede; data curation, A.I. Tayo; writing—original draft preparation, A.I. Tayo.; writing—review and editing, A.O. Onefeli.; visualization, A.O. Onefeli.; supervision, A.O. Onefeli.; project administration, A.I. Tayo.; funding acquisition, A.O Onefeli and A.I. Tayo.

Funding: This research received no external funding.

Acknowledgments: The authors sincerely appreciate the Biodiversity Management unit of the University of Ibadan for enabling the collection of the samples of *Cola millenii*.

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

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