European Journal of Medicinal Plants
7(1): 31-37, 2015, Article no.EJMP.2015.065
ISSN: 2231-0894

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Chemical Composition and Antioxidant Property of Two Species of Monkey Kola (*Cola rostrata* and *Cola lepidota* K. Schum) Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Author EEE designed the study, wrote the protocol, and the first draft of the manuscript. Authors NSP and Author SMA conducted experimental work, managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/15976
Editor(s):
(1) Marcello Iriti, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, Egypt.
(2) Anonymous, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1017&id=13&aid=8121

Original Research Article

Received 30th December 2014
Accepted 17th January 2015
Published 11th February 2015

ABSTRACT

Aims: To determine the total phenols content and antioxidant activity of *Cola rostrata* and *C. lepidota* seeds and fruit pulp methanol extracts.
Study Design: *In vitro* evaluation of antioxidant assays; phytochemical screening, quantitative determination of total phenolics and flavonoids content of seeds and fruit pulp extracts.
Place and Duration of Study: Department of Chemistry, University of Uyo, Nigeria (July – October, 2014).
Methodology: Standard methods were employed in the phytochemical screening, quantitative phenols and flavonoid determination and antioxidant assays (DPPH radical, ferric reducing and metal chelating activity).
Results: Alkaloids, saponins, terpenoids, carbohydrates and flavonoids were detected in the seeds and fruit pulp extracts of the studied plants. Fruit pulp of *C. rostrata* and seeds of *C. lepidota* contained the highest amount of flavonoids (60.5 µgQE/g) and phenolics (72.9 µgGAE/g) respectively. The extracts exhibited significant DPPH radical and ferric reducing activity with IC_{50} values 50-66.5 µg/mL and 60.0-63.0 µg/mL respectively. The *Cola* extracts also demonstrated

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metal chelating activity (11.49-34.83%) at 100 µg/mL.
Conclusion: The results of this study substantiates a probable role of the seeds and edible fruit pulp of *C. rostrata* and *C. lepidota* as natural sources of antioxidants which could be further exploited for their potential biological activity.

Keywords: Sterculiaceae; Cola rostrata; Cola lepidota; antioxidant activity.

1. INTRODUCTION

The genus *Cola* of the family Sterculiaceae is indigenous to tropical Africa and has its centre of greatest diversity in West Africa [1]. About 40 *Cola* species have been described in West Africa. In Nigeria about twenty three (23) species are known and some are used in traditional medicine as stimulant, to prevent dysentery [2], headache [3] and to suppress sleep [4]. *Cola rostrata* and *C. lepidota* (CL) K. Schum are perennial trees popularly known as monkey cola and cockroach kola [5]. Monkey kola is a common name given to a number of minor relatives of the *Cola* spp. that produce edible tasty fruits. Native people of southern Nigeria and the Cameron relish the fruits, as well as some wild primate animals especially monkeys, baboons and other species. Seeds of the monkey kola species are obliquely ovoid with two flattered surfaces, rough and reddish brown or green; but not edible unlike the seeds of kola nut (*C. nitida*). The aril (waxy mesocarp) form the edible portion of the follicle, and varied in colour, with the *C. rostrata* having whitish aril, while *C. lepidota* is characterized by yellowish aril. *Cola lepidota* is reported to be employed in Nigerian folk medicine as febrifuges, for pulmonary problems and cancer related ailments [6,7].

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis and cancer [8,9]. Antioxidant activity [7], anticancer [6,10] and acute toxicity [11] of the leaf and stem bark extracts of *C. lepidota* have been studied. Phytochemical screening and acute toxicity of *C. rostrata* root bark have also been reported by Odion et al. [4]. Literature search reveals that there is paucity of information as regards the antioxidant potential of *C. rostrata* and *C. lepidota* seeds and fruit aril coupled with the increase in demand for herbs and the urgent need to evaluate nature's repository of chemicals in plants for their potential value in health care. This present study was designed to evaluate the phytochemicals, phenolics, flavonoids content and antioxidant activity of the seeds and fruit pulp of *C. rostrata* and *C. lepidota*.

2. MATERIALS AND METHODS

2.1 Samples Collection and Extraction

The fruits of *C. rostrata* and *C. lepidota* were purchased from local markets in Uyo and Essien Udim Local Government Area of Akwa Ibom State in July, 2014. The plants were identified and authenticated by Dr. (Mrs.) M. E. Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria where voucher specimens were deposited. The fruit pulp and seeds were separated, chopped into small pieces and oven dried at 40ºC. The samples were pulverized and extracted with methanol using a Soxhlet apparatus. The extract was concentrated under vacuum using a rotator evaporator. All chemicals and solvents used in this study were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO). Standard antioxidant compounds were obtained from laboratory stock, acquired from commercial sources. All solutions were made in distilled water.

2.2 Phytochemical Screening

Standard methods for phytochemical screening (alkaloids, flavonoids, saponins, tannins, carbohydrates, phlobatannins, sterols and triterpenes) were employed. Alkaloids determination was done using Mayer's and Dragendoff's reagents following the methods of Kapoor et al. [12] and Odebiyi and Sofowora [13]; tannins and phlobatannins [13]. The methods described by Kapoor et al. [12] were used for determining flavonoids. The persistent frothing test as described by Kapoor et al. [12] and Odebiyi and Sofowora [13] were used for saponins. Carbohydrates determination was done using Fehling's reagent following the method described by Harbone [14]. Sterols and triterpenes were determined following the

Eiebemann-Burchard test as described by Odebiyi and Sofowora [13] and Harbone [14].

2.3 Determination of Total Phenolics

The concentration of phenolics was expressed as µg gallic acid equivalent per gram of the extract. The method of Singleton and Rossil was used [15]. Solution (1 mg) containing extract (1 mg) in methanol was added to distilled water (46 ml) and FCR (1 ml) then mixed thoroughly. After 3 mins, sodium carbonate (2%, 3 ml) was added to the mixture and shaken intermittently for 2 hrs at room temperature. The absorbance was read at 760 nm. Gallic acid was used as a standard and a calibration curve was plotted.

2.4 Determination of Total Flavonoids

Measurement of flavonoid concentration of extracts was based on the method of Park et al. [16] expressed as quercetin equivalent. An aliquot of the solution (1 ml) containing the extract (1 mg) in methanol was added to test tubes containing aluminium nitrate (10%, 0.1 ml), potassium acetate (1 M, 0.1 ml) and ethanol (3.8 ml). After 40 mins at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard and a calibration curve was plotted.

2.5 DPPH Radical Scavenging Activity

DPPH radical scavenging activity of each extract was determined according to the method of Blois [17]. DPPH (0.1 mM) in methanol was prepared and the solution (1 mL) was mixed with crude extracts (1.0 mL) prepared in methanol at different concentrations (20, 40, 60, 80, and 100 µg/ mL). The mixture was shaken and kept for 30 mins at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517 nm. Ascorbic acid and Butylated hydroxyanisole were used as the positive control. The DPPH radical scavenging activity was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{\mathrm{A_{control}} - \mathrm{A_{sample}}}{\mathrm{A_{control}}} \mathrm{X}\ \ 100$$

2.6 Ferric Reducing Capacity

The reducing power of each sample was determined according to the method of Oyaizu [18]. Sample solutions of different concentrations were mixed with phosphate buffer (0.2 M, 2.5 mL, pH 6.6) and potassium ferric cyanide (1%, 2.5 mL). After the mixture was incubated at 50°C for 20 mins, trichloroacetic acid (TCA) (10%, 2.5 mL) was added and the mixture was centrifuged for 10 mins. The upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.1%, 2.5 mL), and the absorbance was measured at 700 nm against. Higher absorbance of the reaction mixture indicated greater reducing power. BHA was used as positive control.

2.7 Metal Chelating Activity

The method of Dinis et al. [19] was used. Crude extract (0.5 g) was mixed with $FeCl_2$ (2 mM, 0.05 ml) and Ferrozine (5 mM, 0.2 mL). The total volume was diluted with methanol (2 mL). The mixture was shaken vigorously and left standing at room temperature for 10 mins. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a spectrophotometer. The percentage inhibition of ferrozine Fe^{2+} complex was calculated using the formula:

$$\%\ \text{inhibition of ferrozine} - \text{Fe2+} = \frac{\mathrm{A_{control}} - \mathrm{A_{sample}}}{\mathrm{A_{control}}} \mathrm{X}\ \ 100$$

3. RESULTS AND DISCUSSION

The phytochemical profile of *C. rostrata* and *C. lepidota* extracts are presented in Table 1. The four extracts contained high amount of alkloids, saponins, terpenoids, carbohydrates and flavonoids, while anthraquinones were detected exclusively in *C. rostrata* seed extract. Tannins were also not detected in the edible fruit pulp of both samples whereas the seeds contained appreciable quantity of tannins. Similar phytochemical data for *C. lepidota* seeds and root bark of *C. rostrata* have been reported by Burkill [20] and Odion [4] respectively; *C. nitida* nut varieties [21]. The relative high amount of carbohydrates in *C. lepidota* fruit may be due to the natural sugary taste of the succulent white pulp as compared with the pale yellow fruit pulp of *C. rostrata*. Therefore, the varying degree of phytochemical constituents may confer different levels of antioxidant activity on the studied plant extracts especially, polyphenolic components which have been implicated in recent studies as antioxidants via other mechanisms to prevent disease processes [22]. They are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α-tocopherols and inhibiting oxidases [23].

The Folin-Ciocalteu method is a rapid and widely-used assay in investigating the total phenolic content, but it is known that different phenolic compounds gave different responses with this method [24]. Table 2 indicates the total phenols and flavonoids content of *C. rostrata* and *C. lepidota* methanol extracts. In this study, total phenolics content ranged from 33.1-72.9 µg/GAE/g while flavonoids content was 18.9-51.4 µgQE/g. Fruit pulp of *C. rostrata* and seeds of *C. lepidota* constituted the highest amount of flavonoids (60.5 µgQE/g) and phenolics (72.9 µgGAE/g) respectively. The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [25].

DPPH radical was used as a stable free radical to determine antioxidant activity. Fig. 1 illustrates the concentration of DPPH radical due to the scavenging ability of seed and fruit pulp extracts of *C. rostrata* and *C. lepidota.* Standards BHA and vit C were used as references. Ascorbic acid is a known and potent antioxidant agent used in medicines [26]. The percentage inhibition of the free radical was dose dependent. Increase in concentration gave corresponding increased % inhibition. The DPPH radical scavenging capacity (IC_{50}) of the extracts was found to range from 50.0 – 66.5 µg/ml (Table 3) which is the concentration that decreases the initial DPPH radical concentration by 50% in each extract. On the other hand the (IC_{50}) of vit C and BHT was 22.0 and 16.0 µg/ml respectively. The effectiveness of antioxidant properties is inversely correlated with IC_{50} values. Thus, BHA exhibited higher DPPH scavenging effect than ascorbic acid and extracts in the study.

Table 1. Phytochemical analysis of *C. rostrata* and *C. lepidota* methanol extracts

Test	*C. rostrata* extracts		*C. lepidota* extracts	
	Seeds	Fruit Pulp	Seeds	Fruit Pulp
Alkaloids	++	+++	+++	+++
Flavanoids	+++	++	++	+
Saponins	+++	+++	+	+++
Terpenes/steroids	+++	+++	+	+++
Cardiac glycosides	++	+	++	++
Tannins	++	-	+	-
Phlobatannins	+	-	++	-
Anthraquinones	+++	-	-	-
Carbohydrate	++	++	++	+++
Deoxy sugars	+++	++	++	+++

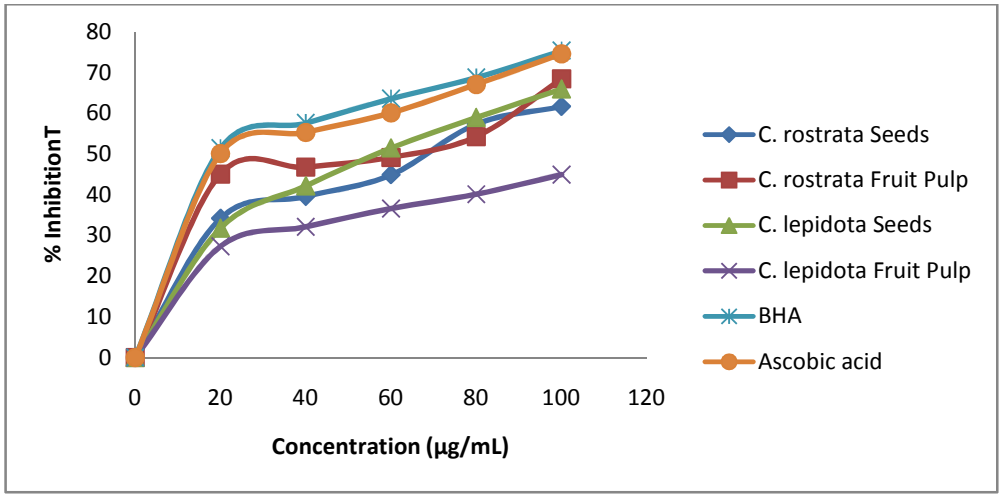

Fig. 1. Percentage DPPH scavenging activity of *Cola rostrata* and *C. lepidota* extracts/standards

The antioxidant power has also been reported by some investigators to be concomitant with the development of reducing power [27]. Reductones, which have strong reducing power, are generally believed not only to react directly with peroxides but also to prevent peroxide formation by reacting with certain precursors [28]. Furthermore, these *Cola* extracts are suggested to act as electron donors, reacting with free radicals and converting them to more stable products, which can terminate radical chain reaction. As shown in Fig. 2, in a concentration of 100 µg/mL of seeds, fruit pulp extracts and standard BHA, the descending order of reducing power is as follows: BHA (6.921) > *C. rostrata* seed (4.955) > *C. lepidota* seed (4.327) > *C. rostrata* fruit (4.174) > *C. lepidota* fruit (3.394). This study also reveals that the *Cola* extracts demonstrated strong metal chelating activity (Fig. 3) compared with standard EDTA at 100 µg/mL. The metal chelating activity of both fruit pulp extracts were similar and about 3-fold that observed for the seed extracts.

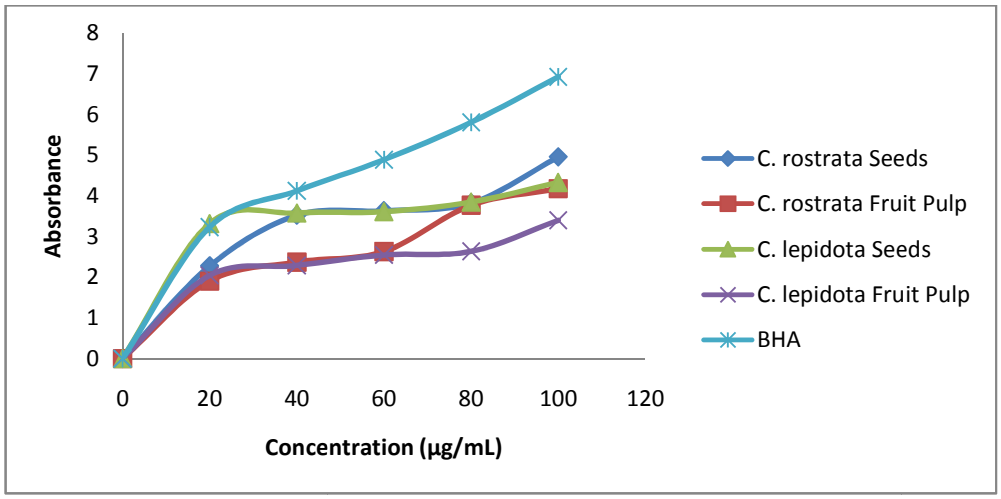

Fig. 2. Ferric reducing activity of *Cola rostrata* and *C. lepidota* extracts/standards

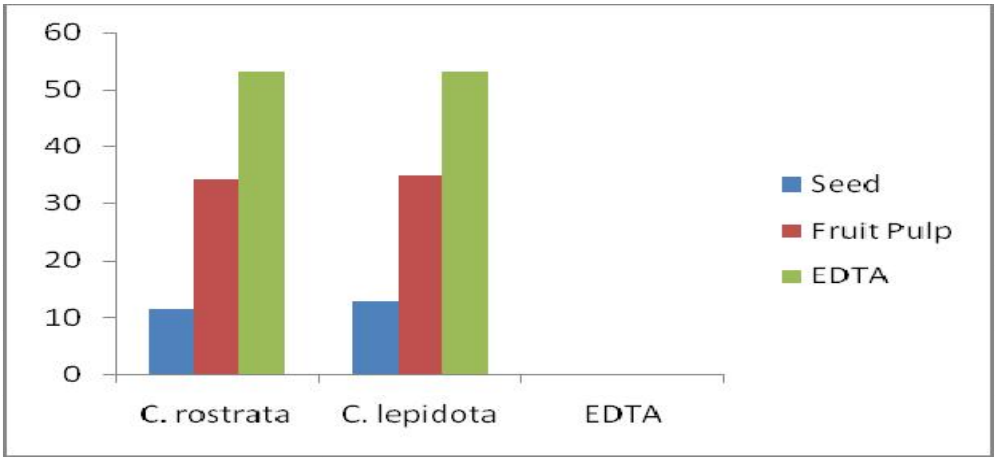

Fig. 3. Metal chelating activity of *C. rostrata* and *C. lepidota* extract/standard (100 µg/mL)

Table 2. Total phenolics and flavonoid content of *C. rostrata* and *C. lepidota* extracts

Methanol extracts	*C. rostrata*		*C. lepidota*	
	Total phenolics (µg/GAE/g)	Total flavonoids (µgQE/g)	Total phenolics (µg/GAE/g)	Total flavonoids (µgQE/g)
Seed	55.00	25.75	72.90	51.40
Fruit pulp	60.50	48.60	30.10	18.90

Table 3. IC_{50} (µg/ mL) of *C. rostrata* and *C. lepidota* extracts

Activity	*C. rostrata* extracts		*C. lepidota* extracts		Standards	
	C. rostrata Seeds	*C. rostrata* Fruit Pulp	*C. lepidota* Seeds	*C. lepidota* Fruit Pulp	BHA	Ascobic acid
DPPH	66.5	50.0	60.0	58.0	16.0	22.0
Ferric Reducing	60.0	62.0	60.0	63.0	50.0	-

4. CONCLUSION

The reducing power of the studied *Cola* extracts correlated well with the DPPH radical scavenging activity. It indicated that the marked antioxidant activity of these extracts may be as a result of their reducing power. It is interesting to note that *C. lepidota* seed and *C. rostrata* fruit pulp extracts with significant amount of polyphenols displayed relative corresponding antioxidant activity, moderately comparable with the pure standard compounds. The results of this study substantiates a probable role of the seeds and edible fruit pulp of *C. rostrata* and *C. lepidota* as natural sources of antioxidants which could be further exploited for their potential biological activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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