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COMPARATIVE PHYTOCHEMICAL AND SPECTROPHOTOMETRIC ANTIOXIDANT EVALUATION OF THE ROOT BARK, STEM BARK, LEAF AND SEED OF *COLA NITIDA* (STERCULACEAE)

Ukwueze Stanley Ejike* and Ugwu Nneka Njideka

Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.

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***Corresponding Author**
Dr. S. E. Ukwueze
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.

ABSTRACT

Cola nitida has been found to be an abundant source of natural antioxidants and also a great therapeutic agent for the prevention and management of free radical-mediated diseases. This study was aimed at evaluating and comparing the phytochemical and *in vitro* antioxidant activity of different parts of *Cola nitida.* Total phenolic and flavonoid contents as well as the antioxidant activities of the methanol extracts were evaluated spectrophotometrically using the Folin-Ciocalteau's reagent, aluminium chloride reagent and 1,1-diphenyl-2-picrylhydrazine (DPPH) methods respectively. Phytochemical screening was done using standard method. The phytochemical screening revealed the presence of alkaloids, tannin, anthraquinone, flavonoids, cardiac glycoside, carbohydrates and phlobatannin in the methanol extract of the various parts of *C. nitida*. The phenolic contents of the leaves, seeds, stem and root barks were 1.591, 6.047, 4.032, and 1.542 (% w/w GAE) respectively while the total flavonoids content of the respective parts were: 0.4733, 0.250, 0.0333, and 0.2367 (% w/w QE). Consequently, the IC_{50} of the leaf, seed, root and stem barks were found to be 0.0219, 0.0019, 0.0193 and 0.0437 mg/ml respectively. There was a positive correlation between the polyphenol contents and the *in vitro* antioxidant activity. These results showed that the various parts of *C. nitida,* especially the seeds, possess high levels of polyphenols with significant antioxidant capacities. Thus, the seeds of *C. nitida* should be preferentially utilized for therapeutic or explorative conditions where the antioxidant activity of the plant might be of primary concern.

KEYWORDS: *Cola nitida*; Phytochemical; Antioxidant activity, Spectrophotometry.

INTRODUCTION

For centuries, plants have been an important part of human diet as well as means of management of various diseases and ailments.[1] Plants owe their nutritional and therapeutic effectiveness to their metabolic products such as alkaloids, polyphenol and glycosides.[2] This has led to the extensive exploration of various species of plants in order to establish an evidence-based report on their phytochemical composition and its relationship to human health. Amongst the important bioactives in plants are the antioxidants or polyphenols.[3] The importance of these compounds is attached to their roles in protecting biological macromolecules from damaging effects of reactive species.

Studies on the role of free radicals in the pathogenesis of some disease conditions and that of antioxidants in the prevention of such diseases have been on a continuous rise in recent years. The involvement of reactive species in disease pathology has been associated with the result of the chemical reactions of these species with biological molecules leading to oxidative stress.[4]

In a lecture delivered by Dr. Boyd Haley[5], he stated that the single chemical abnormality found in essentially all neurological, neuro-degenerative and neuro-behavioral disease is the increased production of oxidative free radicals and low glutathione levels. He further noted that the oxidative stress can be reduced or prevented by maintaining a healthy status. This can be achieved via the utilization of phenolic compounds (phenolic acids, flavonoids, coumarins, lignans and tannins) which have been found to be one of the major radical scavenging molecules in plants.

Cola nitida is a widely distributed plant with many pharmacological attributes such as antioxidant, CNS stimulation, appetite suppression, anti-depressant, anti-inflammatory and trichomonicidal activities[6] The antioxidant activity is thought to be due to the high polyphenol content. Some of the phenolic compounds that have been isolated from *Cola nitida* are tannic acid, catechin, quinic acid, kolatin and theobromine[6] The aim of this study was to comparatively evaluate the phytochemical and *in vitro* antioxidant activities of different parts of *Cola nitida* with a view to identifying the part which can best be utilized in phytotherapeutic application of the plant as antioxidants, and for further research.

MATERIALS AND METHODS

Chemicals and reagents

Freshly prepared Fehling's solution A & B, Dragendorf's reagent, Hydrochloric acid, Molish reagent, Sulphoric acid and Ferric chloride. Others incude: Folin-Ciocalteu's reagent (*Sigma Aldrich*), Gallic acid (*Sigma Aldrich*), Sodium carbonate (*kermel*), Quercetin hydrate (*Sigma Aldrich*), Aluminium chloride (*kermel*), DPPH (*kermel*) and Ascorbic acid (*kermel*). Also, Soxhlet apparatus, UV spectrophotometer, Test tubes, Aluminium foil, measuring cylinder, pipette and weighing balance were used.

Collection of Plant Materials

Plant material was collected from Nsukka town, Enugu state, Nigeria in November 2016. The plant samples were identified taxonomically at the University of Port Harcourt herbarium where the voucher No. 900265 was assigned to it.

Extraction

Fresh leaves, seeds, stem and root barks of the plant were air dried for 14 days after which they were ground into coarse powder. Two hundred gram (200 g) each of the powdered plant materials was defatted by soxhlet extraction with 2.5 litres of n-hexane over a period of 12 hours. The defatted marc was dried and weighed. The dried marc were separately extracted with 2.5 litres of methanol over a period of 24 hours and the methanol extracts from the different plant part were collected, dried and properly stored.

Phytochemical screening

Phytochemical screening was carried out on the methanol extract as described by Harborne[7] to determine the presence of alkaloids, cardiac glycosides, tannins, flavonoids, carbohydrates and anthraquinone.

Determination of phenolic compounds

The concentration of total phenol in each extract was determined using Folin-Ciocalteu's reagent which is a mixture of phosphomoylate and phosphotungstate used for colorimetric assay of phenolic compounds and polyphenols.[8] Standard curve was plotted using different concentrations of gallic acid (0.0625, 0.125, 0.25, 0.5 and 1.0 µg/ml) in methanol. A 0.1 mg/ml solution of the different extracts was made. To each sample, 0.5 ml of 10-fold dilute Folin-Ciocalteu's reagent and 1 ml of 2% sodium carbonate were added. The mixture was incubated for 30 min after which the absorbance at 760 nm was recorded. Total Phenolic

Content (TPC) was calculated as Gallic Acid Equivalence (GAE in % w/w) using the formula;

$$TPC = \frac{GAE \times D.F \times V}{W}$$

Where:

GAE – Gallic Acid Equivalence (obtained from the standard curve)

D.F - Dilution factor

V - Volume of stock Solution

W – Weight (g) of plant used in the experiment

Determination of total flavonoid content

Quercetin concentrations of 6.25, 12.5, 50, 80 and 100 µg/ml were prepared and used to make the calibration curve[9] A volume of 0.5 ml of 2% $AlC1_3$ in methanol solution was added to 0.5 ml of different plant extracts. After 1 hr. incubation at room temperature, the absorbance was read at 415 nm. The extracts were evaluated at a final concentration of 0.1 g/ml.

Total Flavonoid Contents (TFC) were calculated as Quercetin Equivalent (QE in mg/g) using this formula;

$$TFC = \frac{QE \times D.F \times V}{W}$$

Where:

QE – Quercetin Equivalent (obtained from the standard curve)

D.F - Dilution factor

V - Volume of stock Solution

W – Weight (g) of plant used in the experiment

Determination of DPPH scavenging activity

The free radical scavenging activities of the extracts were determined using 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.[10] DPPH in oxidized form gives a deep violet colour in methanol. An antioxidant compound donates the electron to DPPH thus causing its reduction as its colour changes from deep violet to yellow.[11] A fresh solution (0.3 mmol/L) of DPPH was prepared in methanol and its absorbance was recorded at 517 nm. Aliquot of 1 ml of extract solution at different concentrations (0.0078125-0.125 mg/ml) was mixed with 1 ml of DPPH solution. The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance was again recorded at 517 nm. The percentage inhibition of DPPH by extracts was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of pure DPPH in oxidized form while B is the absorbance of sample taken after 30 minutes of reaction with DPPH.

Controls were prepared in a similar way as the test group, using ascorbic acid as the standard. A calibration curve of percentage inhibition of ascorbic acid was made for concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 µg/ml) to determine the IC_{50} values of extracts (that is the concentration at which 50% of DPPH solution is scavenged). IC_{50} was used to determine the level of antioxidant activity.

RESULTS

Table 1.0 Percentage yield of the methanol extracts.

Test	Leave	Seed	Stem	Root
Alkaloid	+	+	+	+
Tannin	+	+	+	+
Saponin	+	+	+	+
Flavonoid	+	+	+	+
Carbohydrate	+	+	+	+
Anthraquinone	-	+	-	+
Cardiac glycoside	-	+	+	+
Phlobatannin	+	+	+	+
Reducing Sugar	+	+	+	+

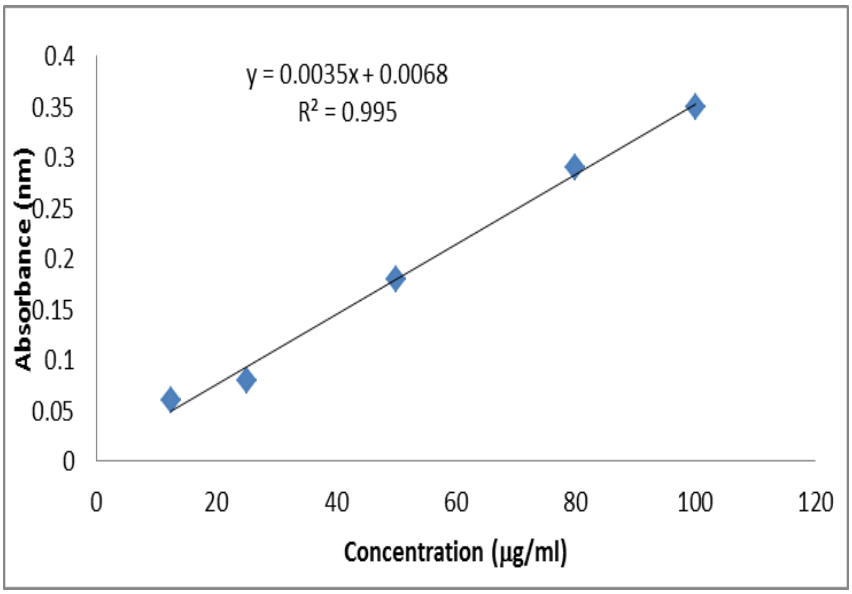

Figure 1.0: Standard quercetin curve.

Table 2: Total flavonoid content in quercetin equivalent per 100g of dry weight (%TFC in grams).

S./NO.	PLANT PART	TFC (g)
1	Leaf	0.4733
2	Seed	0.2500
3	Stem	0.0333
4	Root	0.2367

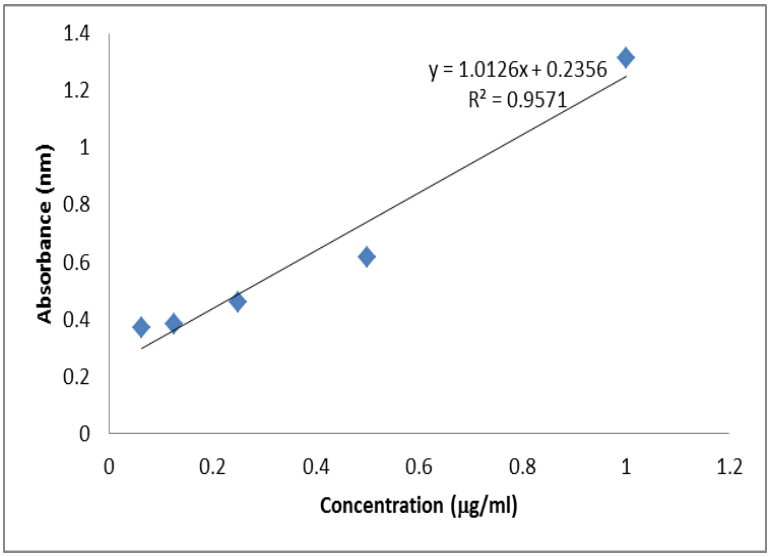

Figure 2: Standard gallic acid curve.

Table 3: Total phenolic content of various parts of *C. nitida.*

S/NO	PLANT PART	GAE (mg/g)
1	Leaves	1.591
2	Seed	6.047
3	Stem	4.032
4	Root	1.542

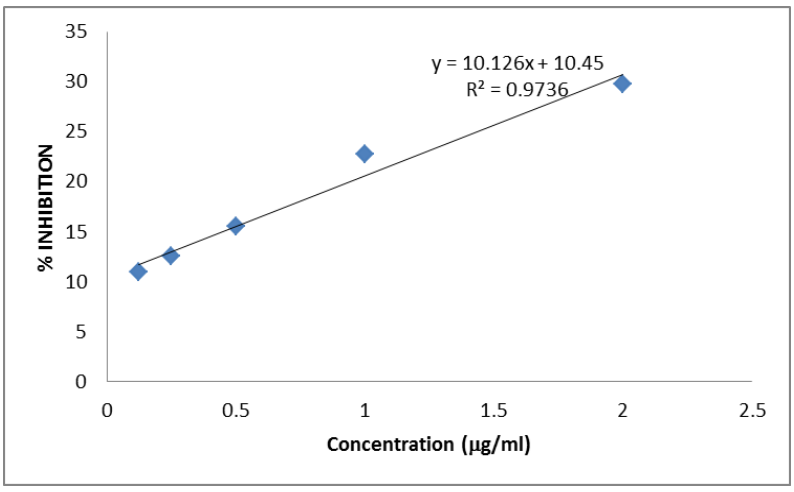

Figure 3: Standard curve for ascorbic acid.

Table 4: DPPH radical scavenging activity of the various parts of *C. nitida.*

	% DPPH Inhibition			
Concentration (mg/ml)	**Leaves**	**Seed**	**Stem**	**Root**
0.009	23.37	55.84	25.19	14.57
0.016	34.29	84.07	40.67	17.45
0.031	62.22	92.11	66.01	30.80
0.063	88.62	92.87	90.59	57.36
0.125	89.38	93.17	90.89	85.74

Table 5: Comparative IC_{50} of ascorbic acid and the various parts of *C. nitida.*

S/NO	PLANT PART	IC_{50}
1	Ascorbic acid	0.0039
2	Leaves	0.0219
3	Seed	0.0019
4	Stem	0.0193
5	Root	0.0437

DISCUSSION

The total flavanoid content of the *C. nitida* was seen to be in the order: leaf (0.4733 % g/g QE) > seed (0.2500 % g/g QE) > root bark (0.2367 % g/g QE) > stem bark (0.0333 % g/g QE). It has been recognized that flavonoids show antioxidant activity through scavenging or chelating process.[12] Flavonoids as antioxidants interfere with the biochemical pathways which are involved in generation of free radical (ROS), quench them, chelate them and make them redox inactive.[13]

The total polyphenols contents on the other hand were: seed (6.047 % g/g GAE) > stem bark (4.032 % g/g GAE) > leaf (1.591 % g/g GAE) > root bark (1.542 % g/g GAE). It has been documented that plant polyphenols are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations.[14] Plant phenolics are a major group of compounds acting as primary antioxidants and free radical scavengers. They are characterized by the presence of several phenol groups and act by donating a hydrogen atom or an electron which makes them very reactive in neutralizing free radicals and chelating metal ions in aqueous solutions.[15] The result of many studies have showed that the total phenolic compounds were the major determinant of antioxidant activity of many plants and this could be attributed to their redox properties.[16,17]

The DPPH free radical scavenging method was used to determine the concentrations of extracts at which they scavenge 50% of the DPPH solution termed as IC_{50} values using gallic (or ascorbic) acid as the standard.[10] The lower the IC_{50} value of an antioxidant, the higher

would be its free radical scavenging power. The antioxidant properties measured as IC_{50} of the various parts starting from the most active were: seed (0.0019 mg/ml) > stem (0.0193 mg/ml) > leaf (0.0219 mg/ml) > root (0.0437 mg/ml). However, the IC_{50} of the standard antioxidant (ascorbic acid) was found to be 0.0039 mg/ml. This result showed that the methanol extract of the seed of *C. nitida* is not only more potent than that of other parts but also more potent than ascorbic acid.

Thus, the *in vitro* antioxidant activities of the various parts showed direct proportionality with the total polyphenol content but the total flavonoids content did not follow similar correlation. This strongly suggests that the free radical scavenging capacity of *C. nitida* extracts is strongly related to the presence of polyphenols and probably to a lesser extent, total flavonoids content. To further confirm this relationship, a correlation analysis was done using the SPSS software. The result showed strong positive relationship between IC_{50} inhibition (mg/ml) and Total Phenolic Content (GAE % g/g) ($r=0.852$) although the ($p=0.15$) suggests that this relationship was not significant. The relationship between IC_{50} Inhibition (mg/ml) and Total Flavonoid Content (QE % g/g) was a very weak non-significant one ($r=0.026$, $p= 0.97$).

CONCLUSION

The flavonoid content of *Cola nitida* was found to be highest in the leaves followed by the seed, the root and the stem. However, the seeds of *C. nitida* contained the highest amount of total phenolic content, followed by the stem, the leaves and then the root. Since the antioxidant properties of different parts of the plant were directly proportional to their total polyphenol content, the seed of *C. nitida* should be preferentially sourced and explored for antioxidant utility of the plant.

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