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**Adebayo Muritala Ayofe**  
Department of Pharmacognosy,  
Professor Dora Akunyili College  
of Pharmacy, Igbidenion  
University, Okada, Edo State,  
Nigeria

**Adedokun Oluwasegun**  
Department of Pharmacognosy,  
Professor Dora Akunyili College  
of Pharmacy, Igbidenion  
University, Okada, Edo State,  
Nigeria

**Ayinde Bunyaminu Adesina**  
Department of Pharmacognosy,  
Faculty of Pharmacy, University  
of Benin, Edo State, Nigeria

**Ume Ogochukwu**  
Department of Pharmaceutical  
Chemistry, Dora Akunyili  
College of Pharmacy, Igbidenion  
University, Okada, Edo State,  
Nigeria

**Correspondence:**  
**Adebayo Muritala Ayofe**  
Department of Pharmacognosy,  
Professor Dora Akunyili College  
of Pharmacy, Igbidenion  
University, Okada, Edo State,  
Nigeria

## Comparative studies on *In-vitro* radical scavenging potential of methanol extracts of *Garcinia kola* heck (Clusiaceae) seeds, *Conyza sumatrensis* retz (Asteraceae) and *Mitracarpus scaber* zucc (Rubiaceae) leaves

**Adebayo Muritala Ayofe, Adedokun Oluwasegun, Ayinde Bunyaminu Adesina and Ume Ogochukwu**

### Abstract

Dating back to history, widespread use of plants as natural herbal remedies has remained a thing of consideration. In this research, the comparative study on antioxidants or free radical scavenging properties of the methanol extracts of *Garcinia kola* nut seeds, *Conyza sumatrensis* and *Mitracarpus scaber* leaves were evaluated in a series of radical (ROS) scavenging assays involving total antioxidants studies using 1,1-diphenyl 2-picrylhydrazyl (DPPH) radical, reducing power assay, total phenolic and flavonoid contents. However, phytochemical screening of the extracts showed the presence of flavonoids, terpenoids, alkaloids, glycosides and reducing sugars. The lowest and highest amount of phenolic compounds were observed in *G. kola* ( $0.31 \pm 0.52$  mg/g) and *M. scaber* ( $0.48 \pm 0.15$  mg/g), which in turn exhibited the greatest amount of flavonoid ( $0.32 \pm 0.15$  mg/g). The extracts of all the examined plants, exhibited a marked antioxidants scavenging effects at the various concentration ranging from 20 - 100 µg/ml using Vitamin C as the reference drug. *M. scaber* exhibited the highest radical scavenging potential in a concentration dependent manner relative to Vitamin C while *C. sumatrensis* exhibited the least activity among the examined samples. Similar activities were observed in reducing power assay with all plants exhibiting a low activity relative to Vitamin C (standard drug) at  $P \leq 0.05$ . In conclusion, findings of this research experimentally justify the ethnopharmacological claim of the usage of *M. scaber* in treatment of oxidative stress induced ailment.

**Keywords:** Antioxidant, *Garcinia kola*, *Conyza sumatrensis*, *Mitracarpus scaber*, Vitamin C

### 1. Introduction

The use of plants as source of medicine in treating disease is an ancient practice [1]. The widespread use of herbal remedies and health care purposes, such as those described in the ancient text like the Bible and the Vedas, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines [2]. The therapeutic power of herbs had been recognized since creation of the universe and botanic medicine is one of the oldest practiced professions by mankind [3]. In recent times, attention has been reverted back to plants as sources of therapeutic agents due to their higher properties. These include among others reduced cost, relative lower incidence of adverse reactions compared to modern conventional pharmaceuticals) and ready availability [4]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [5]. Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive [6].

*Garcinia kola* is an evergreen plant found in the equatorial forest of Sub-Saharan Africa. The plant grows wild and is also domesticated because of the wide medicinal values of the extract of its various components in folk medicine. *G. kola*, (bitter kola) are species of flowering plant in the Clusiaceae or Guttiferae family. The tree is found in moist forest and grows as a medium size tree, up to 12 m high [7]. It is found in Benin, Cameroon, Democratic Republic of Congo, Ivory Coast, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. Its natural habitat is subtropical or tropical moist lowland forests [7]. *G. kola* seeds are considered as a poison antidote in Africa, It has been reported to exhibit aphrodisiac effects on male subjects [7,8] for which reason they are sometimes called “male kola in some parts of Nigeria.

It is reported to suppress ovulation and delay fertility in female subjects [9]. *G. kola* extracts have been shown to possess antipyretic, anti-inflammatory, analgesic<sup>[10]</sup> antiviral, hepatoprotective<sup>[11]</sup>, Central nervous system (CNS) stimulant, antidepressant, antioxidant<sup>[12]</sup>, antidiabetic activities<sup>[13, 14]</sup>

*Conyza sumatrensis* commonly called broad-leaved fleabane is a dicotyledonous herb of the Asteraceae family occurring widely in Nigeria especially in the Niger Delta region. It is an annual or biennial tall herbaceous plant. Traditionally, *C. sumatrensis* is used in the treatment of facial pimples and stomach disorder and also serves as a good source of food for the fowls<sup>[15]</sup>. Others include fever, dysentery, menstrual issues, and act as a diuretic.

*Mitracarpus scaber* is a very common weed of cultivated or fallowed land. Sometimes forms a dense stand on fallowed land, it is a known perennial annual herbs of about 30cm tall or much smaller and possess rough leaves. In Nigeria it is known as Obuobwa in Igbo language and Irawo- ile in Yoruba language. This plant belongs to the family Rubiaceae found throughout the tropical region of the world. Members of the genus *Mitracarpus* Linn are ever green tree or shrubs with greenish leaves with simple whorled arrangements, entire margin with lanceolate shape, acute apex and base. *M. scaber* is used for the treatment of sore throat and also for leprosy especially in Senegal country, but in Nigeria, the juice from the crushed plant is known to be applied topically for treatment of skin diseases such as ringworm, lice, itching, crawl-crawl and other fungal diseases<sup>[16]</sup>. They are also applied to dressing of fresh cuts, wounds and also a treatment of ulcer<sup>[17, 18]</sup>. They can be used as ingredients in fish poison by some pagan tribes, in treatment of upper respiratory diseases and malaria which is recommended by the world health organization (WHO). The leaf extract is widely used in traditional medicine practices in West Africa for the treatment of headaches, toothaches, amenorrhea, dyspepsia, hepatic diseases, venereal diseases as well as leprosy and liver diseases<sup>[19, 20, 21]</sup>. The aim of this research is to compare the radical scavenging potential of some commonly used Nigerian medicinal plants namely: *G. kola*, *C. sumatrensis* and *M. scaber*.

## Materials and methods

### Collection and Identification of plants materials

*G. kola* seeds, *C. sumatrensis* and *M. scaber* leaves were collected in Okada community, Ovia-North East local Government Area of Edo state. And they were identified and authenticated at Professor J. C Okafor of Pax Herbal Ewu in Esan Central Local Government Area, Edo state. Also the herbarium voucher specimens were deposited in the same herbarium.

### Sample preparation

The three plant samples were oven dried at 40°C for 2 days and then grounded into a coarse powder using an Electric milling machine. The powdered samples were exhaustively extracted using Soxhlet extractor with absolute methanol (Analytical grade). The extracts obtained were concentrated under reduced pressure using Rotary evaporator, and then stored in air tight containers, placed in the refrigerator for further use.

### Preliminary phytochemical screening

Phytochemical screening was performed using standard method<sup>[22]</sup>

### Estimation of Total Phenolic Content

Total phenolic assay was measured using the modified Folin-ciocalteau method<sup>[23]</sup>. In order to measure the phenolic content, 1 ml of each plant sample was mixed with 2 ml of 7.5% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and 2 ml of Folin-ciocalteau's reagents. After incubation using water bath at 40°C for 45 min, the absorbance of the reaction mixture was measured at 765 nm on a UV-Visible spectrophotometer (Model SM23A; Micro field®, England). Gallic acid was used as standard. The calculation of total phenol content was based on the calibration curve of the Gallic acid standard and the data was expressed as milligram Gallic acid equivalents (GAE) per gram of plant extract.

### Estimation of Flavonoid Content

The total flavonoid content was determined using the aluminum chloride colorimetric method<sup>[24]</sup>. 1 ml of the plant sample was mixed with 0.1 ml of 10% aluminum chloride hexahydrate (AlCl<sub>3</sub>.6H<sub>2</sub>O), 0.1 ml of 1 M potassium acetate (CH<sub>3</sub>COOK) and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm on a UV-Visible Spectrophotometer (Model SM23A; Microfield®, England). Flavonoid contents were calculated on the basis of the calibration curve of rutin standard.

### Determination of Antioxidant activity

The radical scavenging activity of the plant extract against 2, 2'-Diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) was determined by UV Spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method<sup>[25]</sup>. 20 - 100 µg/ml of crude extract and Vitamin C were prepared in methanol (Analar grade). 1 ml of the extract was placed in a test tube, followed by 2 ml of 0.1 mM DPPH in methanol. A control solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \left\{ \frac{A_b - A_a}{A_b} \right\} \times 100$$

Where A<sub>b</sub> is the absorption of the blank sample and A<sub>a</sub> is the absorption of the extract.

### Ferric reducing power assay

Ferric reducing power was determined by mixing various concentrations of each plant extract and standard ascorbic acid solution (viz. 10, 20, 40, 60, 80 and 100 µg/ml) in 1 ml of methanol with phosphate buffer (2.5 ml, 0.2 M at pH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged at 3000 g for 10 min at room temperature. 2.5 ml of supernatant was mixed with 2.5 ml distilled water and ferric chloride (FeCl<sub>3</sub>) (0.5 ml, 0.1%), and the absorbance of the reaction mixture was measured at 700 nm with a UV-visible spectrophotometer as indicative of increased reducing power. All the tests were performed in triplicate and a graph was plotted for the average of three observations<sup>[26]</sup>.

### Results and discussion

The phytochemical screening result of the three plant samples are shown in Table 3.1 below. However, the presence of flavonoids, reducing sugars, glycosides, alkaloids and terpenoids were observed in all plant sample examined, while phlobatannins, starch and saponins were suspected to be absent in entire samples examined.

**Table 1:** Preliminary phytochemical screening of methanol extracts of the three plants.

Phytochemical constituents	Extracts of plants		
	<i>Garcinia kola</i> (seeds)	<i>Conyzasumatrensis</i> (leaves)	<i>Mitracarpus scaber</i> (leaves)
Steroids	-	-	+
Flavonoids	+	+	++
Reducing sugars	++	++	++
Saponins	-	-	+
Glycosides	++	++	-
Alkaloid	+	+	++
Phenolics	+	+	+
Hydrolyzed tannins	-	-	+
Condensed tannin	-	+	-
Terpenoid	+	+	+
Starch	-	-	+-

Absent (-) Moderately present (++) Present (+) Abundantly Present (+++)

Phenolic compounds are secondary metabolites of plants, these compounds constitute a chemically heterogeneous group, containing a phenol group (a functional hydroxyl group in an aromatic ring) in its basic structure. They differ structurally from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins, comprising different classes. However, the main phenolics in the diet are

the phenolic acids, flavonoids and tannins [27]. From Table 3.2 below, highest and lowest amount of phenolics (Gallic acid equivalent) were observed to be *C. sumatrensis* (0.48±0.15) and *G. kola* (0.31±0.52) respectively. Likewise, least amount of flavonoids (Rutin equivalent) has similarly found in *G. kola* (0.32±0.15), while *M. scaber* possess the highest amount (0.05±0.01)

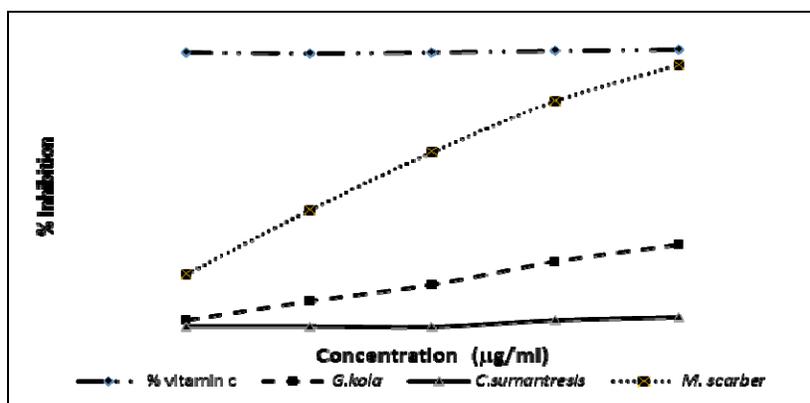
**Table 2:** Total phenolic and flavonoid contents of plant samples

Plant samples	Amount of phenolic content (mg/g)	Amount of flavonoid content (mg/g)
<i>Garcinia kola</i> seed	0.31 ± 0.52	0.05 ± 0.01
<i>Conyza sumatrensis</i> leaves	0.48 ± 0.15	0.18 ± 0.03
<i>Mitracarpus scaber</i> leaves	0.42 ± 0.34	0.32 ± 0.15

The values above are mean of three replicates (MEAN ± SEM)

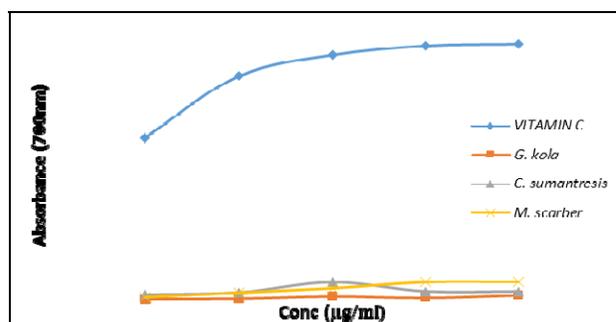
The result for total antioxidant is shown in Figure 3.3 below. *M. scaber* showed an excellent DPPH radical scavenging potential in a concentration dependent manner with the least

and highest activities observed at 20 and 100 µg/ml, with percentage inhibition of 37.64% and 87.27% respectively.



**Fig 3:** Total antioxidant activities of *G. kola* seed, *C. sumatrensis* and *M. scaber* leaves and Vitamin C.

No significant difference at  $P \leq 0.05$  was observed in the DPPH radical scavenging property of *M. scaber* and Vitamin C at 100 µg/ml. Moreover, the least activity was noted in *C. sumatrensis* as shown in Figure 3.3 above. Ample amount of phenolics and flavonoids contents were observed for *C. sumatrensis* as shown in Table 3.2 which scientifically justify the observed radical scavenging activity. The concentration of total phenolics in the grain has been positively associated with the antioxidant activity [28] with potential beneficial effects on health, such as reduction of oxidative stress [29] aid in the prevention of cancer [30] in the control of blood lipids and related diseases, which may help in the prevention of cardiovascular and in the prevention of the complications of diabetes [31]



**Fig 4:** Total reducing power assay of *G. kola* seed, *C. sumatrensis* and *M. scaber* leaves and Vitamin C (standard drug).

Moreover, the result of reducing power assay of the medicinal plant examined is shown in Table 3.4 above. The entire samples showed a low ferric reducing potential relative to Vitamin C. However, despite the weak ferric reducing activity, the highest and lowest activities were still observed in *C. sumatrensis* and *G. kola* respectively.

### Conclusion

This study has established that *G. kola* seed, *C. sumatrensis* and *M. scaber* leaves all possess potent radical scavenging potential, with *M. scaber* exhibiting the highest activity among the medicinal plants examined. These plants have been widely used in traditional medicine for preparation of African herbal medicine. *M. scaber* possess ample of phenolic and flavonoids compounds as a result more excellent radical scavenging potential.

### Conflict of interest

No conflict of interest

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