

Full Length Research Paper

Antisickling activities of extracts of leaf, seed and seed pod of *Garcinia kola* Heckel

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***Garcinia kola* has been hitherto speculated as effective in the management of sickle cell disease (SCD). Investigations into the antisickling activities of both crude methanol extracts and the aqueous fractions of the leaf, seed and seed pod of *G. kola* were carried out using p-hydroxybenzoic acid and normal saline as positive and negative controls respectively. At the tested concentrations of 10.0, 1.0 and 0.1 mg/ml, the leaf extracts exhibited greatest antisickling activity whilst the seed pod had the least antisickling activity. However, it was observed that the activity of methanol extract of the seed pod did not differ significantly ($p > 0.05$) from that of the positive control, p-hydroxybenzoic acid. Results for screened phytochemicals of the investigated parts revealed the presence of saponins, tannins, combined and free anthraquinones. The results infer a preliminary confirmation for the effectiveness and use of *Garcinia kola* in the management of SCD and its implication in drug development.**

Key words: Antisickling activity, *Garcinia kola*, phytochemicals, sickle cell disease.

INTRODUCTION

Garcinia kola Heckel, belong to the family Guttiferae. It is commonly known as bitter kola (English), *orogbo* (Yoruba), *Namijin goro* (Hausa) and *mvule* (Swahili). It is a widespread tree of evergreen forest and it is found from the Democratic Republic of Congo to Ghana, where it occurs in the wet and moist semi deciduous forest zones as well as in the savannah. *G. kola* is well branched, evergreen and grows as a medium size tree, reaching 12 m in height. The specie is one of the most important trees valued in Nigeria for its medicinal seeds and its exploit-

ation in the natural forests has been very heavy (Adegoke et al., 1998; Farombi et al., 2005).

The plant has been reported to have varying uses in folklore medicine. Probably because of its widespread use in folklore medicine, *G. kola* has been a subject of intense scientific investigations. *G. kola* has been proven to exhibit pharmacological uses in treating coughs, throat infections, bronchitis, hepatitis and liver disorders (Farombi et al., 2005). It served as bitter stimulant and as snake repellent when they are placed round the compound (Nair, 1990). Other medicinal uses of this plant include purgative, antiparasitic and antimicrobial. This plant has shown bronchodilator effect (Orie and Ekon, 1993), anti-inflammatory, antibacterial and antiviral properties (Ebana et al., 1991; Akoachere et al., 2002), antihepatotoxic effect (Braid, 1991) and antioxidant

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activity (Adaramoye, 2005). In south west Nigeria, *G. kola* is one of constituents of traditional recipe that is used in the management of sickle cell disease (SCD) (Egunyomi et al., 2009).

SCD is known to be one of the diseases ravaging most world populations cutting across nations and ethnic divide. It has an interesting but alarming statistics. About 89% of sicklers worldwide are in Africa, of which Nigeria alone constitutes 25%. Europe has the least, 0.1%, while the US, Asia and the Mediterranean have 3.5% each (Ameh et al., 2009). The health care cost of the management of SCD patients is disproportionately high compared to the number of people afflicted by the disease. The common people living in the villages are mostly peasant farmers who cannot afford the high cost of treatment by orthodox doctors (Okochi, 2005). Considering all genetic disorders to which man is known to be liable, there is probably no other that presents a collection of problems and challenges quite comparable to SCD and related disorders. Due to the debilitating effect and cost of managing the SCD, research has been on-going to determine the efficacy of the use of medicinal plants to tackle the multiple challenges presented in SCD (Okpuzor et al., 2008). Interestingly, a herbal based drug, Niprisan has been developed from a particular Nigerian traditional recipe by the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The root of *G. kola* is reported to be one of the constituents used in making traditional recipe for the treatment of SCD in south west Nigeria (Egunyomi et al., 2009). In a related development, Elekwa et al. (2003) reported a higher and more effective membrane stabilization effects of aqueous extracts of *G. kola* seeds; that was more than the one exhibited by phenylalanine. This trend seems to depict those different parts/organs of *G. kola* plant need to be investigated for antisickling activity. The present work therefore focused on evaluation of antisickling activities of both methanol and aqueous fraction of leaf, seed pod and seed of *G. kola*.

MATERIALS AND METHODS

Plant collection and preparation

Different plant parts such as leaves, stem and fruits of *G. kola* were collected from the botanical forest in the compound of Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria. The seeds were collected/ purchased from Sabo market in Sagamu, Nigeria. The plant was authenticated by Mr. Felix at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher specimen number given was FHI 108220. The leaf, seed-pod and seed were air dried at room temperature ($28\pm 2^\circ\text{C}$). The dried specimens were reduced to coarse powder using a milling machine. The powdered samples were stored in airtight containers and properly labeled for further studies.

Phytochemical tests

Powdered samples of each of the plant material were used to test

for alkaloids, saponins, tannins, combined and free anthraquinones using established protocols (Adesanya and Sofowora, 1983).

Extraction

500 g of each of the dried powdered material were extracted with methanol (2 L) by cold extraction for 7 days in large amber bottles with intermittent shaking. At the end of seven days, the methanol extract was filtered and the filtrate evaporated to dryness in a water bath. 30 g of crude methanol extract was dissolved in a methanol-water mixture (3:1). The mixture was poured into a separatory funnel and extracted with chloroform (30 ml) in three aliquot portions. This gave chloroform soluble and aqueous fractions of the crude methanol extract. The aqueous fraction obtained was evaporated to dryness under reduced pressure using the rotary evaporator. Thereafter, both crude methanol extract and aqueous fraction were serially diluted with normal saline (0.9% NaCl) to give 10 mg/ml, 1.0 and 0.1 mg/ml solutions which were used for subsequent antisickling assay.

Blood collection and preparation

Five milliliters of blood was obtained by venipuncture from a volunteer sickle cell disease patient (HbSS) in steady state from the Haematology Day Care Unit of the Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria. Blood was collected into sodium EDTA bottles and the content thoroughly mixed by gently rolling the bottle. The blood sample was centrifuged to remove serum and the packed erythrocyte obtained was washed with normal saline as described by Egunyomi et al. (2009). All procedures were carried out in accordance with established University protocols.

Bioassay of plant extracts for antisickling activity

The bioassay of both crude methanol extract and aqueous fraction of the three plant materials for antisickling activity was carried out using two approaches, namely: inhibition of sickling (antisickling) and reversal of sickled erythrocytes. Evaluation of antisickling activities of the extracts/fractions was carried out using a modified method of Sofowora et al. (1979). The washed erythrocyte (0.5 ml) was mixed with 0.5 ml of the concentration of the test extracts/fractions in uncovered test tubes and mixed together. Samples were taken from the different mixtures and the remaining incubated at 37°C for 3 h while shaking occasionally. Five drops of 2% sodium metabisulphite were added to the mixture and mixed thoroughly and sealed with liquid paraffin. Samples were taken in duplicates from the different mixtures at 0 min after which the systems were incubated at 37°C and the samples taken again at 30 min interval until four more readings were taken. The procedure for smear preparation and counting of sickled and unsickled cells was as described by Egunyomi et al. (2009). Two types of controls involving a positive control (p-hydroxybenzoic acid, 5 mg/ml), and a negative control (normal saline) were employed in this bioassay. The percentage inhibition of sickling was calculated using the formula of Moody et al. (2003)

Evaluation of different plant extracts/fractions for sickling reversal activity was carried out according to the procedure of Oduola et al. (2006). The washed erythrocyte (0.5 ml) was mixed with 0.5 ml of freshly prepared 2% sodium metabisulphite in a clean test tube and incubated at 37°C for 30 min. A drop of the mixture was viewed under the microscope.

Equal volume of normal saline/extract/fraction was added to the blood-meta bisulphite mixture in a different test tube and incubated at 37°C for another 30 min. Samples were taken at 0 min and at 30

Table 1. Screened phytochemicals of leaves, seed pod and seed of *Garcinia kola* (Heckel).

Plant part	Combined Anthraquinones	Free Anthraquinones	Alkaloids	Saponins	Tannins
Leaf	+	+	-	+	+
Seed pod	+	+	-	+	+
Seed	+	+	-	+	+

Key: += present, - = absent.

min interval up to 2 h. The procedure described by Egunyomi et al. (2009) was used for smear preparation and counting of sickled and unsickled cells.

Statistical analysis

Data obtained were expressed as means. The statistical significance of differences was assessed using analysis of variance. A two-tailed p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the results for screened phytochemicals of the investigated plant materials. All the investigated organs of *G. kola* contained free and combined anthraquinones, saponins and tannins while alkaloids were absent. The antisickling bioassay revealed that all tested fractions of *G. kola* exhibited antisickling activity in varying proportions. However, the degree of the exhibited antisickling activity was concentration, and plant-part dependent (Tables 2 - 4). For the three investigated plant organs, the activity of the crude methanol extract was significantly higher ($P < 0.05$) than that exhibited by the aqueous fraction.

Also, there was a significant difference between the antisickling values obtained at the different tested concentrations, with those at higher concentrations being significantly higher ($p < 0.05$). Of the three investigated plant organs, the leaf exhibited the greatest antisickling activity and the seed pod the least. However, it should be pointed out that this observed activity of the seed pod (especially the methanol extract at the concentration of 10 mg/ml) compares very well with the activity of positive control.

Both the methanol and aqueous fraction of all the tested plant organs exhibited reversal of sickling activities which favourably compared also with that exhibited by the positive control. The exhibited sickling reversal activity was concentration dependent. There was no significant difference ($p > 0.05$) between the sickling reversal activity of methanol extract and aqueous fraction of leaf, seed pod and the seed of *G. kola*. In a similar trend, results from the present study indicates that the sickling reversal ability of leaf, seed pod and seed of *G.*

kola did not differ significantly ($p > 0.05$).

DISCUSSION

Previous investigations have attributed the antisickling activity of many medicinal plants that are used in the management of SCD to their inherent phytochemicals. Moody et al. (2003) reported that anthraquinones, steroidal and cardiac glycosides of *Cissus populnea* L. contributed to the antisickling properties of *Ajawaron*- a herbal extract made from it. Similarly, Zanthoxylol and 1-hydroxylbenzoic acid were implicated to be responsible for the antisickling activity of *Fagara zanthoxyloides* (Sofowora, 1979). Furthermore, Ibrahim et al. (2007) reported that saponins, carboxylic acids and flavonoids were responsible for the antisickling activity of *Hymenocardia acida* leaves. Meanwhile, combined and free anthraquinones, as well as saponins and tannins were detected in the leaf, seed and seed pod of *G. kola*. The observed antisickling activity of these organs of *G. kola* in the present study could also be attributed to these detected phytochemicals.

Earlier reports indicated that the aqueous extract of *G. kola* seed had a higher membrane stabilization effect than phenylalanine (Elekwa et al., 2003). However, result of the present study had shown that the leaf extract of *G. kola* exhibited greater antisickling activity than the seed extract. The use of *G. kola* root as part of the constituents of recipes used in the management of SCD in south west Nigeria has been documented by Egunyomi et al. (2009). However, results emanating from this study indicated that the use of cheaper and more accessible materials to make recipes for SCD management may have been found in the leaf and seed pod of *G. kola*.

The traditional healers without any scientific background have made use of nature's abundant resources to manage SCD with a great degree of success over time. However, in the present age that is characterized with full understanding of the pathology, physiology and the molecular nature of SCD, duty impresses on the researchers on SCD and the government of the regions in which SCD is endemic to harness their natural abundant resources (such as *G. kola*) in a bid to find a lasting panacea to the problem of sickle cell anemia with a view to developing novel medicinal substances.

Table 2. Antisickling effect (% inhibition of sickling) of methanol and aqueous fraction of *Garcinia kola* (Heckel) leaf.

Incubation time (min)	Normal saline	p-hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous fraction concentration (mg/ml)		
			10.0	1.0	0.1	10.0	1.0	0.1
0	-(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
30	-(2)	60(48)	70(55)	62(53)	54(47)	60(58)	54(55)	48(53)
60	-(4)	68(50)	77(58)	70(55)	54(47)	74(60)	66(58)	54(53)
90	-(5)	54(53)	62(60)	58(57)	49(48)	54(60)	53(60)	49(55)
120	-(8)	48(55)	57(60)	54(59)	45(48)	51(62)	48(60)	45(57)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells.

Table 3. Antisickling effect (% inhibition of sickling) of methanol and aqueous fraction of *Garcinia kola* (Heckel) seed pod.

Incubation time (min)	Normal Saline	p-hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous fraction concentration (mg/ml)		
			10.0	1.0	0.1	10.0	1.0	0.1
0	-(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
30	-(2)	60(48)	54(52)	50(48)	45(43)	50(58)	45(54)	40(48)
60	-(4)	68(50)	60(56)	54(51)	50(46)	54(60)	50(50)	45(50)
90	-(5)	54(53)	53(59)	49(55)	45(49)	49(60)	45(60)	42(53)
120	-(8)	48(55)	51(60)	45(57)	42(52)	45(60)	42(60)	37(55)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells.

Table 4. Antisickling effect (% inhibition of sickling) of methanol and aqueous fraction of *Garcinia kola* (Heckel) seed.

Incubation time (min)	Normal Saline	p-hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous fraction concentration (mg/ml)		
			10.0	1.0	0.1	10.0	1.0	0.1
0	-(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
30	-(2)	60(48)	58(56)	54(54)	48(48)	54(58)	50(53)	45(48)
60	-(4)	68(50)	66(58)	60(56)	54(51)	60(60)	54(56)	50(50)
90	-(5)	54(53)	62(60)	58(58)	49(54)	53(62)	49(57)	47(53)
120	-(8)	48(55)	62(62)	54(59)	45(55)	48(62)	45(59)	42(55)

Values in brackets are percentage sickling reversal of 2% metabisulphite induced sickled cells.

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