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Antioxidant capacity and anti-inflammatory activity of *Garcinia kola* (Heckel) stem bark extracts

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Abstract
Garcinia kola, commonly found in subtropical and tropical forests in West and Central Africa is important in African ethno-medicine for the treatment of asthma, diabetes, cancer, ulcer, infectious diseases, and inflammatory conditions. The present assessed the phytochemical composition, antioxidant and anti-inflammatory activities of the ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* stem barks. The phytochemical screening showed the presence of antioxidant compounds such as Tannins, Flavonoids, Alkaloids, Polyphenols while a more detailed phytochemical characterization by High Performance Thin Layer Chromatography (HPTLC) detected the presence of Gallic acid and Catechin. The polyphenols and flavonoids were higher in the ethanolic extract compared to the aqueous and hydroethanolic extracts. The ethanolic extract equally had the highest antioxidant activity as determined DPPH and ABTS radicals scavenging activity. Similar trend of results was obtained in the anti-inflammatory activity characterized by protein denaturation (BSA), membrane stabilization and anti-proteinase activity with ethanol being better than the other two extracts.

Keywords: *Garcinia kola*, phytochemical characterization, antioxidant, anti-inflammatory

1. Introduction
Reactive oxygen species (ROS) are important composites produced in some degree in the body and are involved in the regulation of biological processes like cell homeostasis, gene expression and signal transduction [1]. In normal situations, free radicals are eradicated by the body's antioxidant defense system. Nevertheless, some cases of disproportion between the production of ROS and their elimination by defense mechanisms (antioxidants) in favor to the ROS results to oxidative stress and chronic inflammation which in a state caused damage or injury to living tissue. In general, inflammation has four major indicators: pain, redness, warmness and swelling, and involves an increase of membrane alteration, permeability of vascular tissue and protein denaturation [2]. Some studies showed the connection between protein denaturation, membrane permeability and the development of inflammatory disorders such as in diabetes, rheumatoid arthritis and cancer [3]. Non-steroidal anti-inflammatory drugs (NSAID) which act by inhibiting both isoforms of cyclooxygenase (COX-1 and COX-2), have been identified for the management of inflammatory disorders. However, they demonstrate quite a lot of adverse side effects like gastric irritation, gastric ulcers [4]. Therefore, the ability of substance to prevent protein denaturation may help prevent inflammatory disorders. This justifies the importance of the search for new molecules from plants with anti-inflammatory and antioxidant properties to reinforce the defense mechanism. Much has been reported about the medicinal benefit of the *Garcinia* genus. More than 300 species belonging to *Garcinia* genus have been identified in Africa and Asia, most of which are endemic and economically significant with huge medicinal properties [5]. In West and Central Africa, *Garcinia kola* (Clusiaceae) is a tropical plant growing in moist forest, well known as a 'traditional' medicinal plant and has a wide range of medicinal uses such as antiparasitic, antimicrobial, antiviral, anti-inflammatory and also reduces menstrual cramps [6]. It is amedium-sized evergreen tree about 15 to 17 m tall, with a fairly narrow crown. Leaves are simple, shiny on both surfaces and spotted with resin glands, flowers covered with short red hairs [7]. *Garcinia kola* is commonly called bitter kola because of the bitter taste of its seeds which are largely consumed and sold by local populations in Africa. Some investigations revealed its range of phytochemicals such as biflavonoids, ameakoflavone, xanthones, kolanone and coumarine [6]. Kolaviron and Garcinoic acidisolated from *Garcinia kola* seeds attenuate inflammatory response [8].

Earlier studies on the biological activity of methanol extract of *G. kola* seeds, showed inhibitory effect on LPS-induced NO production in human macrophage cell line U937and in rats [9] while essential oil from its leaf showed antibacterial and antimicrobial activities effectiveness against Gram-positive and Gram-negative strains [10]. In order to add value to this precious plant, the present study consisted in the phytochemical characterization of the ethanolic, aqueous and hydro-ethanolic extracts of *Garcinia kola* stem bark and the evaluation of their antioxidant power and anti-inflammatory activity.

2. Materials and Methods

2.1 Reagents

Chemicals and reagents were purchased from SIGMA ALRICH Chemical CO, Dinâmica and Merck.

2.2 Plant material

The stem barks of *Garcinia kola* were harvested in the area of Mdom, Log Mbaha village in the Littoral Region of Cameroon in November 2018. The fresh stem bark was cleaned, dried to standard weight for one week at room temperature then crushed into powder using an electric grinder and the powder obtained was subjected to extraction.

2.3 Extraction

Three different extracts were prepared using three different solvents. The method used was earlier described by [11] with slight modifications. The powdered extract of *Garcinia kola* stem barks (20 g) was macerated for 72h in 100 ml of 95% ethanol for the ethanolic extract, fresh 20 g of *Garcinia kola* stem barks was macerated for 72h in100 ml of distilled water for the aqueous extract and another 20 g for 72h in hydroethanolic solvent (mixture of 30 ml of distilled water plus 70 ml of ethanol (30/70)) for the hydroethanolic extract. Samples were then filtered independently and then the filtrate concentrated at 78°C for the ethanolic extract, 100°C for the aqueous extract and 80°C for the hydroethanolic extract using a Rotavapor Büchi R110.

2.4 Phytochemical screening

All three extracts were subjected to phytochemical screening to confirm the presence of secondary metabolites using analytical colorimetric methods earlier described by Harborne in 1998 [12]. The secondary metabolites assessed were tannins, alkaloids, phenols, flavonoids, terpenoids, glucosides, anthraquinones, coumarins, anthocyanins and saponins.

2.4.1 Tannin test: For the determination of the presence of tannins, 20 mg of extract were dissolved in two milliliters (2 ml) of distilled water and the solution obtained was heated in a water bath for five minutes (5 mins), and filtered after cooling. Then 4 drops of ferric chloride (0.5%) were then added and observed for the presence of a blue-black color (dark blue).

2.4.2 Alkaloid test: Briefly, to 0.5 g of extract dissolved in one milliliter (1 ml) of methanol, were added five drops of sulphuric acid (2%). After one minute, four drops of Mayer's reagent were added to the mixture and the white precipitate obtained indicated the presence of alkaloids.

2.4.3 Phenolic compounds test: To 0.5 g of extract dissolved in two milliliters (2ml) of methanol, four drops of ferric chloride (FeCl3) were added and the formation of a blue-purplish complex indicated the presence of phenolic compounds.

2.4.4 Flavonoid test: In a test tube containing two milliliters (2 ml) of methanolic extract solution, three drops of sodium hydroxide solution diluted to 1/10 were added. The appearance of the yellow-orange coloration indicated the presence of flavonoids.

2.4.5 Terpenoid test: To a test tube containing two milliliters (2 ml) of extract, were added six drops of Libermann-Buchard reagents (1 ml of concentrated H_2SO_4, 20 ml of acetic anhydride, 50 ml of $CHCl_3$). The appearance of a bluish-green coloration designated the presence of terpenoids sterols.

2.4.6 Glucoside test: In a test tube, 5 mg of extract were dissolved in one milliliter (1 ml) of hydrochloric acid (5% HCL), and then 2.5 ml of sodium hydroxide (5% NaOH) were added to the medium. Once the mixture was homogenized and filtered, one milliliter (1ml) of hot Fehling's liquor (A and B) was added. The appearance of a brick-red precipitate showed the presence of glucosides.

2.4.7 Anthraquinones test: After dissolving 5 mg of dry extract in 5 ml of ammonia (10%), the mixture was homogenized and the appearance of an orange-yellow color indicated the presence of bound quinones.

2.4.8 Coumarins test: To 5 mg of extract dissolved in 5 ml of methanol, four drops of potash (10%) were added and the mixture was homogenized. The presence of coumarins was revealed by the color variation from blue to purple yellow.

2.4.9 Anthocyanin test: In a solution of 5 mg of extract dissolved in 5 ml of ethanol, four drops of diluted hydrochloric acid (HCl) and four drops of ammonia were added. The appearance of a red color after addition of hydrochloric acid and a blue-purplish-greenish color after addition of ammonia, indicated the presence of Anthocyanins.

2.4.10 Saponins test: 5 mg of extract were dissolved in 5 ml of distilled water and the solution obtained was heated in water bath for five minutes. After stirring, the formation of two centimeter of a thick foam layer revealed the presence of saponins.

2.5 High performance thin layer chromatography (HPTLC) of *Garcinia kola* stem barks extracts

The HPTLC assay was assessed using gallic acid, catechin, quercetin and lupeol as standard. Briefly, 10 µl of each sample (extracts/standard) was plotted in a chromatographic plate (10 cm ×20 cm HPTLC cellulose plates Merck No. 1.05786) which was then introduced in chromatographic glass vat containing an elution system composed by a mixture of Toluene-Ethyl Acetate-Formic Acid (5:4:0,2) for the migration of Quercetin [13] and gallic acid[14], Ethyl Acetate-Toluene-Methanol (70:30:10) for the migration of catechin [15]and Toluene-Methanol (9:1) for the migration of lupeol [16]. After the migration time, the development was done under a UV light and using an eluent such as polyethylene glycol reagent (NP-PEG) 1% for quercetin revelation, FeCl3 0,5% for gallic acid revelation, Ethanol-Phosphoric Acid-Acetic Acid (70:20:10) with 1% of vanillin for catechin revelation and Acetic Acid-Sulphuric Acid (70:30) with 2% of vanillin for lupeol revelation. The retention factor of each compound was calculated using the formula:

$$\text{Retention factor } (R_f) = \frac{\text{compoundmigrationdistanceonchromatographicplate}}{\text{Eluentmigrationdistanceonchromatographicplate}}$$

2.6 Quantification of total polyphenolic, flavonoid content and ferric reducing antioxidant power in *Garcinia kola* stem barks extracts

2.6.1 Evaluation of total polyphenols content (TPC)

The TPC of each extract was determined using Folin-Ciocalteu reagent with catechin as standard following the method described by Li *et al.* in 2007[17]. Into each test tube containing 1 ml of Folin-Ciocalteu reagent (2N diluted 10 times), 200 μl of each extract (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, and 0.03125 mg/ml) were added. After 4 minutes of incubation at room temperature, 800 μl of sodium carbonate (75 mg/ml) were added and the tubes were left at room temperature for 2 hours. The optic density was measured at 765 nm and the results obtained were expressed as mg equivalent of catechin /g of extract (mg Eq Cat / g Ex). All the experiment was performed in triplicate.

2.6.2 Evaluation of total flavonoids content (TFC)

The method as earlier described was used to evaluate the total flavonoids content [18]. Briefly, 500 μl of each plant extract (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, and 0.03125 mg/ml) were mixed with 150 μl of $NaNO_2$ (5%). After 5 minutes, 150 μl of $AlCl_3$ (10%) was added and then 6 minutes later 500 μl of sodium hydroxide (NaOH, 4%) was added. The absorbance of the mixture was measured at 510 nm after 10 minutes of incubation. The TFC was expressed as mg equivalent of quercetin /g of extract (mg Eq Que/g Ex) and the experiment was performed in triplicate.

2.6.3 Evaluation of Ferric Reducing Antioxidant Power (FRAP) of *Garcinia kola* stem barks extracts

The ferric reducing antioxidant power (FRAP) of each extract was determined [19]. The FRAP reagent consisted of ten parts of acetate buffer (300 mM, pH 3.6), one part of 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM in 400 mM of HCl, Sigma) and one part of ferric chloride (10 mM). To 75 μl of each extract solution (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, and 0.03125 mg/ml), were added 2 ml of FRAP reagent and after 15 minutes of incubation, the absorbance of the mixture was read at 593 nm. The standard curve was plotted using gallic acid and the experiment was performed in triplicate. The FRAP results were expressed as mg equivalent of gallic acid/g of extract (mg Eq GA/g Ex).

2.7 Evaluation of radical scavenging activity of*Garcinia kola* stem bark extracts

2.7.1 Evaluation of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Free Radical Scavenging activity

The free radical scavenging power was measured using DPPH free radical test [20]. The optical density of DPPH in methanol was measured using spectrophotometer at 517 nm and adjusted to 0.85. In test tubes, 500 μl of each extract (300 μg/ml, 100 μg/ml, 30 μg/ml, 10 μg/ml, 3 μg/ml, 1 μg/ml) was added to 2 ml of methanol DPPH solution (0.1mM). The mixture was incubated at room temperature in dark for 30 minutes and the variation of absorbance was measured at 517 nm. The IC_{50} value was determined from plots of inhibition percentage versus concentrations of extracts. The experiment was performed in triplicate and the inhibition percentage of the radical scavenging activity was calculated using the formula:

$$\text{Inhibition percentage } (\%) = \left[\frac{(A517\ DPPH - A517\ sample)}{A517\ DPPH}\right] \times 100$$

A517 DPPH = DPPH initial absorbance
A517 sample = absorbance of extract/gallic acid

2.7.2 Evaluation of ABTS (2, 2'-azino-bis (3-éthylbenzothiazoline-6-sulphonique)) Free Radical Scavenging activity

The scavenging activity of $ABTS^+$ radical was measured using ABTS reagent [21]. The initial absorbance of methanol solution of ABTS was measured using spectrophotometer at 534 nm and the optical density adjusted to 1.00. For each extract (300 μg/ml, 100 μg/ml, 30 μg/ml, 10 μg/ml, 3 μg/ml, 1 μg/ml), 200 μl was added to 1.8 ml of ABTS solution (0.1mM). The mixture was then incubated at room temperature in a dark room for 15 minutes and the variation of absorbance was measured at 534 nm. The experiment was performed in triplicate and the IC_{50} values were determined from regression plots of percentage of inhibition versus concentrations of extracts. The percentage inhibition of the scavenging activity was calculated using the formula:

$$\text{Inhibition percentage } (\%) = \left[\frac{(A534\ ABTS - A534\ sample)}{A534\ ABTS}\right] \times 100$$

A534 ABTS= ABTS initial absorbance
A534 sample = absorbance of extract/catechin

2.8 Evaluation of Anti-inflammatory Property of *Garcinia kola* stem bark extracts

2.8.1 Inhibition of Bovine Serum Albumin (BSA) Denaturation

The reaction mixture was comprised of 1.5 ml of phosphate buffered saline (PBS, pH 6.4), 1 ml of varying concentration of extracts and 0.5 ml of bovine serum albumin (4% in PBS). Similar volume of double distilled water served as control. The mixture was incubated at 37 °C for 10 minutes and then heated at 70 °C for 20 minutes in water-bath. After cooling, the absorbance was measured at 660 nm against pure blank; all the experiment was performed in triplicate. Acetylsalicylic acid was used as reference drug [22]. The percentage inhibition of protein denaturation was calculated by the formula mentioned below:

$$\text{Inhibition percentage } (\%) = \left[\frac{(A660\ control - A660\ sample)}{A660\ control}\right] \times 100$$

A660 control = absorbance of control
A660 sample= absorbance of extract/ acetylsalicylic acid

2.8.2 Evaluation of Erythrocytes Membrane Stabilization

To prepare the red blood cells suspension (RBCs), fresh whole blood was collected in heparinized tubes (10 ml) from a healthy patient. The tubes were then centrifuged at 3000 rpm for 10 minutes and washed three times with equal volume of normal saline. The blood volume was measured and reconstituted as 10% (v/v) suspension with normal saline.
The reaction mixture consisted of 1 ml of extract at various concentrations and 1 ml of 10% RBCs suspension. Saline was added to the control test tube and sodium diclofenac was used as standard drug. The tubes were first incubated at 37°C for 10 minutes and then incubated in water bath at 60 °C for 20 minutes. At the end of the incubation, the tubes were cooled, the reaction mixture was centrifuged at 2500 rpm for 5

minutes and the absorbance of the supernatant was read at 560 nm[23]. The experiment was performed in triplicate and the percentage of membrane stabilization activity was calculated by the formula below:

$$\text{Inhibition percentage (\%)} = [\frac{(A560\ control - A560\ sample)}{A560\ control}] \times 100$$

A560 control = absorbance of control
A560 sample= absorbance of extract/sodium diclofenac

2.8.3 Evaluation of anti-proteinase activity

The evaluation of the anti-proteinase activity of *Garcinia kola* stem bark extracts was carried out [24]. The reaction mixture with a total volume of 2 ml consisted of 0.06 mg of trypsin, 1 ml of Tris-HCl buffer (20 mM, pH 7.4) and 1 ml of sample (extract/acetylsalicylic acid) at different concentrations. After 5 minutes of incubation at 37 °C, 0.08% of casein was added and the mixture was re-incubated for 20 minutes at 37 °C. At the end of the incubation period, 2ml of perchloric acid (70%) was added to the medium to stop the reaction and the resulting mixture was centrifuged at 3000 rpm for 10 minutes. The optical density of the supernatant was read at the wavelength of 210 nm against blank. The percentage of anti-proteinase activity was calculated according to the formula mentioned below:

$$\text{Inhibition percentage (\%)} = [\frac{(A210\ control - A210 sample)}{A210 control}] \times 100$$

A210 control = absorbance of control
A210 sample = absorbance of extract/ acetylsalicylic acid

2.9 Statistical analysis

The data are expressed as mean ± SD. All data were analyzed using Graph Pad Prism software 8.0.2 and Microsoft Excel 2013. The statistical analysis of data was performed using analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Differences between extracts and reference molecules were considered significant with $p < 0.05$.

3. Results

3.1 Phytochemical composition of *Garcinia kola* stem barks extracts

The phytochemical screening of ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* stem barks, revealed the presence of several compounds: tannins, alkaloids, phenols, flavonoids, terpenoids, anthraquinones, coumarins, anthocyanins and saponins in the three extracts of *Garcinia kola* stem barks. The presence of glucosides was detected only in ethanolic and hydroethanolic extracts, but not in the aqueous extract (Table 1).

Table 1: Phytochemical composition of ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* stem barks

Compounds	Ethanolic Extract	Aqueous Extract	Hydroethanolic Extract
Tannins	+++	+	++
Alkaloids	++	+	++
Phenols	+++	+	++
Flavonoids	+++	+	++
Terpenoids (Sterols)	++	++	++
Glucosides	++	-	+
Anthraquinones (bound quinones)	+++	+++	+++
Coumarins	+++	+++	+++
Anthocyanins	+	+	+
Saponins	+	++	++

(+++): very colorful; (++): colorful; (+): less coloration; (-): no coloration

3.2 High performance thin layer chromatography of *Garcinia kola* stem barks extract revelation

The HPTLC results revealed that the ethanolic and hydroethanolic extracts of *Garcinia kola* stem barks have identical Retention factor of0.600±0.035close to that of catechin which showed a value of 0.666±0.008 (Figure 1-C). The ethanolic and hydroethanolic extracts also showed identical retention factor of 0.392±0.017substantially equal to that of gallic acid which showed a value of 0.357±0.018 (Figure 1-B). On the other hand, the different extracts showed identical retention factor of 0.285±0.030, 0.285±0.035 and 0.285±0.038 respectively for the ethanolic, aqueous and hydroethanolic extracts which are significantly different from that of quercetin which showed a value of 0.500±0.033 (Figure 1-A). No migration in extracts was noted for the identification of lupeol (Figure 1-D). These results therefore attest to the possible presence of catechin and gallic acid in the ethanolic and hydroethanolic extracts of *Garcinia kola* stem bark.

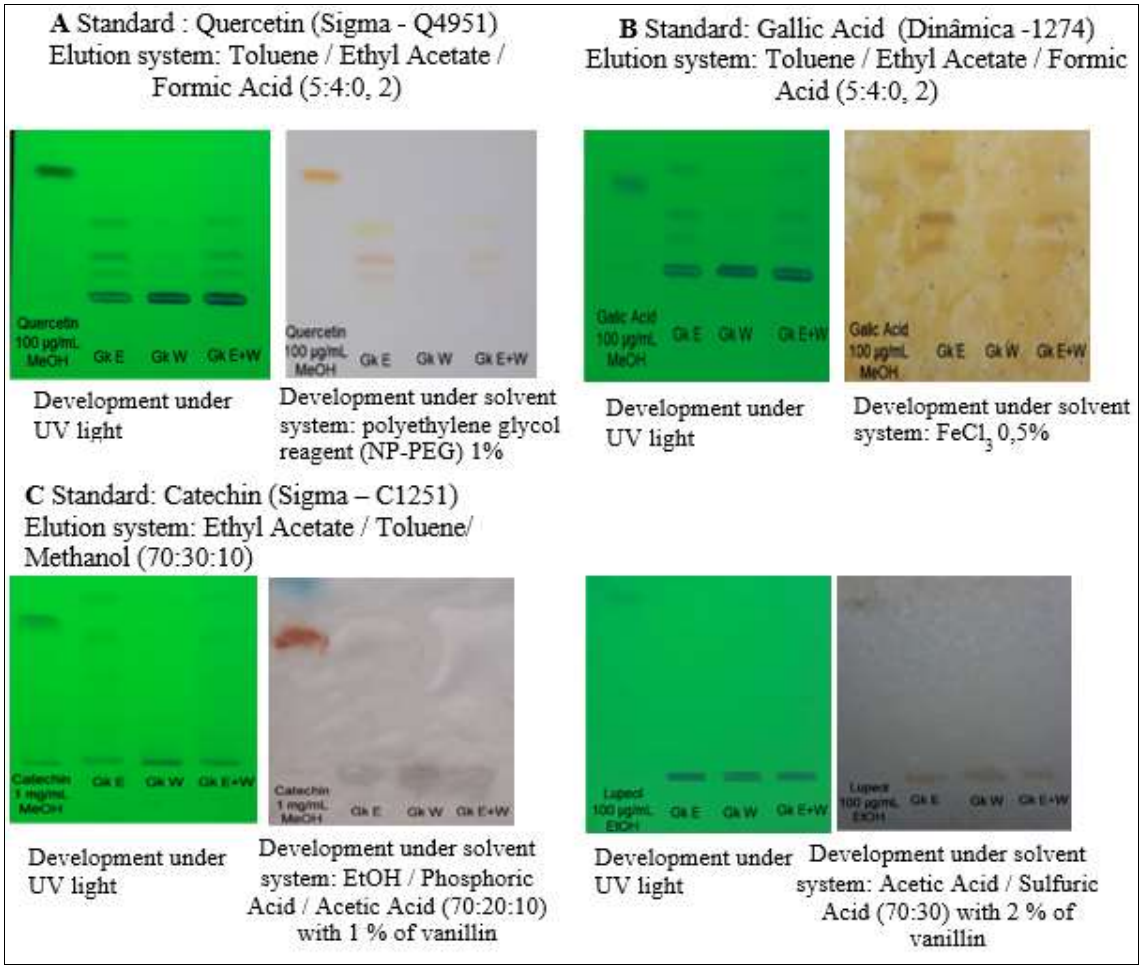

Fig 1: Chromatographic plates after developing under UV light and solvent system

Table 2: Retention factor of extracts compared to standard compounds

	Standard			
	Catechin	**Gallic Acid**	**Quercetin**	**Lupeol**
	0.666±0.008	**0.357±0.018**	**0.500±0.033**	**0.9375±0.013**
Ethanolic Extract	0.600±0.035^{ns}	0.392±0.017^{ns}	0.285±0.030***	0
Aqueous Extract	0.266±0.029****	0.285±0.018**	0.285±0.035***	0
Hydroethanolic Extract	0.600±0.035^{ns}	0.392±0.017 ^{ns}	0.285±0.038***	0

Retention factor with different symbol between extracts (**), (***), (****) are significantly different from the standard $p < 0.05$.**$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared with Tukey's Multiple Comparisons Test. ns: not significant.

3.3. Quantity of antioxidant components

3.3.1. Total Phenolic Content

The ethanolic extract had a significant (p<0.0001) higher phenolic content (0.39 ± 0.00 mg Cat Eq/g Ex) compared to aqueous (0.12 ± 0.00 mg Cat Eq/g Ex) and hydroethanolic extracts (0.166 ± 0.004 mg Cat Eq/g Ex).

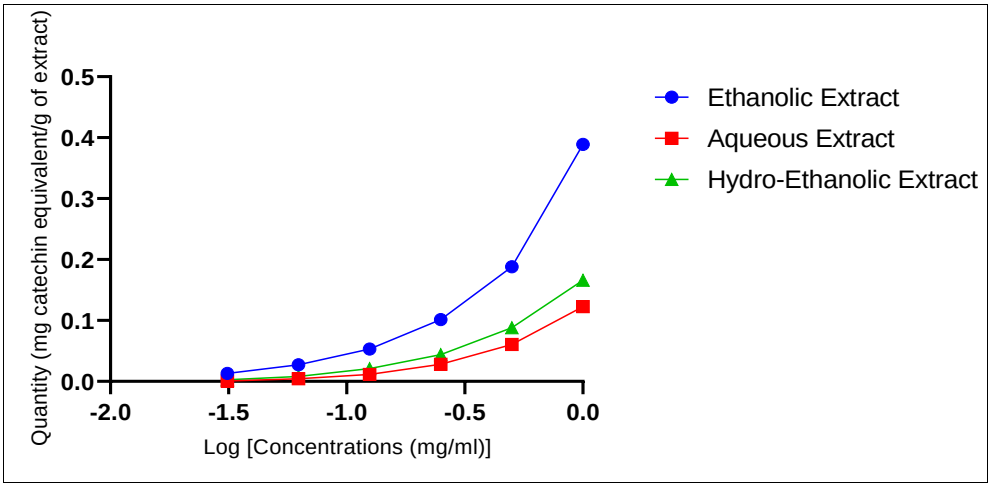

Fig 2: Total phenolic content of ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* stem bark

3.3.2 Total Flavonoids Content

Ethanolic extract of *Garcinia kola* showed a significantly (p<0.0001) higher flavonoids (0.275 ± 0.001 mg Que Eq/g Ex) compared to aqueous (0.068 ± 0.001 mg Que Eq/g Ex) and hydroethanolic extracts (0.098 ± 0.000 mg Que Eq/g Ex).

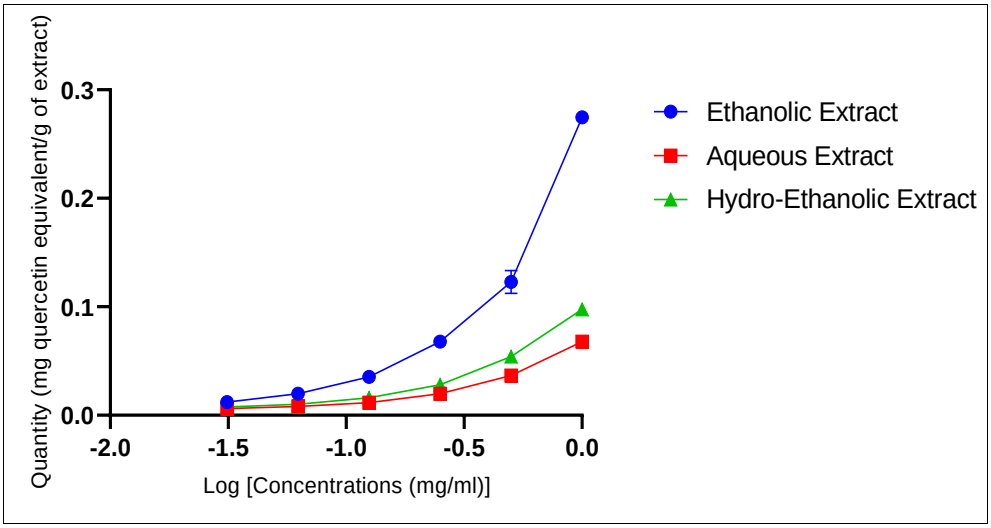

Fig 3: Total flavonoids content in ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* stem bark

3.3.3 Ferric Reducing Antioxidant Power (FRAP)

Ethanolic extract expressed a better ferric reducing antioxidant power of 0.144 ± 0.006of gallic acid equivalent/g of extract (mg GA Eq/g Ex) in comparison to aqueous and hydroethanolic extracts which presented respective of 0.099 ± 0.006 mg GA Eq/g Ex and 0.103 ± 0.006 mg GA Eq/g Ex (figure 4). The FRAP of ethanolic extract was significantly different (p<0.0001) compared to hydroethanolic and aqueous extracts (Table 3).

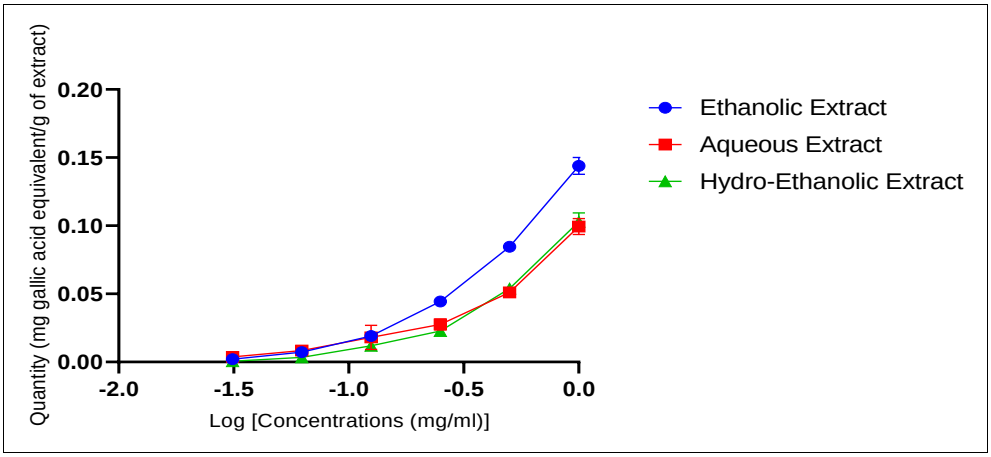

Fig 4: Total phenols of *Garcinia kola* stem bark extracts with ferric reduction antioxidant power

Table 3: Antioxidant components of *Garcinia kola* stem bark extracts

	Concentrations(mg/ml)	Total polyphenols (mg Cat Eq/g Ex)	Total flavonoids (mg Que Eq/g Ex)	FRAP (mg GA Eq/g Ex)
Ethanolic Extract	1	0.389±0.000	0.275±0.001	0.144±0.006
	0.5	0.188±0.002	0.123±0.011	0.085±0.001
	0.25	0.102±0.000	0.068±0.000	0.044±0.002
	0.125	0.053±0.002	0.035±0.001	0.019±0.003
	0.0625	0.027±0.001	0.020±0.001	0.007±0.001
	0.03125	0.013±0.001	0.012±0.001	0.002±0.001
Aqueous Extract	1	0.123±0.002****	0.068±0.001****	0.099±0.006****
	0.5	0.061±0.002	0.037±0.002	0.051±0.002
	0.25	0.028±0.000	0.020±0.001	0.028±0.004
	0.125	0.011±0.001	0.012±0.001	0.018±0.009
	0.0625	0.004±0.001	0.008±0.000	0.008±0.002
	0.03125	0.001±0.000	0.006±0.000	0.004±0.001
Hydro-Ethanolic Extract	1	0.166±0.004****	0.098±0.000****	0.103±0.006****
	0.5	0.008±0.001	0.054±0.000	0.054±0.001
	0.25	0.044±0.001	0.028±0.001	0.023±0.000
	0.125	0.021±0.001	0.016±0.001	0.012±0.002

	0.0625	0.008±0.001	0.010±0.000	0.003±0.000
	0.03125	0.003±0.002	0.008±0.000	0.001±0.000

Results are expressed as means ± SD. Means with different symbol between extracts (****) are significantly different from the ethanolic extract $p< 0.05$. ****$p < 0.0001$, compared with Tukey's Multiple Comparisons Test

3.4 Antioxidant Power

3.4.1 DPPH free Radical scavenging activity

The results showed that ethanolic and hydroethanolic extracts presented good DPPH radical scavenging activity at the concentration of 300 µg/ml with respectively an inhibition percentage of 90.87±4.26% and 87.01± 2.61% compared to gallic acid that showed an inhibition percentage of 94.53±2.45% (Figure 5). The Aqueous extract showed less inhibition percentage compared to ethanolic and hydroethanolic extracts and gallic acid with a value of 56.27± 2.77%. Significant difference was observed between gallic acid and the aqueous and hydroethanolic extracts ($p<0.0001$, $p<0.01$). Though, the difference observed between the ethanolic extract and gallic acid was not significant.

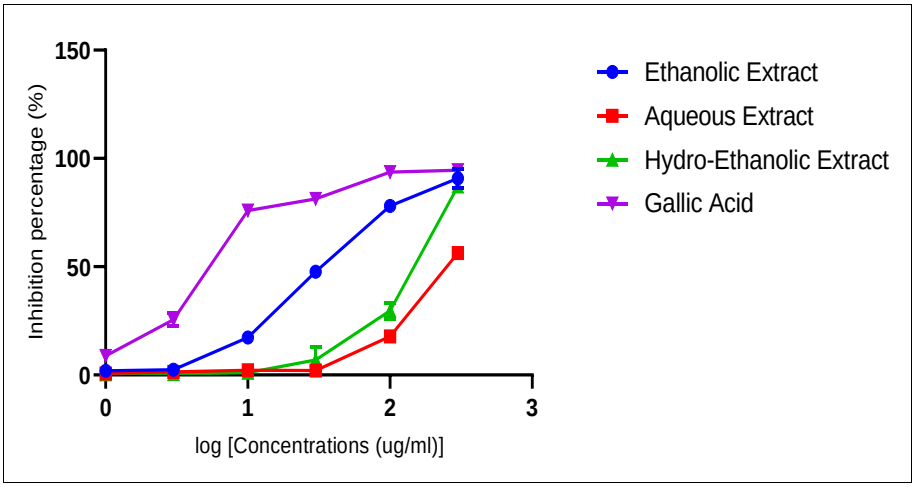

Fig 5: DPPH radical scavenging activity of *Garcinia kola* stem bark extracts

3.4.2 ABTS free radical scavenging activity

The results indicated that, compared to catechin, ethanolic and aqueous extracts showed good ABTS free radical scavenging activity at 300 µg/ml with an inhibition percentage of 95.45±0.40% and 93.82±0.26% (Figure 6) respectively. On the other hand, the hydro-ethanolic extract which showed a significant difference ($p<0.0001$) with catechin, presented less inhibition percentage compared to ethanolic and aqueous extracts with a value of 76.66±2.07%.

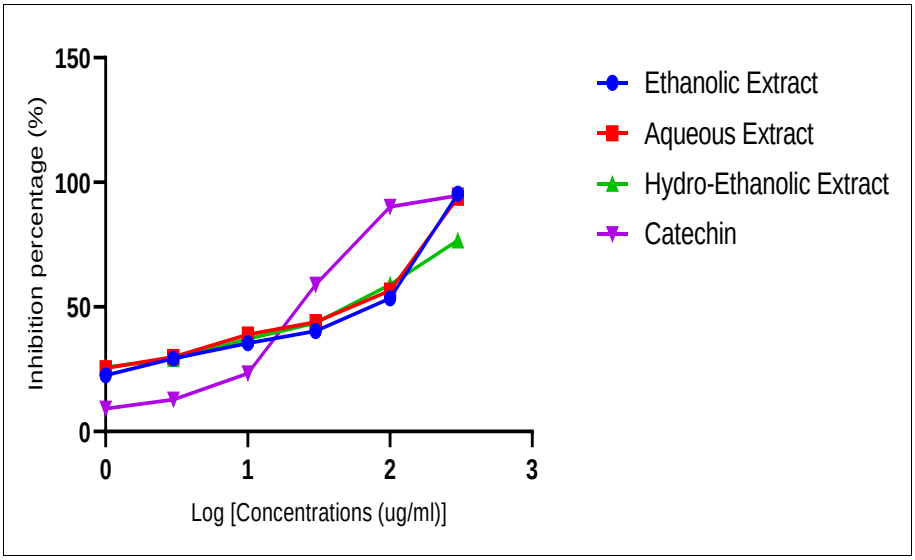

Fig 6: ABTS radical scavenging activity of *Garcinia kola* stem bark extracts

Table 4: Percentage of inhibition and IC_{50} of DPPH and ABTS scavenging activity

		DPPH		ABTS	
	Concentrations (µg/ml)	**Inhibition percentage**	**IC_{50} (µg/ml)**	**Inhibition percentage**	**IC_{50} (µg/ml)**
Ethanolic Extract	300	90.87±4.26ns	113.31	95.45±0.40ns	91.54
	100	78.00±2.09		53.41±0.99	
	30	47.69±0.23		40.33±1.24	
	10	17.31±0.53		35.49±1.54	
	3	2.47±2.04		29.31±2.04	

	1	1.96±0.71		22.54±1.55	
Aqueous Extract	300	56.27±2.77****	268.73	93.82±0.26ns	83.10
	100	17.82±1.83		56.60±1.29	
	30	2.04±1.18		43.92±2.58	
	10	2.16±0.49		38.87±1.38	
	3	1.37±0.47		29.88±0.85	
	1	0.62±0.44		25.43±1.15	
Hydroethanolic Extract	300	87.01± 2.61**	173.06	76.66±2.07****	104.95
	100	29.67±3.76		58.93±0.99	
	30	6.92±5.96		43.52±2.54	
	10	1.06±0.11		37.18±2.45	
	3	0.47±0.11		29.01±0.79	
	1	0.31±0.18		25.76±1.24	
Gallic acid/Catechin	300	94.53±2.45	3.72	94.72±0.10	80.84
	100	93.74±2.35		90.13±0.38	
	30	81.26±2.14		58.95±1.34	
	10	75.99±0.83		23.37±1.95	
	3	25.50±3.18		12.78±0.35	
	1	8.81±1.91		9.12±0.80	

Results are expressed as means ± SD. Means with different symbols between extracts (****), (**) are significantly different from standard $p< 0.05$. **$p < 0.01$, ****$p < 0.0001$ when compared with Tukey's Multiple Comparisons Test. ns: not significant.

The results of DPPH and ABTS tests (table 4), indicate that the ethanolic extract of *G. kola* exhibited IC_{50} of 113.31 µg/ml and 91.54 µg/mlwhich were better than the aqueous and hydroethanolic extracts (268.73 µg/ml and 173.06 µg/ml) for DPPH and for ABTS (83.10µg/ml and 104.95µg/ml). The three extracts of *G. kola* showed poorIC_{50}than gallic acid and catechin which presented of 3.72 and 80.84µg/ml respectively.

3.5 Anti-inflammatory Activity

3.5.1 Bovine serum albumin denaturation inhibitory activity

The maximum inhibitory percentage of protein denaturation of above 60%, was exhibited by the hydroethanolic and ethanolic extracts of *Garcinia kola* (73.17±4.99% and 69.42±1.00%) compared to acetylsalicylic acid (Figure 7) while the aqueous extract showed inhibition percentage of 57.62±5.64%. Significant difference was observed between the three extracts of *Garcinia kola* and the acetylsalicylic acid. The extracts of *Garcinia kola* stem bark were able to inhibit the protein denaturation in a concentration-dependent manner.

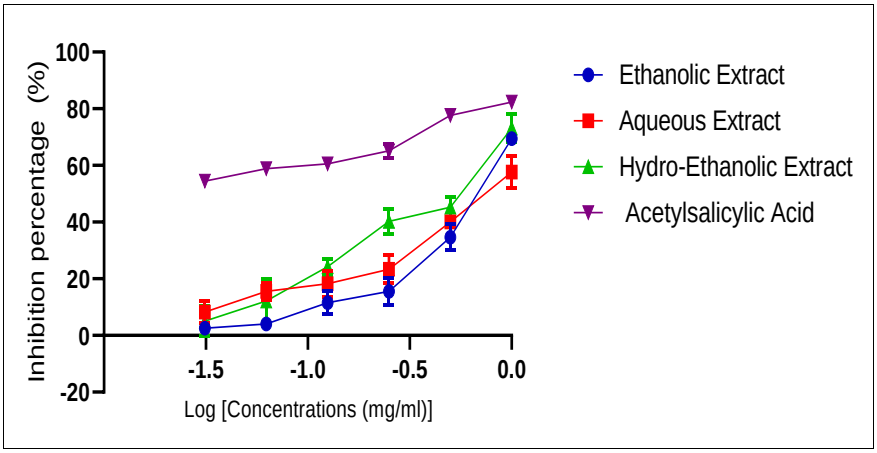

Fig 7: Inhibition percentage of *Garcinia kola* stem bark extracts on BSA denaturation

3.5.2 Erythrocytes Membrane Stabilization activity

The three extracts of *Garcinia kola* exhibited a membrane stabilization activity in a concentration-dependent manner (Figure 8). The ethanolic extract revealed significant membrane stabilization activity with an inhibition percentage within the range from 12.01±0.075% to 91.12±1.75% at the concentration of 0.03125mg/ml-1 mg/ml compared to hydroethanolic and aqueous extracts which showed 22.45±1.01% and 26.72±1.12% values respectively. Likewise, the ethanolic extract showed significantly ($p<0.0001$) higher levels of hemolysis inhibition, compared to sodium diclofenac, while no significant difference was observed between the aqueous extract and sodium diclofenac.

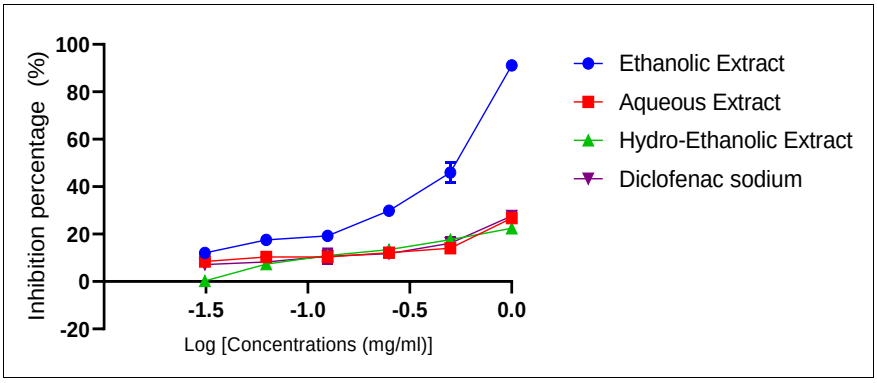

Fig 8: Effect of *Garcinia kola* stem bark extracts on membrane stabilization

3.5.3 Anti-proteinase activity

The three extracts of *Garcinia kola* were effective in inhibiting proteinase activity (Figure 9). The results showed that the maximum inhibition was reached at 1 mg /ml and the ethanolic extract of *G. kola* exhibited the best percentage of inhibition with 95.39 ± 0.91% against 84.48 ± 1.06% for the aqueous extract, 87.44 ± 0.03 for the hydroethanolic extract and 88.54 ± 0.39 for acetylsalicylic acid. The inhibitory effect of *G. kola* stem bark extracts on proteinase activity, increases in a concentration-dependent manner. Significant difference was observed between the activity of the ethanolic and aqueous extracts and the activity of acetylsalicylic acid ($P<0.0001$; $p<0.01$).

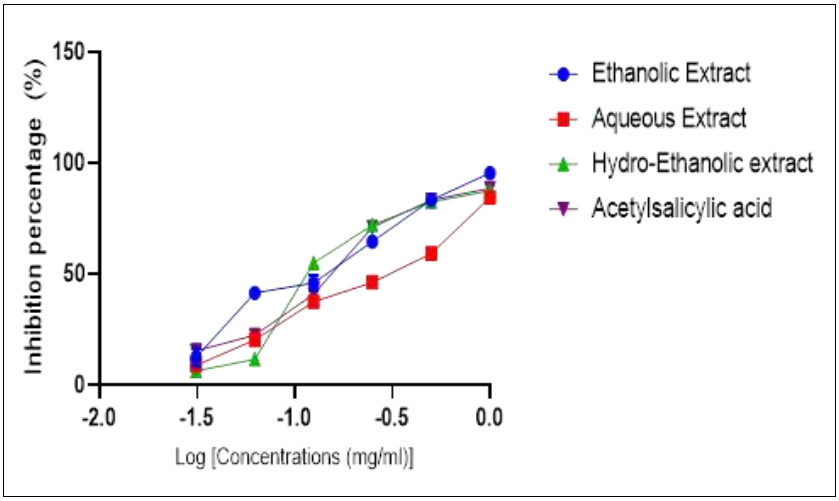

Fig 9: Anti-proteinase effect of *Garcinia kola* stem bark extracts

Table 5: Percentage of inhibition and IC_{50} of protein denaturation, membrane stabilization and anti-proteinase activity

		Protein denaturation		Membrane stabilization		Anti-proteinase	
	Concentrations (mg/ml)	Inhibition percentage	IC_{50}(mg/ml)	Inhibition percentage	IC_{50} (mg/ml)	Inhibition percentage	IC_{50} (mg/ml)
Ethanolic Extract	1	69.42±1.00***	0.721	91.12±1.75****	0.504	95,39±0.91****	0.227
	0.5	34.61±4.66		45.91±4.23		83.29±0.73	
	0.25	15.54±4.92		29.83±2.02		64.60±1.23	
	0.125	11.50±4.03		19.25±1.40		45.95±3.34	
	0.0625	4.03±1.09		17.53±0.17		41.48±0.60	
	0.03125	2.56±1.23		12.01±0.75		12.45±4.18	
Aqueous Extract	1	57.62±5.64****	0.796	26.72±1.12ns	2.395	84,48±1.06**	0.432
	0.5	40.09±2.10		14.03±0.47		59.19±0.57	
	0.25	23.30±5.09		12.19±0.49		46.18±0.39	
	0.125	18.20±4.63		10.35±1.29		37.46±0.25	
	0.0625	15.51±3.10		10.30±0.25		20.31±1.53	
	0.03125	8.26±3.87		8.42±0.61		8.78±1.66	
Hydro-ethanolic Extract	1	73.17±4.99*	0.586	22.45±1.01**	2.361	87.44±0.03ns	0.295
	0.5	45.25±3.72		17.68±0.57		82.43±0.95	
	0.25	40.28±4.42		13.48±1.42		72.11±0.63	
	0.125	24.29±2.90		10.92±0.27		54.96±0.81	
	0.0625	12.14±7.69		7.35±1.30		11.53±0.59	
	0.03125	5.09±5.48		0.25±0.73		6.30±0.52	
Acetyl salicylic Acid - Sodium	1	82.30±1.61	0.012	27.65±1.50	2.123	88,54±0.39	0.278
	0.5	77.62±0.58		16.12±2.49		83.33±1.44	
	0.25	65.12±2.53		11.76±1.16		71.11±0.89	

Diclofenac	0.125	60.51±1.05		10.69±3.12		40.75±0.39	
	0.0625	58.81±0.33		8.28±1.22		22.48±0.48	
	0.03125	54.45±0.53		7.19±1.46		15.26±3.07	

Results are expressed as means ± SD. Means with different symbols between extracts (****), (***), (**), (*) are significantly different from standard at $p< 0.05$. *$p < 0.05$, **p<0.01, ***$p < 0.001$, ****$p < 0.0001$ when compared with Tukey's Multiple Comparisons Test. ns: not significant.

The results showed that the ethanolic extract of *G. kola* exhibited the best IC_{50} values for membrane stabilization and anti-proteinase assays with respective values of 0.504 mg/ml and 0.227 mg/ml compared to the aqueous and hydroethanolic extracts. On the other hand, the best IC_{50} value for protein denaturation assay was presented by the hydroethanolic extract with 0.586 mg/ml compared to the ethanolic and aqueous extract.

4. Discussion

Oxidative stress, resulting from an imbalance between free radicals and antioxidants, has been identified as the starting point for several disease conditions including diabetes, cancer, Alzheimer's disease, Parkinson's disease and inflammatory disorders in general. Inflammation is a response of an organism or living tissue caused by injury or damage and whose mechanisms consist of a series of metabolic events [25] such as the denaturation of proteins that results in a loss of the biological properties of protein molecules [3]. To prevent or treat these diseases, molecules with antioxidant properties are widely researched. Antioxidants are substances found in the body and/or in food that significantly scavenge free radicals which in high concentrations are harmful to the body. Among these substances with strong reducing power, there are polyphenols, thiols, ascorbic acid [26], thus justifying the growing interest in current research on these molecules. The various studies carried out have proven the presence of a multitude of antioxidant molecules (alkaloids, flavonoids, tannins, polyphenols, etc.) in various plant organs (leaves, flowers, seeds, bark, stems, etc.) making them an immense reservoir of antioxidants and many secondary metabolites that can treat bacterial, viral, parasitic or inflammatory diseases[26].The evaluation of free radical scavenging activity of *Garcinia kola* extracts, revealed a high inhibition percentage by the ethanolic extract compared to the aqueous and hydroethanolic extracts. This difference in radical scavenging activity between the extracts could be correlated with the difference in the concentration of their antioxidant compounds. Studies have shown that biological activity of plants extracts are due to the presence of a multitude of bioactive molecules in plants especially with polyphenols owing to their antioxidant potentials [27]. Hence, the antioxidant activity of *Garcinia kola* reveals the potential of these extracts to limit or neutralize the process of oxidation in living cells. Phenolic acids and flavonoids are classes of polyphenols that have been associated with activity against dyslipidemia, cardiovascular disease and the essential mechanisms of other disease processes such as inflammatory diseases [27]. Food-derived Flavonols have also been reported to exhibit numerous biological functions and medicinal properties such as antioxidant, antithrombotic, anti-inflammatory, anti-atherogenic, cardioprotective and anti-atherosclerotic properties [28]. Indeed, catechin, possesses antioxidant activity by preventing cardiovascular diseases by increasing vascular reactivity and decreasing inflammatory processes in the atherogenesis process [29].

Inflammation is a complex biological response to injury by the activation of several metabolic pathways increasing vascular permeability, membrane damage and protein denaturation [4]. Following exposure of red blood cells to harmful substances (hypotonic medium, heat, etc.), lysis of the membrane with hemolysis and oxidation of hemoglobin may occur making membrane stabilization an important element in the establishment of the inflammatory response [30].Non-steroidal anti-inflammatory drugs (NSAIDs) protect against protein denaturation [31] by inhibiting the release of lysosomal enzymes and stabilizing lysosomal membranes. As protein denaturation, membrane alteration and proteinase activity are correlated with the formation of inflammatory disorders, the ability of natural substances to prevent or inhibit this denaturation and promote membrane stabilization may also help to prevent inflammatory disorders [3]. The anti-inflammatory activity of the ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* on bovine serum albumin (BSA) denaturation, membrane stabilization and proteinase activity, revealed a significant medicinal property of this plant. Likewise, the results of an in-vivo study of Morabandza *et al.* in 2014 [32], showed the anti-inflammatory activity of *Garcinia kola* mesocarp fruit of 19.90% at a dose of 800 mg/kg. It has been shown that in setting up the inflammatory response, the migration of leukocytes from the venous system to damaged sites and the release of cytokines (IL-1, IL-8, etc.), play an important role. Leukocytes as a defense mechanism, release their lysosomal enzymes (proteinase), which will damage already irritated tissue. The precise mechanism of action of membrane stabilization has not been clearly established. However, some studies suggest that plant extracts inhibit the release of elements contained in the lysosomes on the inflammatory site, including bacterial enzymes and proteases which, once found in the extracellular environment, cause lesions and accentuate the inflammatory process in tissues [33], [34]. Thus, it is still believed that an interaction between the bioactive compounds of plant extracts and the constituent elements of the membrane play a role as reported by Aitadafoun *et al.* [35]. Extracts of *G. kola* could therefore act via the process of inhibiting the rupture of the membrane, thus preventing the release of cell constituents. Proteases of leukocytes, play an important role in the development of tissue damage during inflammatory processes. According to Das and Chatterjee [36], an important protection of tissue injury during inflammation is due to the action of protease inhibitor molecules. The various extracts of *G. kola* showed a concentration-dependent inhibition of protease activity and the ethanolic extract exhibited the best inhibition percentage.

The phytochemical screening of ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* stem barks, revealed the presence of bioactive compounds such as polyphenols, alkaloids and flavonoids which were previously highlighted [37]. High-performance thin layer chromatography of the ethanolic and hydroethanolic extracts of *Garcinia kola*, suggested the presence of catechin and gallic acid which are molecules of the flavonoid and phenolic acids family respectively. It has been noted that phenolic compounds receive considerable attention due to their physiological functions, such as scavenging free radicals [38]. Their

antioxidant action is mainly due to their redox properties which make them reducing agents, hydrogen donors and singlet oxygen deactivators [39]. According to earlier studies, many flavonoids contribute significantly to the antioxidant and anti-inflammatory activity of plant extracts [4].

In the present study, the ethanolic extract of *Garcinia kola* bark, presented the highest polyphenols and flavonoids content compared to the aqueous and hydroethanolic extracts and this could be explained by the affinity between solvents used for the extraction and the nature of these compounds. The ethanolic extract also showed the best antioxidant and anti-inflammatory activity. These results indicate a significant correlation between the anti-inflammatory activity, the antioxidant activity and the estimated amount of antioxidant compounds such as alkaloids, phenols and flavonoids present in the ethanolic extract.

5. Conclusion

The study carried out to evaluate the antioxidant and anti-inflammatory activities of ethanolic, aqueous and hydroethanolic extracts of *Garcinia kola* stem bark, showed that the ethanolic extract had a better antioxidant capacity in both DPPH and ABTS radical scavenging and a good anti-inflammatory activity in BSA denaturation, membrane stabilization and anti-proteinase activity. The quantification of antioxidant components, total polyphenols and total flavonoids, also revealed that the ethanolic extract of *Garcinia kola* showed the best amount. Thus, *Garcinia kola* could be an interesting source of secondary metabolites for the treatment of inflammatory diseases.

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