

***In vitro* Antioxidant, Anti-inflammatory and Antimicrobial Activities of *Garcinia kola* Seeds (Clusiaceae)**

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Abstract: The use of plants in health promotion is increasingly noteworthy in the face of the re-emergence of diseases and the limitation of conventional drugs. *Garcinia kola* (Clusiaceae) is used in traditional medicine in Togo. The seeds, very used, are renowned for their anti-infective and antioxidant properties. The objective of this study was to contribute to the valuation of *Garcinia kola* through the determination of its phytochemical groups and the evaluation of some of its biological activities. The supernatant was obtained by maceration ethanol/water (80/20) of powder from the seeds after separation. The phytochemical screening was carried out by staining and precipitation test. Antioxidant activity was determined by DPPH and FRAP test. Assessment of anti-inflammatory activity was performed by test of denaturation of bovine serum albumin protein (BSA) and denaturation of egg albumin protein (EA). The evaluation of the antibacterial activity of the extract was carried out by the agar diffusion and micro dilution method. The antibacterial effect was assessed by the MBC/MIC ratio. The phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, phenolic compounds, triterpenes, sterols and cardiotonic heterosides. The extract has shown an important antioxidant and anti-free radical activity concomitantly with its phytochemical makeup. The anti-inflammatory test, meanwhile, showed the ability of the extract to inhibit protein denaturation. Furthermore, antibacterial test revealed that the supernatant has activity on *Staphylococcus aureus* ATCC 29213 (MIC=3.125mg/mL). The result showed that the seeds of *Garcinia kola* have antioxidant, anti-inflammatory and antibacterial activities, which would justify phytodrug research.

Keywords: *Garcinia kola*, Supernatant, Antioxidant, Anti-inflammatory, Antibacterial

1. Introduction

In our developing countries, traditional medicine have an important place in the treatment of various pathologies [1].

Despite advances in modern medicine, some of people in developing countries can not access to necessary and sufficient health care because of their poverty [2].

In Africa and Asia, eighty percent of the population continue to use traditional medicine for primary health care. In developed nations, people are showing renewed interest in traditional, complementary and alternative medicine [1].

If some think that the traditional pharmacopoeia appears as an alternative to modern medicine, others believe that a combination of the two medicines must be promoted [3]. In order to be able to overcome scourges such as resistance to

antibacterials. Indeed, the use of antibiotics to treat infections presents in recent years some problems related to resistance developed with the proliferation of multidrug-resistant strains such as Beta-Lactamase with Expanded Spectrum and *Staphylococcus aureus* Resistant to Meticillin.

In addition, the scientific society, biologist and chemists, highlights the tragic role of the uncontrollable oxidative process induced by reactive oxygen species. Free radicals are one of the major public health problems. The current Coronavirus-19 pandemic shows the importance of exogenous antioxidants in strengthening the immune system [4]. Indeed, people with a strengthened immune system are more resistant to infection than people with a weak immune system whether related to age or other pathologies.

In Togo, several studies on medicinal plants have been

conducted in this direction [5, 6] and specially at this current Coronavirus-19 pandemic. *Garcinia kola* is one of them. However, very few studies have focused on this species, which is nevertheless used everywhere, particularly in Africa, Asia and South America [7]. And the sale of *Garcinia kola* seeds by hawkers in public places, traffic lights and markets at Lomé is a matter of course. It is therefore necessary to value this species used in traditional medicine for its therapeutic virtues specially in sore throat. The aim of this study is the valorization of *Garcinia kola* seeds through the evaluation of its antioxidant, anti-inflammatory and antibacterial activities.

2. Materials and Methods

2.1. Type and Study Framework

Our study is experimental. It took place from June 2019 to July 2020. The work was carried out in the chemistry and microbiology laboratories of the Faculty of Health Sciences and in the animal physiology laboratory of the Faculty of Sciences of the University of Lomé.

2.2. Plant Material

The fresh seeds of *Garcinia kola* were purchased at the Atikpodji market at Lomé. Plant material was identified by one of the authors and confirmed by a botanist, Koffi Akpagana (Botany Department, University of Lomé). A Voucher specimen was deposited in the department Herbarium Center under the number 15696.

After washing the seeds were dried under air conditioning at 20°C in the Chemistry Laboratory of the Faculty of Health Sciences, then reduced to powder. A relatively fine powder with a beige color was obtained. The extractions were carried out with this powder.

2.3. Chemicals and Reagents

Ethanol, distilled water were used for sample extraction. Analytical standards were gently provided by the laboratories.

Quercetin, gallic acid, DPPH (2,2'-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu, ascorbic acid, trichloroacetic acid have been used for antioxidant assays.

Sterile anti-inflammatory tablets were powdered: aspirin 500mg (ASPIRIN WINTHROP®), diclofenac 50mg (DICLO-DENK®) and ketoprofen 100mg (KETOPROFENE UBI®) purchased in a pharmacy were used as controls for anti-inflammatory assays.

Sterile antibiotic powders (chloramphenicol 250mg, tetracycline 250mg, amoxicillin 500mg) were used as controls for the antibacterial assays.

The 0.5 Mc Farland solution was used as a comparator to prepare bacterial suspensions.

2.4. Preparation of Extracts

The powder obtained (1000g) was macerated in 10 liters of ethanol-water mixture (80/20) for 72 hours at room

temperature of 20°C. The hydro-ethanolic extracts of the *Garcinia kola* seeds were obtained by maceration. It is an operation that consists in leaving the powder of the plant material in prolonged contact with the solvent to extract the active ingredients. At the maceration of the powder with the solvent ethanol-water (80/20) it was noticed a separation of two phases that we called supernatant (superficial part) and pellet (part below constituting a deposit). The supernatant was filtered on filter paper and the pellet was pressed and dried under air conditioning. The filtrate was evaporated under vacuum at a temperature of 45°C with Rotavapor R210.

The supernatant fraction was weighed and used for chemical and biological assays. Its yield was calculated by the formula:

$$R = (\text{mass of supernatant} / \text{mass of powder}) \times 100.$$

The yield of our extraction is 19.2% and the supernatant fraction has a brown color and an amorphous appearance.

The extracts were recovered and stored at 4°C in the refrigerator in labeled glass vials.

The pellet fraction was discontinued after phytochemical tests.

2.5. Phytochemical Tests

It consisted in the search for major chemical groups such as: flavonoids, alkaloids, phenolic compounds, total tannins, condensed tannins, saponosides, sterols, triterpenes, cardiogenic heterosides and anthracenic derivatives. The Sukhdev *et al.* method was used [8].

2.6. Antioxidants Assays

2.6.1. DPPH Test

The method used to measure this activity was that described by Popovici *et al.*, with some modifications [9]. A calibration curve was first established using Quercetin as the reference substance. A volume of 2mL of DPPH (19.716mg per 500mL of methanol) was mixed with 100µL of Quercetin (concentrations ranging from 5 to 30mg/L). The absorbance was read after 30 minutes away from light at 517nm using a spectrophotometer. To assess the anti-radical activity of the *Garcinia kola* supernatant, 100µL of sample of increasing concentrations (50 to 800µg/mL), are added to 2mL of the DPPH solution. The absorbance was read after 30 minutes in the dark at 415nm using a spectrophotometer.

2.6.2. FRAP Test

The method of Dieng *et al.*, with some modifications was used [10]. The ferric reducing antioxidant power (FRAP) consisted of mixing 1mL of the *G. kola* supernatant at different concentrations with 2.5 mL of phosphate buffer (0.2M, pH=6.6). Then 2.5mL of a solution of potassium ferricyanide (at 1%) were added. The mixture obtained was incubated at 50°C for 20 minutes and then 2.5mL of trichloroacetic acid was added to stop the reaction. The 3000mg mixture was centrifuged for 10 minutes. To 2.5mL

of *G. kola* supernatant collected are added 2.5mL of distilled water and 0.5mL of iron chloride (FeCl_3) at 1%. The absorbance was read by the UV spectrophotometer at 700nm.

The increase in absorbance indicates an increase in the reducing power of the supernatant tested. The activity of *G. kola* supernatant was compared to ascorbic acid activity.

2.7. Anti-inflammatory Assay

2.7.1. BSA Test

The inhibition of denaturation of bovine serum albumin protein (BSA) of *G. kola* supernatant has been studied according to the method of Saleem et al., [11].

To 0.45mL of bovine serum albumin (BSA) solution (5% w/v) was added 0.05mL of extract or reference drug (Aspirin, Diclofenac and Ketoprofen) at different concentrations (200, 150, 100, 50, 25 and 0 $\mu\text{g/mL}$). After 20 minutes of incubation at 37°C, was then incubated at 70°C for 5 minutes. After cooling 2.5mL of PBS (pH 6.3) were added to each sample. The test was performed in three replicates for each concentration and the percentage of inhibition of protein denaturation that reflects anti-inflammatory activity was determined after reading the optical density (DO) at 660nm.

The percentage of inhibition was calculated using the following formula:

$$\text{Anti-inflammatory activity (\%)} = [(A_0 - A_t) / A_0] \times 100$$

Where A_0 was the absorbance of the control (without extract) and A_t the absorbance in the presence of the *G. kola* supernatant or reference drugs.

2.7.2. EA Test

The inhibition of denaturation of egg albumin protein (EA) was performed according to the previous Saleem method [11]. The reaction mixture (5mL) consisted of 0.2mL of EA (from fresh chicken eggs), 2.8mL of PBS (pH 6.4) and 2mL of the extract of reference drugs (Aspirin, Diclofenac and Ketoprofen) at different concentrations (200, 150, 100, 50, 25 and 0 $\mu\text{g/mL}$). The control and test samples were incubated at 37°C for 25 minutes followed by heating to 70°C for 5 minutes. After cooling to 37°C, the absorbance of the test and control samples was determined at 660nm. The percentage of inhibition of protein denaturation was calculated by the formula:

$$\text{Anti-inflammatory activity (\%)} = [(A_0 - A_t) / A_0] \times 100$$

Where A_0 was the absorbance of the control (without extract) and A_t the absorbance in the presence of the *G. kola* supernatant or reference drugs.

2.8. Antibacterial Assay

2.8.1. Biological Materials for Antibacterial Test

In order to perform the antibacterial test, we used Petri dishes, MH agar, the nutritious broth, Glycerin and an oven from the microbiology laboratory for incubations, the autoclave, the use of a bunsen spout connected to a gas cylinder allowed us to carry out the manipulations in a sterile

atmosphere and to sterilize the handles, a graduated ruler was used to measure inhibition zones, microplates have been used in the determination of Minimum Inhibitory Concentration (MIC). An oven was used to make the incubations at a constant temperature.

2.8.2. Test Organisms

The organisms used for the test were *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 29213 and *E. coli* hospital strain. A total of one hospital strain and three references wild strains were used for this experiment. The hospital strain was isolated from urine samples analyzed at the Bacteriology Laboratory of the CHU Campus of Lomé. The strains were stored in sterile glycerin at 4°C prior to the study.

2.8.3. Culture Media

Nutrient agar and nutrient broth were used for the investigation.

2.8.4. Preparation of Inocula

The inoculum size of all bacterial tested was standardized by the use of overnight broth cultures prepared by inoculating 3 loopfuls of well- isolated colonies of test bacteria in 10mL of nutrient broth which was incubated at 37°C for 24 hours. A loopfuls of the overnight broth culture was diluted in 4mL of sterile physiological saline (0.8% w/v), such that its turbidity marched with that of 0.5 Mc Farland standard (a Barium sulphate standard) considered to have a mean bacterial density of 1 to 2.108 CFU/mL. This was gauged by comparing the turbidity of the test suspension with the turbidity 1% (w/v) Barium sulphate solution against the background of a printed white paper [12].

2.8.5. Solubilization of Extract

The supernatant of *G. kola* was dissolved in sterile physiological water. The stock solution of 100mg/mL was prepared.

2.8.6. Control Antibiotics

We used as control antibiotics amoxicillin (AMITRON® capsule), tetracycline (TETRACYCLINE CREAT® capsule) and chloramphenicol (CHLORAMPHENICOL GGIA®) purchased in pharmacy. A stock solution of 100 mg/mL is prepared for each control antibiotic. The stock solutions were prepared by letting the antibiotic powder contained in the capsule dissolve in sterile physiological water. For amoxicillin 500mg, the contents of one capsule were dissolved in 5mL of physiological water while for tetracycline 250mg and chloramphenicol 250mg, two capsules were dissolved in the same amount of physiological water.

2.8.7. Determination of Inhibition Zones

The method of diffusion in agar medium made it possible to determine the inhibition zones [13]. It provides access to essentially qualitative results. To perform the test, the MH agar surface (poured into 90mm diameter Petri dishes) was seeded with 100 μL of each bacterial suspension and then spread over the entire agar using a handle. Then micro-wells

were dug within the agar. In each well was introduced 50µL of antibiotic solution or *G. kola* supernatant.

The boxes are then incubated at 37°C for 18 to 24 hours and the inhibition zones have been determined. Indeed, a clear area or halo has appeared around a microwell if the antibiotic or *G. kola* supernatant inhibits microbial development.

2.8.8. Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined as the lowest concentration of various extracts that inhibit the growth of the tube isolates. The bacteriological peptone was poured into test tubes in appropriate volumes. The tubes were thoroughly mixed and incubated at 37°C for 24 hours, after which they were examined for visible turbidity. Tubes which showed turbidity were tubes which had microbial growth, while those which were undisturbed (no turbidity) did not have microbial growth. The MIC was reported as the lowest concentration that prevented visible growth [12].

2.8.9. Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by sub-culturing all the tubes in each set in which no visible turbidity was observed during the test for MIC. A loop full of the contents of the tubes showing no microbial growth were sub-cultured by streaking over the surface of already set nutrient agar plates without extracts. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration which no growth was observed after sub-culturing. All plates showing no growth on the nutrient agar indicated bactericidal effect of the extract concentration [12].

2.9. Processing of Results

All the results obtained were processed by Excel software and Graph Pad Prism 8 software (San Diego, California, USA) from a Spline analysis.

3. Results

3.1. Phytochemical Screening of *Garcinia Kola* Seeds Extracts

Table 1. Phytochemical composition of *Garcinia kola* seeds extracts.

	Supernatant	Pellet	Powder
Flavonoids	+	-	+
Alkaloids	+	-	+
Condensed tannins	+	-	+
Total tannins	+	-	+
Saponosides	-	-	-
Anthracenes	-	-	-
Phenolics compounds	+	-	+
Sterols	+	+	+
Triterpenes	+	-	+
Cardiotonic heterosides	+	-	+

presence=+; absence=—

The phytochemical compositions of supernatant revealed

the presence of flavonoids, alkaloids, tannins, phenolics compounds, sterols, triterpenes, cardiotonic heterosides while saponosides and anthracenes were absent. The pellet revealed only the presence of sterols, what justifies the aborting tests (Table 1).

3.2. Antioxidant Tests Results

3.2.1. DPPH

The inhibition of the *G. kola* supernatant was low compared to that of Quercetin used as a reference (Figure 1).

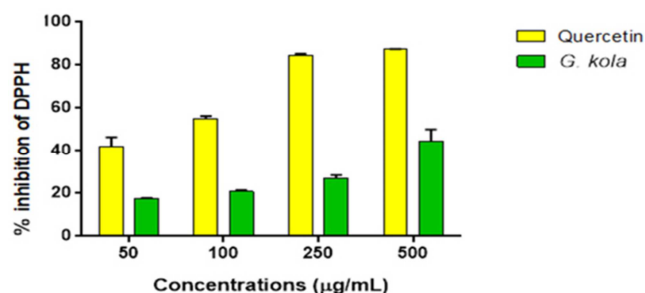


Figure 1. Percentage of inhibition of *G. kola* supernatant compared to Quercetin.

3.2.2. FRAP

The reducing power of iron Fe^{3+} to Fe^{2+} induced by the supernatant of *G. kola* was significant compared to reference (Figure 2).

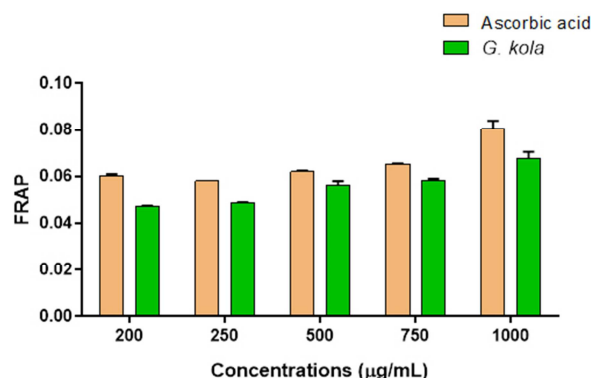


Figure 2. Reducing power of *G. kola* supernatant compared to ascorbic acid.

3.3. Anti-inflammatory Test Results

The *G. kola* supernatant exhibits anti-inflammatory activity but low compared to references (Table 2).

Table 2. Inhibition of denaturation of egg albumin (EA) and bovine serum albumin (BSA).

Substances	IC ₅₀ (µg/mL)	
	EA	BSA
<i>Garcinia kola</i>	460,35±1,11	502,37±0,88
Ketoprofen	98,83±4,59	97,83±1,28
Aspirin	106,98±4,73	116,98±2,37
Diclofenac	64,18±1,57	58,08±1,23

3.4. Antibacterial Test

3.4.1. Sensitivity Test

The *G. kola* supernatant caused a 26 mm inhibition zone

on the *S. aureus*. As for antibiotics they showed inhibition zones for all germs except amoxicillin for which we did not note inhibition on *E. coli* hospital strain (Table 3).

Table 3. *G. kola* supernatant and antibiotics inhibition zones (in millimeter).

	<i>E. coli</i> ATCC 25922	<i>E. coli</i> hospital strain	<i>K. pneumoniae</i> ATCC 13883	<i>S. aureus</i> ATCC 29213
Supernatant of <i>G. kola</i>	-	-	-	26
Amoxicillin	30	-	18	35
Chloramphenicol	36	34	30	32
Tetracycline	36	26	36	40

—: no zone of inhibition

3.4.2. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)

The antibacterial activity of supernatant of *G. kola* showed that the MIC was 3.12 mg/mL and the MBC was 12.5 mg/mL for *S. aureus*, very significant and our extract has a bacteriostatic effect (Table 4).

Table 4. MIC and MBC of *G. kola* supernatant on bacterial strains.

	<i>E. coli</i> ATCC 25922	<i>E. coli</i> Hospital	<i>K. pneumoniae</i> ATCC 13883	<i>S. aureus</i> ATCC 29213
MIC	6.25	12.5	6.25	3.12
MBC	>25	50	≥25	12.5
MBC/MIC	4	4	4	4

4. Discussion

The ethanol-water binary (80/20) promoted the extraction at the high yield of 19.2% of *G. kola* seeds. Our results were similar with those found by Yété et al., in Bénin [14].

The phytochemicals obtained were similar to Yété et al., who reported the presence of flavonoids, alkaloids, condensed tannins, total tannins, phenolic compounds, tri terpene, sterols and cardiotonic heterosides in *G. kola* seeds. The results were also similar to Adesuyi et al., who reported the presence of cardiotonic heterosides [15].

We reported the absence of saponosides and anthracenes. Our results contrasts with Adeyusi et al. and Yété et al. who reported a high percentage of saponins but they are similar with those reported by Diallo et al., in Togo [16]. This would be due to the type of extraction done and the geographical distribution of *G. kola*.

G. kola supernatant clearly demonstrated antioxidant activity against DPPH radicals with an IC₅₀ of 726.99 µg/mL. These results are similar to those reported by Yété et al., who found a value of 870 µg/mL. On the other hand these results contrasts with those found by Ouéméla et al., [17] who reported an IC₅₀ of 65.86±1.17 µg/mL with aqueous and hydroalcoholic extraction carried out in Côte d'Ivoire. In addition, the estimation of the reducing power of ferric ions (FRAP) revealed a reducing effect of *G. kola*. These results are similar to those found by Ouéméla et al. Polyphenols including flavonoids would have a very important role in the chelation of transition metals involved in the Fenton reaction.

Substances that can prevent protein denaturation would be considered potential for the development of anti-inflammatory drugs. In our study, *G. kola* supernatant seeds prevented denaturation of bovine serum albumin and egg albumin opposite to heat. These results lead to the conclusion

that *G. kola* would be able to slow down the production of autoantigens, which would reduce protein denaturation and thus relieve organ damage in inflammatory processes [18]. These results are similar to those reported by Olaleye et al., [19] which showed strong anti-inflammatory power compared to the standard reference drug.

The assessment of antibacterial activity is usually carried out by determining the minimum inhibitory concentrations (MIC) of the extract. However, an initial sensitivity test was performed beforehand. This test made it possible to identify the sensitivity of the extract on the different bacteria used for this study. As reported in the literature, an extract is active when it induces an inhibition zone greater than or equal 10mm [20]. Thus, in view of the sensitivity test, the supernatant was found to be active on *S. aureus* ATCC (26mm) but not active on *K. pneumoniae*. These results on *K. pneumoniae* contrast to those reported by Akoachere et al., and Chomini et al [21, 22]. The difference would be due to the extraction method.

Traditionally, the seeds are used in the treatment of respiratory infections. The inhibition of the growth of *S. aureus* seems to justify, at least partially, these traditional uses.

The standard antibiotics used (amoxicillin, chloramphenicol, tetracycline) were more active than the extract. They trained inhibition zones between 18 mm and 40 mm. These results could be explained by the fact the extract is crude drug whereas standard antibiotics are pure molecules.

The MIC of the *G. kola* supernatant is 3.125mg/mL on *S. aureus*. These results contrast those found by Akoachere et al., 2002 [21] who had noted an MIC between (1 µg/mL and 1.8 µg/mL) on *S. aureus*. Also those reported by Morabandza et al., who found an MIC of 9mg/mL [23]. This difference could be explained by the geographical distribution of the

species or solvents used for extraction.

Thus, *G. kola* extract exhibits activity against free radicals and their damage. Antioxidant and anti-inflammatory activities of *G. kola* have a relationship with the antibacterial power and suggest its probable antimicrobial potential.

5. Conclusion

This study is a contribution to the valorization of *Garcinia kola* seeds through the evaluation of the antioxidant, anti-inflammatory and antimicrobial activities. The results first showed through phytochemical screening that the hydro-ethanolic extract (20/80) of *Garcinia kola* seeds contains secondary metabolites that justify antioxidant, anti-inflammatory and antimicrobial properties. The supernatant showed its antimicrobial potential against *S. aureus*.

The observed inhibitory effect of the seeds extract of *G. kola* explained their utilization in traditional medicine. *Garcinia kola* would be a good alternative source of drugs for the treatment of throat infections. Moreover, it will be necessary to investigate the identification of bioactive compounds and toxicity assay to promote phytodrugs.

Conflicting Interest

All the authors do not have any possible conflicts of interest.

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