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Antioxidant activity and efficacy of *Garcinia kola* (bitter kola) oil on pathogenic and alteration microorganisms of attiéké

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ABSTRACT

Attiéké, the most widely consumed cassava product in Côte d'Ivoire, has a short shelf life. This is largely due to microbial contamination. The aim of this study was therefore to assess the antioxidant activity and effectiveness of *Garcinia kola* seed oil against pathogenic and spoilage strains of attiéké. This was possible through an extraction of Garcinia kola oil by maceration in hexane. The antimicrobial activity of the oil was assessed using an agar diffusion method. The antioxidant activity of *Garcinia kola* oil was assessed using the DiPhenyl Picryl Hydrazyl (DPPH) free radical scavenging test and the Ferric Reducing Antioxidant Power (FRAP) test. *Garcinia kola* oil showed significant antioxidant potential. A high percentage of DPPH radical inhibition was observed, with an IC50 of 2.57 mg/mL. Iron-reducing power was highest when the oil was used at a concentration of 100 %. *Garcinia kola* oil was able to inhibit the bacterial growth of *Staphylococcus aureus*, *Escherichia coli* at a concentration of 100, 50 and 25 % respectively from 22 ± 0.05 to 16 ± 0.00 mm and 20 ± 0.05 to 14 ± 0.08 mm and *Salmonella typhimurium* (12 ± 00 mm) at a concentration of 100 %. *Candida albicans* (20 ± 0.07 to 18 ± 0.01 mm), *Aspergillus flavus* (28 ± 1.41 to 16 ± 0.00 mm) and *Aspergillus niger* (21 ± 1.01 to 15 ± 0.02) were inhibited at concentrations ranging from 100 to 12.5 %. *Bacillus cereus*, on the other hand, was resistant to *Garcinia kola* oil. Based on the findings of this study, *Garcinia kola* seed oil could be used to extend the shelf life of attiéké.

1. Introduction

Attiéké is a food made from steamed cassava semolina with a slightly tart taste [1]. Consumed two to three times a day accompanied by meat, or fish and raw vegetables, it is the most consumed food in urban centers in Côte d'Ivoire. However, its conservation is a major concern for vendors and consumers. Indeed, the lifespan of attiéké sold in the city of Abidjan is only 3 days at room temperature (25 °C ± 3). From this time on, undesirable colourings appear [2]. In addition, studies show that after steaming, when attieke sold for consumption is poorly conserved (packaging or protection) it can be re-contaminated. Indeed, the presence of resistant spore-forming germs of Bacillus can be observed when attiéké is not well cooked [2]. A high amount of *Staphylococcus aureus* has been found in attiéké [3]. The unsatisfactory microbiological quality of attiéké in Côte d'Ivoire has been demonstrated by Ref. [4] who

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assessed the presence of *Staphylococcus aureus*, *Escherichia coli* and Salmonella spp. As demonstrated in a study on spoilage moulds and aflatoxin detection [5], the main spoilage fungi of attiéké are mainly *Aspergillus niger and Aspergilus flavus* and *Candida albicans*.

Contamination by pathogens and spoilage microorganisms result in food loss, reduced economic value and health risk to consumers [6,7]. Pathogens also damage food and cause food-borne illnesses [8]. Several methods have been used to inhibit microbial growth. However, most of the techniques involved are heat treatments, which can damage the natural flavour and aroma of foods [6,9]. In addition, heat treatment is frequently used for inactive microorganisms and might not be sufficient for complete degradation of mycotoxins [7]. Therefore, the development of non-thermal decontamination technologies is necessary to improve food safety [7].

In the aim of inhibiting microbial proliferation, researchers are exploring various new natural plant-based ingredients, such as the by-products of fruit and vegetable processing, namely peel, pomace, seeds and trimmings. Indeed, natural plant-based products are rich in bioactive compounds, phytochemicals, antioxidants and nutrients and are readily available and affordable [10,11]. Forest trees and shrubs are primary sources of secondary metabolites, which include several bioactive chemical compounds, such as phenolic compounds, glycosides, lignans, flavonoids, saponins, alkaloids, essential oils, fixed oils, fatty acids, organic acids, and others [12]. Among them, the literature reports that fixed oils are known to have excellent antimicrobial properties. Similarly, fixed oils contain a significant amount of antioxidants, which play an important role in the human defence system and overcome oxidative stress caused by reactive oxygen species [13]. For example, Quercus infectoria seeds are used for their free radical scavenging activity [14]. The fixed oil from the stem bark of *Tamarix aphylla* has been shown to have a chemical composition rich in bioactive secondary metabolites, giving it antimicrobial and antioxidant properties [13].

Garcinia kola seeds contain a wide range of useful phytochemicals, including high levels of tannins and flavonoids, proteins, lipids, carbohydrates [15,16]. In addition to flavonoids, *Garcinia kola* seeds contain biflavonoids (GB-1, GB-1a and GB-2, garcinal acid and garcinoic acid) and amentoflavonones (presence of hydroxybiflavonols in the ethyl acetate fraction). Many researchers have reported that the bioactivity of *Garcinia kola* seeds is linked to the presence of bioflavonoids, which are well-known antioxidants [16,17]. These elements explain the antifungal, antibacterial, antiviral and antioxidant properties of *Garcinia kola* seed extracts [16]. The growth of Streptococcus sp, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus* could be inhibited with the extract of *Garcinia kola* seeds [18]. Studies have also demonstrated that the aqueous extract of *Garcinia kola* seeds produced in Nigeria has a significant inhibitory action against bacterial and fungal isolates [19]. Similarly, Aqueous extract of *Garcinia kola* inhibited the DPPH radical and hydrogen peroxide with IC50 values of 1.02 ± 0.2, $3.2.10^{-2} \pm 0.12$ and 1.01 ± 0.5 mg/mg respectively [20].

In Côte d'Ivoire, few studies have been conducted on *Garcinia kola* extracts. For example, in 2018, the effect of the aphrodisiac plant *Garcinia kola* was evaluated on zootechnical performance and sexual inversion rate in *Oreochromis niloticus* by Ref. [21]. [22] studied the in vitro antioxidant activities of the aqueous extract and alcoholic extract of *Garcinia kola* seeds collected in Abidjan (Côte d'Ivoire). Recently in 2022, a study was conducted on the evaluation of the haemostatic and antihaemolytic effects of the aqueous extract of fresh *Garcinia kola* seeds by Ref. [23]. However, no studies have been carried out on *Garcinia kola* oil. To this end, this work aims to evaluate the antioxidant activity and effectiveness of *Garcinia kola* seed oil on pathogenic strains and alterations of attiéké for use in the protective packaging design of attiéké produced in Côte d'Ivoire.

2. Material and methods

2.1. Material

2.1.1. Plant material

The *Garcinia kola* seeds were collected at the market of Anyama in Abidjan (Côte d'Ivoire) and then packaged in a cooler and transported to the Laboratory of Biochemistry and Technicology of Tropical Products of Université Nangui Abrogoua in Abidjan.

2.1.2. Microbial material

Reference pathogenic strains selected according to the prescriptions of the French Society of Microbiology (2009), and pathogenic strains isolated from local foods in the laboratories of Institut Pasteur of Cote d'Ivoire, were the subject of this study. The bacterial strains include Gram-negative bacilli (*Escherichia coli* and *Salmonella typhimurium*), a Gram-positive coccus (*Staphylococcus aureus*), Gram-positive bacilli (*Bacillus cereus*), a yeast (*Candida albicans*) and moulds (*Aspergillus flavus* and *Aspergillus niger*). These strains were chosen for their high frequency in food contamination and their ability to cause health problems.

2.2. Methods

2.2.1. Oil extraction from the Garcinia kola seed

The oil contained in the *Garcinia kola* (bitter kola) seeds was extracted by maceration in hexane at room temperature (25 °C) according to the method described by Ref. [24]. In this process, *Garcinia kola* seeds were ground using a blender (Silver crest blender) and then dried at 50 °C for 72 h in an oven (Biobase model BOV-T105F). Pulverised seeds (1500 g) were placed in a sealed container with 1000 mL of n-hexane and allowed to stand at room temperature (25 ± 3 °C) for a period of 3 days with momentary agitation until the soluble material was fully dissolved. The mixture was then filtered, the pomace (the wet solid) was pressed and the oil extract was removed by mechanical filtration using a 100 μm sieve (haver & boecker d-59302 oelde made in Germany) after a rest period. The oil is recovered from the mixture (oil and solvent) using a rotary evaporator (Heidolph, Hei-VAP Ultimate - G1, Germany) at 40 °C. The oil was collected in a smoked glass bottle, labelled, and placed in a refrigerator (4 °C) for analysis.

2.2.2. Yield of Garcinia kola oil

The extraction yield was determined according to the method described by Ref. [25]. The yield was obtained by relating the amount of oil extracted to the dry mass of *Garcinia kola* seeds. The yield is expressed as a percentage according to the following equation (1):

$$R = (MH \ / \ MS) \times 100 \quad (1)$$

where R: the yield of the oil extracted from *Garcinia kola* seeds; MH: the mass of the oil extracted from *Garcinia kola* seeds; MS: the dry mass of *Garcinia kola* seeds.

2.2.3. Relative density of Garcinia kola oil

The procedure for determining the relative density (d) was done according to the method of [26]. A density bottle was used to determine the relative density of *Garcinia kola* oil. A clean, dry bottle with a capacity of 25 mL was weighed (m0) then filled with the *Garcinia kola* oil, cap inserted and weighed again to give (m1) at 20 °C. The oil was replaced with distilled water after the bottle was washed and dried and weighed at a temperature of 20 °C to give (m2). The relative density was calculated by applying equation (2):

$$d = (m1 - m0 \ / \ m2 - m0) \times 100 \quad (2)$$

Where m0 = Weight of container (g), m1 = Weight of container and oil (g) and m2 = Weight of container and water (g).

2.2.4. Antioxidant activity of Garcinia kola oil

2.2.4.1. Determination of phytochemicals in Garcinia kola oil. The phytochemicals were extracted with methanol according to the method of [27].

One (1) mL of the oil was mixed with 10 mL of 80 % methanol and placed in a centrifuge tube. The mixture was then homogenised and centrifuged at 42,000 rpm for 5 min using a SIGMA 3–16 P centrifuge, Spain. After centrifugation, the supernatant of the mixture was collected and stored in a 50 mL Erlenmeyer flask. The volume was adjusted to 20 mL with distilled water. The resulting solution was the methanolic extract.

a. Determination of polyphenol content

The total polyphenol content of *Garcinia kola* oil was determined using the Folin-Ciocalteu reagent method [27]. A volume of 500 μL of the methanolic extract was mixed with 300 μL of Folin-Ciocalteu reagent (1/10). The mixture was thoroughly homogenised by manual shaking. After 3 min, a volume of 500 μL of 20 % aqueous sodium carbonate solution was added and the final volume of the mixture was adjusted to 10 mL with distilled water. The mixture was placed in the dark for 30 min. The Optical Density (OD) reading was taken at 765 nm using a spectrophotometer of the brand (MS-A 5100, Spain, Europe) against a blank not containing *Garcinia kola* oil. A calibration curve was established with a range of concentrations of gallic acid solution (1 mL/mL).

b. Determination of flavonoid content

The method for the determination of flavonoid content was developed as described by Ref. [28]. A 0.5 mL sample of the methanolic extract was introduced into a test tube. To the contents of the tube, were successively added 0.5 mL distilled water, 0.5 aluminium chloride (10 % w/v), 0.5 mL potassium acetate (1 M) and 2 mL distilled water. The tube was left to stand for 30 min in the dark and the optical density (OD) was read at 415 nm using a spectrophotometer (MS-A 5100, Spain, Europe) against a blank not containing *Garcinia kola* oil. The flavonoid content of the samples was determined using a standard range established from a stock solution of quercitrin (0.1 mg/mL).

c. Free radical scavenging activity DPPH (1,1-diphenyl-2- picrylhydrazyl)

The ability of *Garcinia kola* oil to scavenge free radicals was assessed using the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) spectrophotometric method [29] with slight modifications.

A 0.4 mM DPPH methanolic solution (1 mL) was added to a methanolic solution of the oil sample (2.5 mL) at different concentrations (1.56; 3.1 2; 6.25; 12.5; 25; 50 and 100 mg/mL) and allowed to stand for 30 min at 30 °C in the dark. Different concentrations of vitamin C standard solutions (0.156; 0.312; 0.625; 1.25; 2.5; 5 and 10 mg/mL) were prepared and treated in the same way as the sample. The absorbance was measured at 517 nm with a Spectrometer (MS-A 5100, Spain, Europe) using the methanolic solution of DPPH as control. The antiradical activity of the oil corresponding to the percentage of inhibition (PI) of the DPPH radical is calculated according to the following equation (3):

$$PI = (A0 - A \ / \ A0) \times 100 \quad (3)$$

Where A0: the absorbance of the negative control; this contains DPPH and methanol without extract; A: the absorbance of the oil, This test was performed in three trials for each concentration.

The IC_{50} value representing the concentration of the sample that inhibits 50 % of the DPPH free radical was extrapolated from the

standard calibration curve.

d. Essai FRAP (Ferric-ion reducing antioxidant power)

The FRAP method is based on the reduction of ferric ion (Fe3+) to ferrous ion (Fe2+). It was determined by the method described by Thaipong *et al* [29]. Different concentrations (12.5; 25; 50 and 100 mg/mL) of *Garcinia kola* oil were mixed with 2500 μL of FRAP reagent and incubated in the dark for 30 min. The FRAP solution was prepared by mixing 25 mL of acetate buffer pH 3.6 (3.1 g of C2H3NaO2·3H2O and 16 mL of C2H4O2), 2.5 mL of TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution (10 mM TPTZ solution in 40 mM HCl), and 2.5 mL of FeCl3·6H2O solution (20 mM), and then warmed to 37 °C prior to use. The absorbance of the coloured product (tri-pyridyltriazine iron complex) was read at 593 nm.

The antioxidant capacity based on the ability of *Garcinia kola* oil to reduce iron ions was calculated from the linear standard curve (25 and 800 μM Trolox) and expressed in Trolox equivalents (in micromolar).

2.2.5. Antimicrobial activity of Garcinia kola oil

The reference strains were grown in nutrient broth for bacteria and Chloramphenicol sabouraud broth for yeasts and moulds respectively at 37 °C and 30 °C for 24 h.

Antibacterial and antifungal activities were evaluated by the agar diffusion method [30]. Mueller-Hinton (MH) agar was inoculated by flooding with 2 mL of the inoculum previously prepared for antimicrobial activity evaluation. For the evaluation of antifungal activity, Sabouraud Chloramphenicol Agar was inoculated. The seeded petri dishes were dried in a laminar flow hood for 15 min. Then wells were carefully dug in the shape of a disc using sterile Pasteur pipettes. The excavated wells were filled with 80 μL of *Garcinia kola* oil diluted to different concentrations (100 %, 50 %, 25 %, 12.5 %, 6.5 %) in acetone (v/v) for each strain. One well contained 80 μL of acetone and was used as a negative control. The Petri dishes were kept at 4 °C for 1 h (to allow the diffusion of the active substances contained in the oil without the microorganisms starting their growth). They were then incubated at 37 °C for bacteria and 30 °C for yeasts and moulds for 18–24 h.

Finally, the antibacterial and antifungal activities were evaluated by measuring the diameter of the oil-induced inhibition zone with a caliper. Each test was performed 3 times.

2.2.6. Statistical analysis

Analyses were performed by trials of 3. The values are means ± standard deviation. The results of the analyses were subjected to an analysis of variance (ANOVA) at a significance level of 0.05 using STASTISTICA 7.1 software. In case of significant differences in the samples, Tukey's Test was used to determine which samples differed from each other.

3. Results

3.1. Physical and chemical properties of Garcinia kola oil

Table 1 shows the yield and relative density of *Garcinia kola* oil. The yield and relative density of *Garcinia kola* oil extracted from the seed are 7.13 % w/v and 0.93 ± 0.05 respectively.

3.2. Antioxidant activity

3.2.1. Phytochemicals of Garcinia kola oil

Table 2 shows the flavonoid and polyphenol contents of *Garcinia kola* oil. The flavonoid content was 21.97 ± 0.15 and the polyphenol content was 18.4 ± 0.6.

3.2.2. DPPH free radical scavenging activity

The percentage of DPPH free radical inhibition as a function of concentration of *Garcinia kola* oil is shown in Fig. 1. The oil scavenged DPPH radicals in a concentration-dependent manner. Indeed, the higher the concentration of the oil, the greater its ability to scavenge DPPH free radicals ($P < 0.05$) compared to the previous concentration. The ability of the oil to inhibit DPPH free radicals was reflected in its IC50 value, which was 2.57 mg/mL.

3.2.3. FRAP (ferric-ion reducing antioxidant power)

Fig. 2 shows the evaluation of the antioxidant activity of *Garcinia kola* seed oil by the FRAP method. The content of active iron reducing molecules in the oil increases with the concentration of *Garcinia kola* oil from 12.5 to 100 mg/mL. However, the 100 mg/mL

Table 1
Physical and chemical properties of *Garcinia kola* oil.

Sample	R (%)	d
***Garcinia kola* oil**	7.13	0.93 ± 0.05

R: Yield and d: Relative density.

Table 2
Polyphenol and flavonoid content of *Garcinia kola* oil.

Sample	Polyphenols (mg/g)	Flavonoid (mg/g)
***Garcinia kola* oil**	18.4 ± 0.6	21.97 ± 0.15

Data are expressed as the mean ± standard error of three replicates.

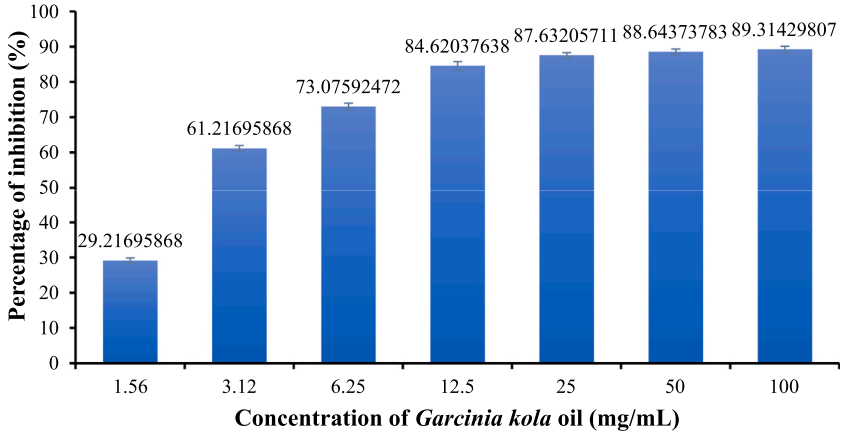

Fig. 1. Percentage of DPPH radical inhibition as a function of *Garcinia kola* oil concentration.

oil had a significantly ($P < 0.05$) higher percentage inhibition of iron ions than the other concentrations used.

3.3. Antibacterial and antifungal activity of Garcinia kola oil

The results of the antibacterial and antifungal analysis of *Garcinia kola* seed oil are presented in Table 3. The results showed that the oil had inhibitory activity against *Escherichia coli*, *Staphylococcus*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. However, *Bacillus cereus* was resistant to all concentrations of the oil used. The 25 % (V/V) oil was able to inhibit a broad spectrum (28 mm) of the growth of the fungal strain *Aspergillus flavus*.

4. Discussion

The oil content of *Garcinia kola* seeds is an important criterion for its future use. The yield of the oil obtained from the seed of *Garcinia kola* is 7.13 %. The yield of *Garcinia kola* oil obtained in this study is a little lower than that obtained by Ref. [31] in Nigeria which is 10 %. This quantitative difference may be due to various factors, in particular, the geographical region, the genotypic factor and the pedoclimatic conditions as well as the extraction method and time, the choice of solvent and the size of the extraction particles [32]. The determination of the relative density is one of the purity criteria of an oil. It is a function of the chemical composition of the oil and the temperature. The value of the density obtained in this study is of the order of 0.93. This value is consistent with that of pure vegetable oils whose relative density values are between 0.9 and 0.93 [33]. The relative density of vegetable oils is a parameter that provides information on the length of the carbon chains of the fatty substance, the presence of unsaturation and secondary functions.

Phytochemical tests revealed the presence of secondary metabolites such as polyphenols and flavonoids. These results show that the *Garcinia kola* seed oil contains a significant amount of these phytochemicals. Indeed, they are known to have very important

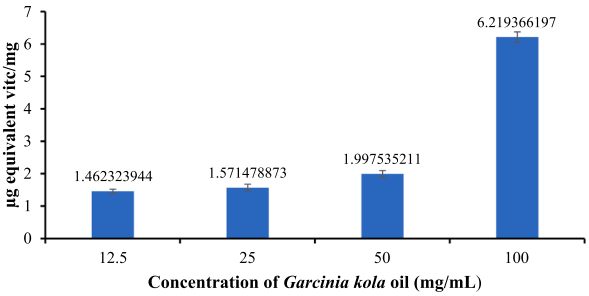

Fig. 2. Evaluation of the antioxidant activity of *Garcinia kola* oil by the FRAP method.

Table 3
Diameter of the inhibition zones (mm) of microorganisms by different concentrations of *Garcinia kola* oil.

Microorganisms	Oil concentration (%)				
	100	50	25	12.5	6.25
Escherichia coli	20 ± 0.05^{a}	18 ± 0.15^{b}	14 ± 0.08^{c}	R	R
Salmonella typhi	12 ± 00^{a}	7 ± 0.2^{b}	R	R	R
Bacillus cereus	8 ± 0.02^{a}	R	R	R	R
Staphylococcus aureus	22 ± 0.05^{a}	21 ± 1.09^{b}	16 ± 0.00^{c}	R	R
Candida albicans	19 ± 00^{ab}	20 ± 0.07^{a}	20 ± 0.02^{a}	18 ± 0.01^{b}	8 ± 00^{c}
Aspergillus flavus	20 ± 0.7^{b}	22 ± 1.41^{b}	28 ± 0.01^{a}	16 ± 00^{c}	R
Aspergillus niger	21 ± 1.01^{a}	20 ± 00^{a}	17 ± 0.07^{b}	15 ± 0.02^{c}	R

Data are expressed as the mean ± standard error of three replicates. R = resistant.

antioxidant and antimicrobial functions. Polyphenols and flavonoids are widespread metabolites in plants. They are present in almost all parts of plants at different concentrations [34]. A previous study showed the presence of polyphenols and flavonoids in *Garcinia kola* seeds [35].

The free radical scavenging activity of *Garcinia kola* seed oil may be due to its richness in phytochemicals such as flavonoids and polyphenol contents [36]. Indeed, these compounds are known to exert a significant antioxidant effect even in vivo [37]. This oxidative potential could give *Garcinia kola* oil the ability to prevent or fight against certain diseases and ageing. Indeed, according to a study [38], olive oil is of great interest in the prevention of cardiovascular diseases, cancers, diabetes, inflammation and ageing thanks to its antioxidant capacity. The ability of *Garcinia kola* oil to reduce iron could be due to the presence of phenolic compounds. Indeed, flavonoids play a very important role in the chelation of transition metals involved in the Fenton reaction (formation of hydroxyl radicals resulting from the reaction of iron with hydrogen peroxide) [39].

Regarding the antibacterial activity of *Garcinia kola* oil, *Staphylococcus aureus* and *E. coli* were sensitive to *Garcinia kola* oil with diameters of 22 mm and 20 mm respectively. These results could be explained by the mechanism of action of oils on the membrane wall of bacteria. In general, the effect of the oil is closely linked to the ability to permeabilize and/or disrupt membrane integrity, resulting in the leakage of intracellular material [40]. In addition, the antibacterial power could be due to its flavonoid content. *Bacillus cereus* was resistant to *Garcinia kola* seed oil from Côte d'Ivoire. This result is due to the fact that most strains of Bacillus produce β-lactamase and are therefore considered resistant to antimicrobial agents [41].

Regarding the antifungal activity of *Garcinia kola* seed oil, all tested fungal strains were extremely sensitive or very sensitive to the oil. These results can also be explained by the high flavonoid content in *Garcinia kola* seed oil from Côte d'Ivoire. Some flavonoids are characterised by the absence of a hydroxyl group on the B cycle. Flavonoids without hydroxyl groups on their B rings are more active against microbial membranes. This is should to the negative correlation between the relative hydrophobicity of flavonoids and the number of hydroxyl groups present on ring B. In addition, other authors suggest that lipophilic flavonoids that are highly hydroxylated can further disrupt the structure of fungal strains [42].

5. Conclusion

This study revealed that *Garcinia kola* oil has good antioxidant activity. This activity is dependent on the oil concentration. The antimicrobial activity of *Garcinia kola* oil was positive on bacterial strains (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*) and fungal strains (*Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*). The largest inhibition diameter was observed on the *Aspergillus flavus* strain (28 mm) at a concentration of 25 %. *Garcinia kola* seed oil could be used to extend the shelf life of attiéké. To this end, the oil could be incorporated into the starch matrix to produce an antimicrobial plastic wrapper that will enable attiéké to be packaged and stored for a long time.

Author contribution statement

Doh Amenan Aline: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper. Adjouman Yao Désiré;: conceived and designed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper. Nindjin Charlemagne: conceived and designed the experiments; analyzed and interpreted the data; wrote the paper. Kouamé Kohi Alfred: analyzed and interpreted the data. Gbezo Aka Solange: analyzed and interpreted the data; wrote the paper. Ouattara Kolo Boubacar: performed the experiments; wrote the paper. Amani N'Guessan Georges: contributed reagents, materials, analysis tools or data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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