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Effects of teguments on phytochemistry and antimicrobial activities of *Garcinia kola* seeds

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Abstract
Many studies have been done on *Garcinia kola* seeds. They have not indicated the importance of the coats on biological activities of these seeds. Thus, we studied the impact of the use of *Garcinia kola* seeds with its teguments. We studied the comparative phytochemical and antibacterial activities of *Garcinia kola* seeds with and without tegument. First, the qualitative analysis of ethanolic extract of peeled and unpeeled seeds revealed the presence of large families of molecules such as alkaloids, saponins, flavonoids and phenolic compounds consisting of catechic and gallic tannins in both extracts. Additional compounds namely anthocyanins and O-heterosides had been identified in unpeeled seeds extract. The difference noted during the quantitative analysis of the two extracts is not significant ($p > 0.05$). Then, antimicrobial tests had shown higher inhibition diameters with peeled seeds in 70% of the cases. The largest inhibition diameter was 35 mm obtained against *Proteus vulgaris*. The minimum inhibitory concentrations of the two extracts were identical in 83% of the cases. These concentrations varied from 0.625 mg/mL to 5 mg/mL with the two extracts. The minimum bactericidal concentrations of the two extracts were also identical in 75% of the cases. They varied from 1.25 mg/mL to 20 mg/mL. In the remaining 25% of cases, unpeeled seeds extract has more beneficial bactericidal activity than peeled seeds extract. In light of these results, it appears that the skin of *Garcinia kola* seeds qualitatively provides additional compounds. However, the presence of these additional compounds has no significant influence on the antimicrobial activity of seeds.

Keywords: Peeled seeds, unpeeled seeds, *Garcinia kola*, phytochemical composition, antibacterial activity

1. Introduction
Traditional medicine continues to make an important contribution to the health system in many developing countries. It is a source of primary health care for 80% of the world's population [1, 2]. Endogenous medical knowledge of plants is useful not only for the conservation of cultural traditions and biodiversity, but also for community health care and drug development [3]. In Africa, medicinal plants constitute a rich but still largely untapped reservoir of natural products. From roots to leaves, plants are used in powders, decoctions, macerations, alcohol, food porridges, etc. to cure various ailments [4]. *Garcinia kola* (G. kola) is a species of the Clusiaceae or Guttiferae family. It's an oleaginous species of the African forests, usually known as bitter kola for its seeds. Authors termed these seeds as "miracle drug"[5, 6]. The seeds of the plant have several activities. The literature has described their anti-inflammatory, antioxidant, antitussive, antidiabetic, antimicrobial activities, to name a few [7,8,9,10]. These applications require only pulp, while the hull is discarded. Yet, the envelope of the *Garcinia kola* seed is used as a substitute for hops in the development of certain native alcoholic beverages. It is also used as a taste enhancer. The presence of beneficial bioactive compounds has been reported in the envelopes of almond seeds, peanuts (*Arachis hypogea*), lotus seeds (*Nelumbo nucifera*) and African yam beans [11, 12, 13]. The chemical composition of the Garcinia seed coat has already been studied by gas-liquid chromatography and High-Performance Liquid Chromatography. These envelopes contain fatty acids and amino acid derivatives, pentadecanoic, margaric, pentadecanoic, myristoleic, cis-palmitoleic, cisvaccenic and eicosadienoic, methionine, tyrosine, histidine, and arginine [14]. Eleyinmi *et al.* (2006) described the chemical composition of *Garcinia kola* seeds and hulls. They quantified the raw proteins, lipid extracts, ashes, raw fibers of these elements [15]. To our knowledge, no or very few studies have evaluated the comparative phytochemistry and antimicrobial activity of peeled and unpeeled *Garcinia kola* seeds. Yet, in Benin, rumour has it that the use of seeds with the envelope has better virtues than the use of peeled seeds. Some people believe that these seeds are more effective in alcoholic decoctions. The main objective of this study is then to evaluate the phytochemistry and antimicrobial activity of peeled *Garcinia kola* seed's vs unpeeled *Garcinia kola* seeds, both extracted in alcohol.

2. Material and Methods

2.1 Materials

Seeds of *Garcinia kola* (*G. kola*) were collected at Dantokpa's main Market (South of Benin). They were separated on two parts. The one were peeled to obtain their skin. Skins, unpeeled (UGK) and peeled (PGK) G. kola were all cleaned with tap water to avoid any undesirable substances.

Unpeeled and peeled seeds were separately crushed into smaller pieces with the help of manual grater. Thereafter, they were air dried for 3 weeks under regular turning to enhance even drying. Obtained dry seeds were separately grounded into fine powder using a mechanical grinder.

2.2 Extract Preparation

Due to its capacity to extract maximum chemical compounds, ethanol was chose to extract G. kola organs [16]. 50 g of both powders (peeled and unpeeled seeds of G. kola) were separately crushed and recovered in 500 mL of cold ethanol 96°C. After agitation and homogenization, the mixture is filtered on Whatman paper and the filter is concentrated in a rotary evaporator at a temperature between 55°C and 60°C with help of vacuum pump to obtain the extract. The dry, watery triturated extract obtained was stored in a refrigerator at 4°C.

2.3 Phytochemical Screening

The qualitative phytochemical screening was performed based on colouring or precipitation reactions. It is made directly on the ethanolic extract of UGK and PGK by following methods described by previous authors [17, 18]. Quantitative phytochemical tests were carried out according to the method of Harbon, (1973) and Umeaku *et al*., (2018) [19, 20].

2.4 Antimicrobial activity assessment methods

Twelve references strains such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilus* A24974, *Micrococcus luteus* ATCC10240, *Proteus vulgaris* A25015, *Streptococcus oralis*, *Enterococcus foecalis* ATCC 29212, *Candida albicans* MHMR, *Salmonella typhi* R 30951401 and *Escherichia coli* O157 were used.

2.5 Sensitivity test

The antimicrobial sensitivity test was done according to the disc method inspired of those described previously [21]. Briefly, 1 mL of pre-culture of 18-24 h (10^6 UFC/mL) enabled planting a box of Petri dishes containing agar Mueller Hinton by flood. After seeding, the sterile Whatman paper discs (with 5 mm of diameter) were deposited with sterile pince. These discs have been carefully impregnated with 30 µL of plant extract (20 mg/mL). The dishes were kept for 15-30 min at room temperature before incubation at 37°C. The inhibition zones diameters were measured after 24 to 48 hours using a calibrated meter rule [22]. For each extract, the experiment was performed in duplicate.

2.6 Determination of the Minimum Inhibitory Concentration (MIC)

The MIC has been determined by macrodilution method with visual assessment of the growth of microorganisms. Briefly, nine concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 µg/mL) was performed in screw tube. To 1 mL of the above concentrations was added 1 mL of the bacteria inoculum (10^6 UFC/mL). After 24 h of incubation, turbidity tubes was examined relative to the control tube containing distilled water and the inoculum (10^6 UFC/ml) [23].

2.7 Determination of the Minimum Bactericidal Concentration (MBC)

The MBC was determined by solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37 ° C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC [24].

2.8 Data treatment and analysis

The spreadsheet Microsoft Excel version 2013 has been used for the capture and encoding the data. Minitab (version 17) software was used for the variance analysis (ANOVA).

3. Results and Discussion

3.1 Extraction performance

By using cold ethanol, the extraction performance of PGK and UGK were respectively 8.84% and 9.47%. So, by following our method, unpeeled seeds have a higher extraction yield than that of peeled seeds. Eleyinmi *et al*., 2006 have shown that hulls of *Garcinia kola* have more fibers than the pulp. This explain the importance of the yield of the alcoholic extract of unpeeled seed than that of peeled seeds. Indeed, the important role of fibers in the extraction of seed substances had already been highlighted.

3.2 Composition of different extracts

The qualitative analyze of phytochemical constituents of both extracts are shown in Table 1.

Table 1: Qualitative analyze of phytochemical constituents of peeled (PGK) and no peeled (UGK) ethanolic extracts of seeds of *Garcinia kola* (mg/100g)

Compounds	UGK	PGK	Others [25]
Reducing compound	+	+	-
Alkaloids	+	+	-
Flavonoids	+	+	na
Tanins catechic	+	+	na
Tanins gallic	+	+	na
Saponin	+	+	-
Anthocyanins	+	-	+
O-heterosides	+	-	na
Leuco-anthocyanins	-	-	na
Quinonics compounds	-	-	na
Coumarin	-	-	na
Terpenoids	-	-	na
Mucilages	-	-	+
Cartenoids	-	-	+
Free Anthracenics	-	-	na

PGK: Peeled seed of *Garcinia kola*, **UGK:** Unpeeled Seed of *Garcinia kola*; (+) = Presence; (-) = Absence; na : not available

The ethanolic extracts of peeled (PGK) and unpeeled (UGK) seeds of *G. kola* had qualitatively shown the presence of reducing compound, alkaloids, flavonoids, saponins and phenolic compounds consisting of catechic and gallic tannins in the two extracts. Anthocyanins and o-heterosides were present in the unpeeled seeds and absent in the peeled seeds. Other compounds such as leuco-anthocyanins, quinonics compound, coumarin, terpenoids, mucilages, cartenoids, free anthracenics are not present in the two extracts.

In contrast to the results of our work, Hounmenou *et al*., 2018 detected the presence of mucilages and carotenoids in the watery decoctions of *Garcinia kola* seeds, harvested in

Nigeria. They also reported the absence of reductive compounds and alkaloids. This difference in composition may be due to extraction solvents or intrinsic conditions (climate, soils of the harvest site) to the plants.

We quantify the phytochemistry compounds of both extracts (Table 2). Quantitative analysis of the two extracts shows have no significant difference ($p > 0.05$).

Table 2: Quantitative phytochemical constituents of peeled (PGK) and no peeled (UGK) seeds of *Garcinia kola* (mg/100g)

	PGK	UGK	Others [26, 27, 28]
Reducing compound	1.65 ± 0.03	1.68 ± 0.01	na
Alkaloids	2.11 ± 0.02	2.14 ± 0.4	0.64±0.20-2.30±0.05
Flavonoids	2.16 ± 0.04	2.11 ± 0.2	2.05±0.03-10.8 ± 0.2 mg/g (GAE)
Tanins catechic	3.62 ± 0.01	3.68 ± 0.01	0.35±0.03
Tanins gallic	0.34 ± 0.01	0.38 ± 0.03	na
Saponin	nd	nd	2.47±0.04
Anthocyanins	nd	nd	na
O-heterosides	nd	nd	na
Glycosides	nd	nd	3.42±0.00 (methanol extract)
Leuco-anthocyanins	nd	nd	na
Quinonics compounds	nd	nd	0.08±0.00
Coumarin	nd	nd	na
Terpenoids	nd	nd	na
Mucilages	nd	nd	na
Cartenoids	nd	nd	na
Free Anthracenics	nd	nd	na

PGK: Peeled seed of *Garcinia kola*, **UGK:** Unpeeled Seed of *Garcinia kola*; (na) not available, nd: not determined

Tanins (≈ 3.9 mg/100g) are the most quantified compounds of both types of extract. Their values are followed by flavonoids (≈2.13mg/100g), alkaloids (2.12 mg/100g) and reducing compounds (1.67mg/100g). The values of secondary plant metabolites are generally low. However, those quantified in ethanol extracts of both peeled and unpeeled seeds studied here are quite close to the values reported by other authors. It should also be noted that a significant amount of glycosides has been reported in methanol extracts (3.42mg/100g) of *Garcinia kola* seeds, unlike our samples that do not contain any [27]. At the same time, the reductive compounds quantified by our work could not be quantified by the literature consulted.

The chemical compounds quantified in our extracts have been cited as izing important biological properties. As an illustration, catechic tannins, often suspected of being the origin of the astringent taste of food products, are recognized for their ability to trap free radicals. They could also speed up the healing rate of superficial wounds by 50% [29].

3.3 Antimicrobial activities of *Garcinia kola* extracts

3.3.1 Sensitivity test

The inhibition diameters of the two extracts on the twelve bacteria tested are illustrated in Figure 1.

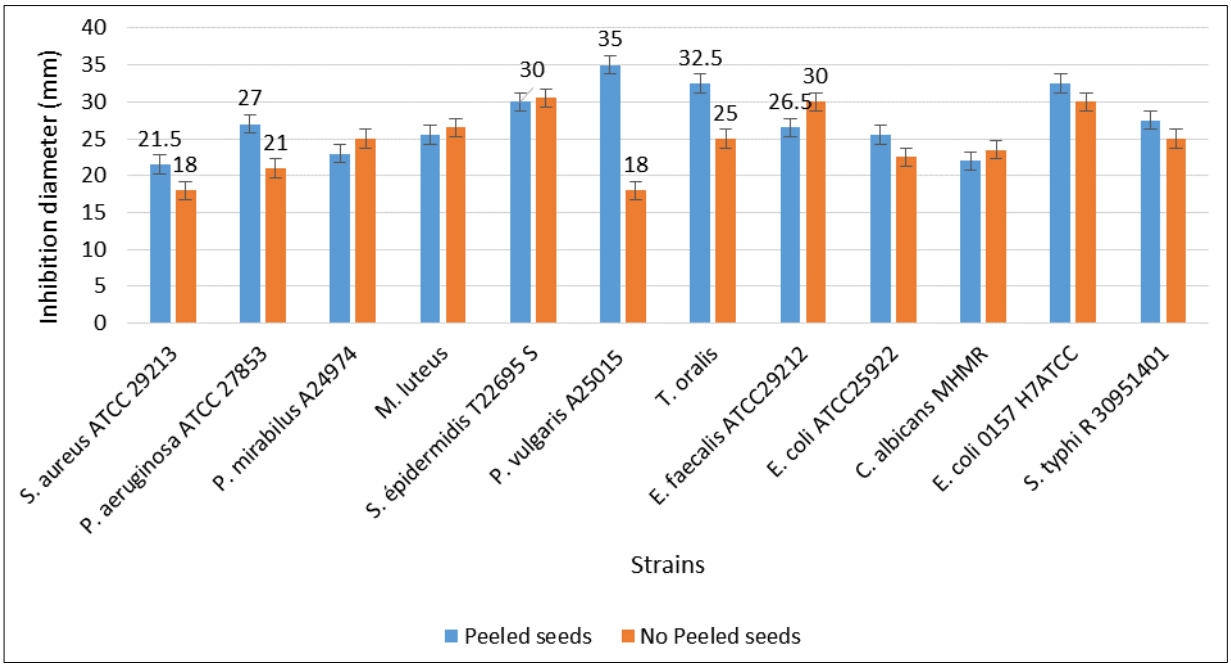

Fig 1: Diameters of inhibition of peeled and unpeeled ethanolic seeds extracts (20 mg/mL) of *Garcinia kola*

The sensitivity test performed with extracts on these bacteria varies from one strain to another depending on the type of extract. The observed variation is not significant ($p > 0.05$). The two extracts tested showed a pronounced effect by inhibiting the growth of 100% of the pathogenic strains tested (Figure 1). The largest inhibition diameters were obtained with the peeled seeds extract. In general, the inhibition diameters are higher in 70% of cases with peeled seeds extract

against 30% of cases with unpeeled seeds extract. The largest inhibition diameter (35 mm) was obtained against P. vulgaris strain and the following inhibition diameter (32.5 mm) against Staphylococcus aureus ATCC 29213 strain. Intermediate inhibition diameters ranging from 30 to 22 mm have been obtained against different germs. The smallest diameter of inhibition observed is 21 mm against the microorganism S. aureus ATCC 29213. In the case of unpeeled seeds extract, the largest diameter of inhibition is 30.5 mm against the strain S. epidermidis T22695 S. Intermediate inhibition diameters ranging from 30 mm to 21 mm have been observed against different microorganisms. The smallest inhibition diameter observed with unpeeled seeds extract is 18 mm against S. aureus ATCC 29213 and P. vulgaris A25015. In both cases, there is a very interesting inhibitory activity since in our previous work the largest inhibition diameters were around 25 mm [30]. The different compounds noted in the two extracts are irrefutably the basis of the antimicrobial activity noted in the two extracts. But, one would have thought that unpeeled seeds extract of G. kola would be more active, but the opposite has been noted. The additional compounds observed would then have produced an antagonistic effect in the inhibition of germs. Or some germs would be insensitive to the additional compounds noted in unpeeled seeds. In this section, it appears that the skin of G. kola seeds has no influence on the inhibition of certain bacteria.

3.3.2 Minimum Inhibitory Concentrations (MIC) of peeled and unpeeled seeds extract of *G. kola*

The two extracts were inhibited the proliferation of all pathogenic bacteria with variable minimum inhibitory concentrations (MIC) (Table 3). The smallest (0.625 mg/ml) MIC was obtained with peeled seeds extract. It was obtained against *Staphylococcus aureus* ATCC 29213. The highest concentration with this extract is (5 mg/mL) against *S. typhi* R 30951401. Intermediate concentrations of 1.25 and 2.5 were also obtained with this extract (Table 3). In the case of unpeeled seeds extract, the smallest minimum inhibitory concentrations is (1.25 mg / ml) obtained against 7 microorganisms. The greatest concentration is (2.5 mg / ml) obtained against 5 strains. As in the previous paragraph, the two extracts showed good activities against the different strains. This can be explained by the presence of the different compounds since the antibacterial activities of these different compounds have been described in the literature [31, 32, 33]. In most cases, the activities are identical. They only differ in two cases. In the case of *Staphylococcus aureus* ATCC 29213, peeled seeds are more active than unpeeled seeds. As in the previous section, the additional compounds would have produced an antagonistic effect or the microorganisms tested would be insensitive to these compounds. In the second case (*S. typhi* R 30951401), we notice the opposite effect. Unpeeled seeds are more active than peeled seeds. *S. typhi* R 30951401 would be more sensitive to the additional compounds seen in this extract. The good activity of these seeds has been described by other authors [34, 35]. This section shows us that the skin of the seed does not have a major influence on the majority of microorganisms. The activity of the two extracts is almost identical in more than 83% of the cases.

Table 3: Minimum inhibitory concentrations (mg/mL) of the extracts on the studied reference strains

Extracts	*S. aureus ATCC 29213*	*P. aeruginosa ATCC 27853*	*P. mirabilus A24974*	*M. luteus*	*S. épidermidis T22695 S*	*P. vulgaris A25015*	*T. oralis*	*E. faecalis ATCC29212*	*E. coli ATCC25922*	*C. albicans MHMR*	*E. coli 0157 :H7ATCC*	*S. typhi R 30951401*
PS	0.625	2.5	2.5	1.25	1.25	1.25	1.25	2.5	2.5	1.25	1.25	5
US	1.25	2.5	2.5	1.25	1.25	1.25	1.25	2.5	2.5	1.25	1.25	2.5

PS: Peeled Seeds; **US:** Unpeeled Seeds

3.3.3 Minimum Bactericidal Concentration (MBC) (mg/ml) of peeled and unpeeled seeds extract of *G. kola*

The two extracts indicated a very interesting bactericidal effect. The bactericidal effect is identical in 75% of the cases. Of the three remaining cases, unpeeled seeds showed greater bactericidal activity. The lowest bactericidal concentration is 1.25 mg/ml obtained on four different microorganisms (*P. mirabilus* A24974, *M. luteus*, *P. vulgaris* A25015, *E. coli* 0157: H7ATCC) with the two extracts (table 3). The highest concentration with unpeeled seeds is 10 mg/ml. The largest observed with peeled seeds is 20 mg/ml. Concentrations of 2.5 mg/ml were also noted on five microorganisms (*S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *S. epidermidis* T22695 S, *T. oralis*, *E. faecalis* ATCC29212) with the two extracts (Table 4). Peeled seeds has no bactericidal effect on *E. faecalis* ATCC29212. Likewise, the two extracts have no bactericidal effect on *S. typhi* R 30951401. In cases where the effects are identical, the bacteria are insensitive to the additional compounds. On the other hand, one might think that the additional compounds in unpeeled seeds have an effect on certain bacteria (*E. faecalis* ATCC29212, *C. albicans* MHMR and *E. coli* 0157: H7ATCC). Compared to our previous work, this is one of the rare times when extracts have a very interesting bactericidal effect [34, 35]. The bactericidal effect of *Garcinia kola* extracts has also been described by other authors. This time, unpeeled seeds extract showed a more interesting effect than peeled seeds extract.

Table 4: Minimum Bactericidal Concentrations (mg/ml) of extracts with reference strains

Extracts	*S. aureus ATCC 29213*	*P. aeruginosa ATCC 27853*	*P. mirabilus A24974*	*M. luteus*	*S. épidermidis T22695 S*	*P. vulgaris A25015*	*T. oralis*	*E. faecalis ATCC29212*	*E. coli ATCC25922*	*C. albicans MHMR*	*E. coli 0157 :H7ATCC*	*S. typhi R 30951401*
PS	2,5	2,5	1,25	1,25	2,5	1,25	2,5	-	10	20	10	-
US	2,5	2,5	1,25	1,25	2,5	1,25	2,5	2,5	10	10	1,25	-

PS: Peeled Seeds; **US:** Unpeeled Seeds

4. Conclusion

From the qualitative analysis of peeled seeds and unpeeled seeds of *Garcinia kola* extracts, the presence of large families of compounds such as reducing compound, alkaloids, flavonoids, phenolic compound, saponins is evident. Two additional compounds are contained in unpeeled seeds extract: Anthocyanins and O-heterosides. The antimicrobial tests which indicate a very good activity of the two extracts are identical in most of the cases. Unpeeled seeds extract has a more interesting bactericidal effect on certain bacteria. The skin of *Garcinia kola* seeds does not significantly increase the antibacterial activity of the seeds. Perspectives studies should be done to improve the properties of the skins.

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