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Phytochemical screening and study of in vitro antioxidant activities of the aqueous extract and the alcoholic (*koutoukou* extract) of *Garcinia kola* seeds (Guttiferea) collected in Abidjan (Ivory Coast).

[redacted]

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ABSTRACT

Objectives: *Garcinia kola* (Guttiferea) is a plant commonly found in West and Central Africa. Many biological activities such as anti-inflammatory, antimicrobial, antiviral and antioxidant are conferred because of its chemical composition. This work aimed to perform the triphytochemistry of two (2) extracts i.e.the aqueous extract and *koutoukou* (alcoholic extract) of *G. kola* seed, and to evaluate their antioxidant activities.

Methodology and results: The triphytochemical screening of the aqueous extract and the *koutoukou* extract were carried out. The DDPH radical scavenging test and the determination of the ferric ion reducer (FRAP) provided evidence of antioxidant activity. The average alcoholic degree of *koutoukou* samples varied between 42.33 ° ± 4.04 and 62 ° ± 3.00. Phytochemical screening revealed the presence of flavonoids, saponosides, polyphenols, polyterpenes, sterols, Catechin tannins and the absence of Gallic tannins, quinone substances and alkaloids in both extracts (aqueous extract and *koutoukou* extract). The profile of the antioxidant activity of the study, using the DDPH radical trapping test and the FRAP method, respectively revealed that Ci 50 (65.86 ± 1.17 µg /mL) and the reducing power of the Ferric ion (125. 4 ± 4. 91 mg / mL) of *koutoukou* extract from *G. kola* seeds is statistically significant.

Conclusion and applications of the results: Catechin tannins, saponosides, sterols, polyterpenes, flavonoids and polyphenols were found in the aqueous extract and *koutoukou* extract of *Garcinia kola* seeds. Thus, the antioxidant activity of the *koutoukou* extract of *G. kola* seeds was greater than that of the aqueous extract. The *koutoukou* extract of seeds of *G. kola*, still keeps the biological properties conferred on *G. kola*. The doses and mechanism of action of this cocktail deserve to be highlighted in order to propose it as an Improved Traditional Medicine (MTA). However, this cocktail can be used and recommended to combat oxidative stress and its consequences for good health.

Keywords*:* Phytochemical screening, antioxidant activities, *Garcinia kola*, *koutoukou*

RESUME

Objectif **:** *Garcinia kola* est une plante qu'on retrouve généralement en Afrique de l'Ouest, en Afrique Centrale. Plusieurs activités biologiques lui sont conférées à cause de sa composition chimique et est souvent utilisée en association avec certaines boissons. Le but de cette étude est de réaliser le triphytochimique de deux (2) extraits (extrait aqueux et extrait au *koutoukou*) des graines de *G. kola,* et d'évaluer leurs activités antioxydants.

Méthodologie et résultats : Le titre alcoométrique des différents échantillons de *koutoukou* et le screening triphytochimique de l'extrait aqueux et de l'extrait au koutoukou ont été réalisés. Le test de piégeage du radical DDPH et la détermination du pourvoir réducteur de l'ion ferrique (FRAP), ont permis de mettre en évidence l'activité antioxydant. La moyenne du degré alcoolique des échantillons de *koutoukou* variait entre 42, 33° ± 4,04 et 62° ± 3,00. Le criblage phytochimique a révélé la présence des flavonoïdes, des saponosides, des polyphénols, des polyterpènes, des stérols, des tanins et l'absence des tanins galliques, des substances quinoniques et des alcaloïdes dans les deux extraits (extrait aqueux et extrait au *koutoukou*). Le profil de l'activité antioxydant de l'étude, utilisant le test de piégeage du radical DDPH et la méthode FRAP, a révélé respectivement que la Ci 50 (65,86 ± 1,17 µg/mL) et le pouvoir réducteur de l'ion ferrique (125, 4 ± 4, 91 mg/mL) de l'extrait au *koutoukou* des graines de *G. kola* sont statistiquement significatifs.

Conclusion et applications des résultats **:** Les tanins catéchiques, les saponosides, les stérols, les polyterpènes, les flavonoïdes et les polyphénols ont été mis en évidence dans l'extrait aqueux et dans l'extrait au *koutoukou* des graines de Garcinia *kola*. Ainsi, l'activité antioxydant de l'extrait au *koutoukou* des graines de *G. kola* a été plus importante que celle de l'extrait aqueux. L'extrait au *koutoukou* des graines de *G. kola*, garde encore plus les propriétés biologiques conférées à *G. kola*. Les doses et mécanisme d'action de ce cocktail méritent d'être mis en évidence afin de le proposer comme un Médicament Traditionnelle Amélioré (MTA).Cependant, ce cocktail peut être recommandé et employé pour lutter contre le stress oxydatif et ses conséquences pour une bonne santé.

Mots clés **:** Screening phytochimique, activités antioxydants, Garcinia kola, *koutoukou*

INTRODUCTION

The use of plants as a therapy (phytotherapy) is very old and is currently experiencing a renewed interest in the public especially in Africa (Fleurentin *et al.,* 2002). This enthusiasm for plants or traditional medicine could be explained by various reasons such as the lack of geographical and economic accessibility to modern health care, the inefficiency and poor allocation of health personnel, as well as socio-cultural behaviour (Sangaré, 2003). All the organs of these plants can be used in the same way as their extraction products (Marc, 2001). Many medicinal plants like *Garcinia kola* continue to be the aim of lot of researches because of their abilities to cure many diseases (Fleurentin *et al.,* 2002). In fact, *Garcinia kola* is a plant, which generally found in west and central Africa (Adedeji *et al.,* 2006). Its seed is elliptical or oval, hard and has a slightly aromatic odour (Mazi *et al.*, 2013). The plant is commonly referred to as "bitter cola" or "male cola", respectively because of its bitter taste or claimed aphrodisiac effect. The bark of the stem is used as a purgative in the native people of eastern Nigeria and the latex is applied externally to fresh wounds to prevent sepsis, thus helping to heal the wound (Uko *et al.,* 2001). *Garcinia kola* has been identified as having properties of biochemical and physiological interest such as antibacterial, hepatotoxic, hypoglycaemic and antioxidant (Farombi *et al.,* 2002; Okunji *et al.,* 2007). The activities that are conferred on it are due to its chemical composition in several secondary metabolites such as polyphenols, quinone substances, tannins, alkaloids (Uko *et al.,* 2001). Moreover, it is increasingly used in combination with other plants including Moringa and/or some alcoholic beverages such as *koutoukou*. *Koutoukou* is an artisanal liquor resulting from the

fermentation and then the distillation of several sweetened juices (water-sugar-yeast, sugar cane, palm wine). This artisanal brandy has shown a lot of damage in the body (Diboh, 2015), but also some benefits when taken moderately. The general objective is to perform the triphytochemical of two (2) extracts (aqueous extract and *koutoukou* extract) of *G. kola* and evaluate their antioxidant activities. Specifically, it will be: (i) to determine the alcoholic degree of the *koutoukou* samples used; (ii) perform the DDPH radical trapping test; (iii) measure the reducing power (FRAP) of the two extracts (aqueous and *koutoukou*).

MATERIALS AND METHODS

MATERIAL

Plant: The fresh seeds of *G. kola* were obtained on the markets of the agglomerations of Abidjan. They were then taken to the National Floristic Center (CNF) of Félix Houphouët Boigny University, Abidjan, to verify and confirm the species and the desired genus.

Technical material: The technical equipment consisted of screening equipment, extraction equipment (knives, spatulas, porcelain capsules, glass funnel, hydrophilic cotton, wattman paper No. 1), apparatus (electronic grinder, magnetic stirrer, sand bath, rota vapour, oven at 50 ° C, freeze dryer, alcohol meter, UV spectrophotometer) and glassware (test tubes, Erlenmeyer flask and test tube ...).

Solvents: The extracts were made with two solvents namely distilled water and *koutoukou* traditionally made in the suburbs of Abidjan (Ivory Coast). *Koutoukou* samples are of two (2) types: Non-consumable samples and consumable samples.

Reagents: Phytochemical screening required various reagents depending on the chemical group. Catechin tannin research was performed using stiasny reagent. Characterization of Gallic tannins used Stiasny's reagent, sodium acetate and ferric chloride. Hydrochloric alcohol, magnesium chips and isoamyl alcohol were used to search for flavonoids. Acetic anhydride and concentrated sulfuric acid were needed for sterols and polyterpenes. The alcohol solution of ferric chloride at 2% allowed the characterization of polyphenols. Bornstraëgen's reagent, chloroform, 2-fold diluted ammonia and hydrochloric acid have been used to search for quinone substances. The reagents of dragendorff (potassium iodobismuthate reagent), bouchardat (iodine-iodide reagent) and valser-mayer (potassium iodomercurate reagent) and alcohol at 60 ° C have been alkaloids.

METHODS

Determination of alcoholic degree: The determination of the alcoholic degree of the different samples of *koutoukou* was carried out using an alcoholmeter. 500 mL of *koutoukou* was poured into a test tube. Then, the alcoholmeter equipped with a thermometer is introduced into the test tube. When the temperature of the measurement indicates 20 ° C, the graduation indicated on the stem is read and corresponds to the alcoholic strength of the studied *koutoukou*.

Extracts: After reception and identification of *G. kola* seeds, the latter are cut into small pieces with a knife and then dried in ambient air, away from the sun for 3 days. They are then ground using an electronic mill at 2500rpm 3 times in succession, in order to obtain a fine powder. Two types of solvents (distilled water and *koutoukou*) are used to make extracts. The extraction was thus carried out according to the method of Zirihi *et al.*, 2003.

Aqueous extract: 100 g of *G. kola* powder were added in 1000 ml of distilled water in an Erlenmeyer flask. After maceration with a magnetic stirrer continuously for 24 hours, the mixture is filtered with hydrophilic cotton, then wattman paper No. 1. The filtrate is heated in a condenser at 20 ° C for 24H and freeze-dried. The lyophilisate obtained called aqueous extract is used for the tests.

Extract at *koutoukou:* 100 g of *G. kola* powder was added in 1000 mL of *koutoukou* in an Erlenmeyer flask. After maceration with a magnetic stirrer continuously for 24 hours, the mixture is filtered with hydrophilic cotton. The supernatant is brought to the rota-vapour for evaporation. Then it is introduced into an oven at 50 ° C until a completely dry extract.

I-2-3-Phytochemical Screening: The identification of the different chemical groups was carried out using the techniques described in the work of Ronchetti and Russo (1971), Hegnauer (1973), Wagner (1983), Békro *et al.* (2007). Sterols and polyterpenes were searched for by the Liebermann reaction. Five (5) mL of each of the two extracts were evaporated without charring on a sand bath. The residues are dissolved in hot in 1 ml of acetic anhydride. 0.5 ml of concentrated sulfuric acid was added to the triturate. The appearance, at the interphase, of a ring purple or purple, turning blue then

green, indicated a positive reaction. The reaction with ferric chloride (FeCl3) made it possible to characterize the polyphenols. To 2 ml of each extract (aqueous and *koutoukou*), an addition of a drop of alcoholic solution of ferric chloride 2% was carried out. The appearance of a blue-blackish or green colour more or less dark reflected the presence of polyphenols. Flavonoids were evidenced by the cyanidin reaction. Two (2) mL of each extract was evaporated and the residue was taken up in 5 mL of 2-fold diluted hydrochloric alcohol. An addition of 2 to 3 magnesium chips leads to a release of heat and then a pink-orange or purplish colour. The addition of 3 drops of isoamyl alcohol intensifies this coloration and confirms the presence of flavonoids. The search for catechin tannins was carried out using Stiasny's reagent. Five (5) ml of each extract was evaporated to dryness in capsules. After adding 5 ml of the Stiasny reagent to the residue, the mixture was kept in a water bath at 80 ° C for 30 min and then allowed to cool. The observation of a precipitate in large flakes characterized the catechin tannins. For Gallic tannins, the previous solution is filtered. The filtrate is collected and saturated with sodium acetate. The addition of drops of 2% FeCl3 causes the appearance of an intense blue-black colour attesting the presence of Gallic tannins. Quinone substances were searched for from the Bornstraëgen reagent. Two (2) mL of each of the two extracts were evaporated to dryness in a capsule. The residue is triturated in 5 mL of 1: 5 hydrochloric acid. The triturate is poured into a test tube and brought to a water bath for 30 minutes. After cooling, it is extracted with 20 ml of chloroform. Ammonia diluted 2-fold (0.5 mL) was added to the chloroform solution. A red or purple colour showed the presence of quinones. Bouchardat reagents (iodine-iodide reagent), Dragendorff (potassium iodo-bismuthate reagent) and valser-mayer (potassium iodomercurate reagent) were used to characterize the alkaloids. Six (6) mL of each solution was evaporated to dryness in a test tube. The residue is taken up in 6 mL of alcohol at 60 ° and distributed in three (3) different tubes. In the first tube, the addition of 2 drops of Dragendorff reagent to the alcoholic solution causes a precipitate or an orange colouring. The addition of 2 drops of Bouchardat reagent to the alcoholic solution in the second tube causes a reddish-brown precipitate and indicates a positive reaction. In the third tube, an addition of 2 drops of valser-mayer reagent causes the appearance of a precipitate or a cream-white colour reflecting a positive reaction. For saponosides, 15 mL of aqueous extract is poured into a test tube 15 cm in height and 15 mm in diameter. Then the tube is shaken until the appearance of foam, and then is left for 10 minutes. The persistence of the foam at a height of more than 3cm confirms the presence of Saponosides.

Determination of antioxidant activity: The antioxidant activity of the two extracts (aqueous extract and *koutoukou* extract) of *G. kola* was demonstrated by two methods, namely the DDPH radical scavenging test (For anti-free radical activity) and the FRAP method (For reduction activity).

Trapping test of the radical DDPH: The effect of extracts (aqueous and *koutoukou*) on DDPH was determined according to the method of Parejo *et al.*, (2002) with some modifications. Two (2) ml of a methanolic solution of DPPH (100 µM) is mixed with 1.5 ml of different dilutions of the extracts (0-100 µg / ml) and vitamin C (used as reference). The mixtures obtained are protected from light at room temperature for 30 minutes. The absorbance is measured at 517 nm against a control consisting of 2 ml of the DPPH solution and 1.5 ml of the methanolic solution.

Measurement of Total *in Vitro* Antioxidant Activity (FRAP Test): The FRAP (Reducing Iron Power) test for this study was carried out according to the method described by Pulido *et al.* (2000). 3500 µl of the FRAP reagent are added to 140 µl of the test compounds (aqueous extracts and kutuku extract), dissolved in a methanolic solution. After 30 min of incubation in the dark, the absorbance is read at 593 nm.

Statistical Analysis: Averages and standard deviations were obtained using the EXCEL version 2013 software and the statistical analyses were processed with the GRAPHPAD 5. 1.

RESULTS AND DISCUSSION

Determination of alcoholic degree: The alcoholic strengths of the different samples (consumable and non-consumable) used during the study are shown in Table 1. The average alcoholic degree of the first distillate or non-consumable sample is 62 ° ± 3.00 and the average of the Alcoholic degree of the consumable sample is 42.33 ± 4.04.

Obtaining Extracts: The extraction gave two types of *G. kola* extracts namely the aqueous extract (EAGK) and the ethanolic or *koutoukou* extract (EKGK). The aqueous extract (EAGK), obtained using a lyophilizer,

have a thinner and lighter texture than that of the *koutoukou* extract obtained from a rota-vapour.

Phytochemical Screening: Flavonoids, saponosides, polyphenols, polyterpenes, sterols, tannins and quinone substances are the secondary metabolites that have been identified in this study. Their identification was carried out by the classical methods of characterization and identification of Nemlin and Brunel (1995) (Table 2). Gallic tannins, quinone substances and alkaloids are absent in both extracts (aqueous extract and *koutoukou* extract). In addition, sterols, catechin tannins, saponosides and alkaloids are abundantly present in the aqueous extract.

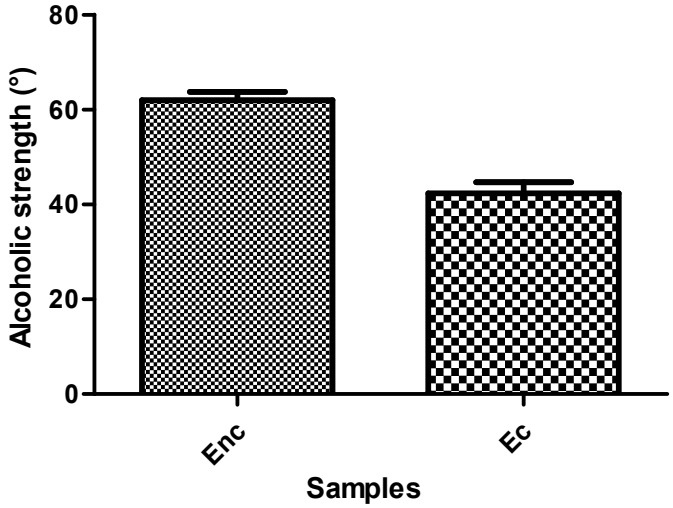


Figure 1: Alcoholic strength of samples of artisanal alcohol (*koutoukou*) from the suburbs of Abidjan.
-Enc: Non-consumable samples taken from different sites in the suburbs of Abidjan.
-Ec : Consumable samples taken from different sites in the suburbs of Abidjan.
-Data are expressed as mean ± SEM, n = 3
** p <0.001: very highly significant difference compared to Enc

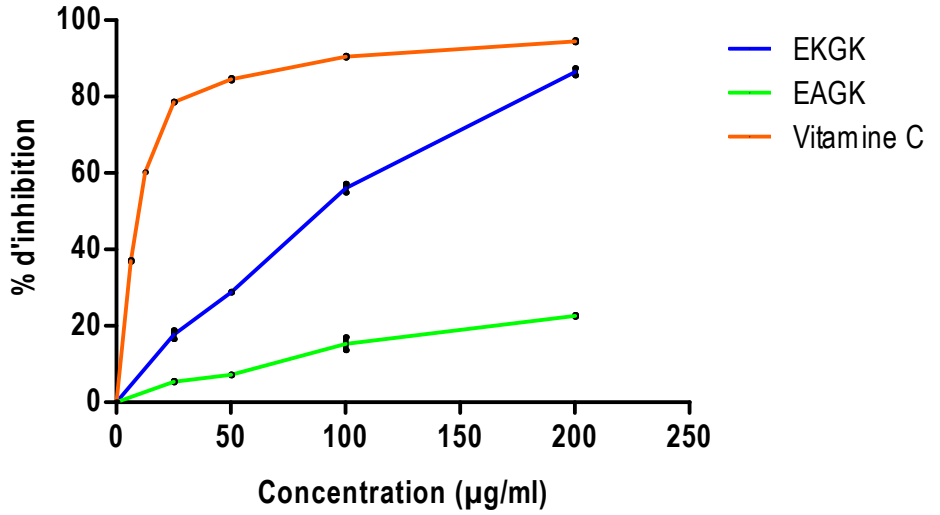
Table 1: Constitutions in secondary metabolites of *Garcinia kola* extracts

Metabolites / Extracts	Sterols Polyterpenes	Polyphenols	Flavonoids	Tannins		quinone Substances	Alkaloids			Saponosides
				Gal	Cat		D	B	V M	
EAGK	+++	++	++	-	+++	-	+++	++	-	+++
EKGK	++	++	++	-	++	-	++	++	-	++

Positive test: (+)
Negative test: (-)
Significantly present: (++)
Abundantly present: (+++)
EAGK): Aqueous extract of *G. kola*
EKGK): Extract at *Koutoukou* from *G. kola*
Gal: Gallic, **Cat:** Catechistic, **D**: DRAGENDORFF, **B**: BOUCHARDAT, **V-M**: VALSER-MAYER.

Antioxidant activity: The antioxidant activity of the two extracts (aqueous extract and *koutoukou* extract) of *G. kola* was demonstrated by two (2) methods that are: the DDPH radical trapping test and the determination of the reducing agent of the ion Ferric (FRAP). The DDPH scavenging test revealed that the IC50 of *G. kola*

koutoukou extract (65. 86 ± 1.17 µg / mL) was higher than that of the aqueous extract (not determined because it was extremely low) and vitamin C (8.64 ± 0.13 µg / mL). At the level of the FRAP method, the reducing power of the *koutoukou* extract of *G. kola* (125. 4 ± 4. 91 mg / mL) is greater than that of the aqueous extract (19.28 ± 2.86 mg / mL) (Figure 1 and Table 3).



EAGK: Aqueous extract of *G. kola*
EKGK: *Koutoukou* extract from *G. kola*

Figure 2: Representative Curves of Antioxidant Activity (DPPH)

Table 2: Antioxidant Activity of the Aqueous Extract and *Koutoukou* Extract by the FRAP Method

	Reduction Potency µMol Eq trolox / g Ext. sec	
Extracts / **Tests**	**E1 (EAGK)**	**E2 (EKGA)**
1	17.2044928	120.6097344
2	18.09591728	125.0668568
3	22.55303976	130.4154038
Average	**19.28 ± 2.86**	**125.4 ± 4.91*** **

Data are expressed as mean ± SEM, n = 3; *** p <0.0001: very highly significant difference compared to EAGK

DISCUSSION

The use of plants such as *Garcinia kola* is of increasing interest. This is because this plant participates in the primary health needs of humans. Its use being sometimes in association with drinking places like *koutoukou* would attribute therapeutic virtues. The aim of this study was to perform the triphytochemistry of two (2) extracts (aqueous extract and *koutoukou* extract) *G. kola* seeds, and to evaluate their antioxidant activities. The alcoholic strengths of the non-consumable *koutoukou* samples used for the study averaged 67 ° ±

3.00 while the consumable samples averaged 42.33 ± 4.04 (figure 1). Non-consumable samples and consumables made in the strict traditional practice, have an alcoholometry corresponding respectively to vodka, which goes from 45 to 70 ° and whiskey 40 to 60 ° (Pequignot and Trémolières, 1984). Indeed the average alcoholic degree of the non-consumable sample is very statistically significant ($p < 0.001$) than that of the consumable sample .Consumable samples remain the most popular because of its low alcohol content, which according to consumers causes less damage than that called 1st degree. Indeed, the *koutoukou* resulting from the distillation of fermented sweetened juices including sugar cane, sugar-water-yeast mixture and oil palm sap, as in our case, has various alcoholic strengths due to several factors, mainly to the origin of the raw material and the fermentation time. Thus, the non-consumable sample or even the first degree of *koutoukou*, which is the very first distillate directly derived from the fermentation of palm sap, is the part of *koutoukou* that has the highest alcoholic strength of all other distillates. During this study, it has an average of 67 ° ± 3.00. This result is similar to that of Yao, 2012, who showed in his work by another method (CPG) that the 1st degree had an ethanolic percentage that went up to 69 °. In addition, a significant alcoholemia by *koutoukou* would lead to serious consequences such as damage to vital organs or even sudden death due to its high composition of methanol, which is a very toxic product (Yao, 2012) for the body. Phytochemical screening of the aqueous extract and *Koutoukou* extract of *Garcinia kola* revealed the presence of flavonoids, saponosides, polyphenols, polyterpenes, sterols, tannins and quinone substances. Gallic tannins, quinone substances and alkaloids are absent in both extracts (aqueous extract and *koutoukou* extract). In addition, sterols, catechin tannins, saponosides and alkaloids are abundantly present in the aqueous extract (table 1). The results for the ethanolic extract are similar to those of Denen *et al.*, 2015, which revealed the secondary metabolites (saponosides, polyphenols, polyterpenes, sterols, tannins) with an absence of quinonic substances in their ethanolic extract of *G. kola*. However, the chemical groups found in the aqueous extract during the study differ from those of Osemwegie *et al.*, 2017, by the absence of alkaloids in their aqueous *G. kola* extract while Dah-Nouvlessounon *et al.*, 2015, did not highlight the catechin tannins. This difference could be explained mainly by the period of grain harvest or the location (country, regions) of harvest (Dah-Nouvlessounon *et al.,* 2015). Indeed, the therapeutic nature of plants like *Garcinia kola* is due to its particular chemical composition in secondary metabolics. Flavonoids are chemical compounds that are involved in inflammation, allergies, pain and oxidative stress (Hodek *et al.,* 2002). Alkaloids are also involved in the treatment of oedema (Kerharo & Adam, 1974). According to them, the punarnavine alkaloid family causes a marked and persistent increase in blood pressure with a strong diuresis, by action on the renal epithelium. N'guessan *et al.,* (2015), reported that polyphenols (catechols) and catechin tannins contained in Petersianthus macrocarpus stem bark give it bactericidal properties such as anticholera. According to them, the presence of saponosides, sterols, (tropanic) alkaloids, in the leaves of Boerhavia diffusa L. (Nyctaginaceae), would play an antiasthmatic effect while polyphenols (coumarins) are used against haemorrhages. In addition, sterols and polyterpenes have bactericidal properties and allow the use of this plant against shingles and cholera (N'guessan *et al.,* 2015). These various compounds listed are of great importance because associated with many biological activities: anti-inflammatory (Middleton *et al.,* 2000), anti-hepatotoxic, anti-tumour (Milane, 2004), antihypertensive, anti-thrombic, antibacterial antiviral, antiallergic and antioxidant agents (Lagnika *et al.,* 2012). The profile of antioxidant activity in the study, using the DDPH radical scavenging test and the FRAP method, revealed several aspects of the aqueous extract and *koutoukou* extract of *G. kola* (figure 2 and table 2). The method of radical of DDPH shows that the aqueous extract of *G. kola* (EAGK) has the lowest IC50 (not determined), while the *koutoukou* extract (EKGK) has the highest value (Ci 50 = 65 , 86 ± 1.17 µg / mL) and statistically significant ($p < 0.001$) . With the FRAP method, the greatest ferric ion reducing power (125, 4 ± 4, 91 mg / mL), which is very highly significant, was obtained with the *koutoukou* extract, while that of the aqueous extract was 19.28. ± 2, 86 mg / mL. The results of the antioxidant activity of the *koutoukou* extract are consistent with those of Esomonu *et al.*, (2005), which demonstrated the effect of the ethanolic extract of *G. kola* on the erythrocytes of the wistar rat. However, the antioxidant activity of the aqueous *G. kola* extract obtained by Emerole *et al.*, 2005, is at odds with that of the present invention. They showed in their work a remarkable activity of the aqueous extract of *G. kola* due to a strong presence of flavonoids in this extract. The high antioxidant activity of the *koutoukou* extract in this study is explained by the majority composition of

koutoukou in ethanol, known as a good solvent for the extraction of certain secondary metabolites.

CONCLUSION AND APPLICATION OF RESULTS

The present study made it possible to determine the average alcoholic degree of the different samples of *koutoukou* employed. Catechin tannins, saponosides, sterols, polyterpenes, flavonoids and polyphenols were found in the aqueous extract and *koutoukou* extract of *Garcinia kola* seeds collected in Abidjan. The intensity of the presence of the compounds differs from one extract to another because of the extraction solvent used. Thus, the antioxidant activity of the *koutoukou* extract of *G. kola* seeds was greater than that of the aqueous extract.

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