academicJournals

Vol. 9(28), pp. 1716-1727, 15 July, 2015
DOI: 10.5897/AJMR2015.7611
Article Number: 8B94D2F54434
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Phytochemical screening and biological activities of *Garcinia kola* (bark, leaves and seeds) collected in Benin

Durand Dah-Nouvlessounon[1], Farid Baba-Moussa[2], Adolphe Adjanohoun[3], Haziz Sina[1], Pacôme A. Noumavo[1], Hubert Adoukonou-Sagbadja[4], Nafan Diarrassouba[5], Christine N'tcha[1], François S. Anago[4] and Lamine Baba-Moussa[1]*

[1]Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, FAST, Université d'Abomey Calavi 05 BP: 1604 Cotonou, Bénin.
[2]Laboratoire de Microbiologie et de Technologie Alimentaire, FAST, Université d'Abomey-Calavi, 01 BP: 526 ISBA-Champ de foire, Cotonou Bénin.
[3]Centre de Recherches Agricoles Sud, Institut National des Recherches Agricoles du Bénin, Attogon BP: 884 Cotonou, Bénin.
[4]Laboratoire des Ressources Génétiques et d'Amélioration des Espèces, Département de Génétique et des Biotechnologies, FAST, Université d'Abomey Calavi, BP: 526 Cotonou, Bénin.
[5]Université Péléforo Gon Coulibaly de Korhogo UFR des Sciences Biologiques BP: 1328 Côte d'Ivoire.

Received 6 June, 2015; Accepted 10 July, 2015

Garcinia kola **is a tropical plant which grows in moist forest. It seeds are edible and are consumed for their multiple property. This study aimed at making phytochemical screening and evaluating some biological activities of *G. kola* organs (bark, leaves and seeds) extracts. The selected organs powdered were used for phytochemical screening and extractions. The antibacterial activity was tested on 10 references strains and 28 meat isolated *Staphylococcus* strains by agar perforation method. The antifungal activity of three fungal strains was determined on the potato-dextrose agar medium mixed with the tested extract. The antioxidant activity was determined by both DPPH and ABTS methods. The results reveal that 40% of studied secondary metabolites were present in the seeds against 28.57% in the leaves as the bark. The reference and meat isolated strains inhibitory diameter ranged from 28 ± 2.3 mm (*S. haemolyticcus*) to 7.5 ± 0.70 mm (*E. coli*). All extracts had a minimum inhibitory concentration (MIC) lower than 20 mg/ml, while the minimum bactericidal concentration (MBC) was ≥ 20 mg/ml for some tested extracts. The lowest MIC and MBC was 0.039 mg/ml with *C. albicans*. The fungal strains susceptibility varied ($p < 0.001$) depending on extracts. The seeds ethyl acetate extract displays the better antioxidant activity with DPPH (25.03 ± 3.64 µg/µl) and ABTS (22.99 ± 1.34 µmol EqAA/g) methods. The LD_{50} of the extracts was greater than 0.1 mg/ml and not exhibiting toxicity. The leaves extract had the best bactericidal effects while the seeds extract presented better antifungal and antioxidant activity.**

Key words: Antifungal, *Staphylococcus*, food strains, antioxidant, cytotoxicity, Benin.

INTRODUCTION

The Benin forest agro-ecosystems shelter over 162 forest plant species, with social, religious and/or cultural meaning, used for many purposes such as domestic food and commercial (Codjia et al., 2009). Apart from feeding

and commercial uses, many of these species are currently used in traditional medicine all over the country. Among the plants uses in traditional medicines, we can cite *Garcinia kola* (*Clusisaceae*), a large tree with fattening base (Akoegninou et al., 2006). This species is present in tropical Africa, from Sierra Leone to the DR Congo (Adesuyi et al., 2012).

Nowadays, infectious diseases are responsible for a high morbidity and mortality rate and are consider as a public health problem because of their frequency and their severity (Bourgeois, 1999). For the treatment of these diseases, people often use synthetic drugs such as beta-1actamines. But, bacteria developed a resistance mechanism to fight against most of the synthetic family of antibiotics. There are four main mechanisms used by bacteria to resist beta-1actamines molecules: i) enzymatic inactivation, ii) the modification of the cellular target of the antibacterial agent, iii) the cellular efflux and, iv) the decrease or absence of penetration of the antibiotic in the bacteria. The combination of these mechanisms in a bacterium makes its multi-resistant to many antibiotics (Gangoue-Piéboji et al., 2004). Similarly, several cases of fungi resistant to conventional antifungal agents have been reported. Moreover, the production of free radicals in living organisms is a vital phenomenon for the cell regulated through various biochemical or enzymatic detoxification process (Salem, 2009). The free radicals are reported to be involved in many serious illnesses and constitute even aggressive form factors to DNA (Boumaza, 2009).

To face all these health problems, the formalization of endogenous knowledge would be a reliable asset in the control of not only resistant microbial strains but also diseases caused by free radicals. It is known that several medicinal plant synthesize a wide variety of phytochemicals which include alkaloids, tannins, flavonoids, steroids, saponins, and phenols, which have antimicrobial proprieties. Moreover, potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables fruits, leaves, barks, roots and crude plant drugs. Antioxidants are vital substances which protect the body from damage caused by free radical inducing oxidative stress (Ozsoy et al., 2008). Therefore, many plants were used as a source of traditional medicine to treat various diseases and conditions (Razali et al., 2008). *G. kola* belongs to the plant used in traditional pharmacopeia in Benin. This plant various parts extracts are reported to be used in the treatment of cough, sore heart, liver problems (Kanmegne and Ndoumou Omokolo, 2007), diarrhea (Braide, 1991) inflammatory diseases (Iwu and Igboko, 1982). *G. kola,* one of the preferred seed in West Africa is deemed to have an antidote power (Kabangu et al., 1987), anti-hepatotoxic properties (Akintowa and Essien, 1990), antinephrotoxic, antimicrobial (Adefule-Ositelu et al., 2004), hypoglycemic (Odeigah et al., 1999) and aphrodisiac effects (Ajibola and Satake, 1992). Other scientific studies of *G. kola* by other authors (Farshori et al., 2013; Okunji et al., 2002; Pietta, 2000) showed the importance of the plant. In Benin, there is few scientific work on this plant species. So, the aims of this study were to investigate the phytochemical on one hand and on the other hand evaluate some biological activities of *G. kola* collected in Benin.

MATERIALS AND METHODS

Collection of plant material

The bark, seed and leaves were collected in the village of Anagbo (commune of Adjarra: 6°29'35"N, 2°40'28"E) department of Oueme, southern Benin. The plants materials were air dried at 25 to 30°C for two weeks, ground and sieved into a bark powder. The smooth powder was stored in airtight glassware and kept in darkness at -20°C until use.

Phytochemical profiling

The phytochemical profiling of bark, seed and leaves powders of *G. kola* to determine the major constituents (nitrogenous, polyphenolic, terpenic compound and glycosides) was done according to the method describe by Houghton and Raman (1998).

Preparation of aqueous

The aqueous extract was obtained according to the method describe by Dah-Nouvlessounon et al. (2015). Briefly, the powder (50 g) of *G. kola* appropriate organ (bark, leaf or seed) obtained above was macerated into 500 ml of distilled water under magnetic agitator for 72 h at room temperature. The homogenate was then filtered two times on absorbent cotton and once on Whatman N°1 paper (125 mm ø, Cat No 1001 125). For the aqueous extract, the filtrate was dried in the oven at 40°C; the obtained powder is considered as the total extract ready to use for the biological activities. All extracts were stored in labeled sterile bottles and kept at -20°C until further use.

Preparation of ethanol and ethyl acetate extracts

These extracts were made using an adapted methods described by Sanogo et al. (2006) and N'Guessan et al. (2007). We macerated 50 g of *G. kola* powders (bark, leaf or seed) in 500 ml of 96% ethanol for 72 h. The obtained extract was filtered thrice using Whatman N°1 filter paper (125 mm ø, Cat No 1001 125). Half of the filtrate was directly dried at 40°C to obtain the ethanolic extract of *G. kola*. To the second half of the filtrate, 200 ml of H_2O and 100 ml of ethyl acetate were added. The solution was gently mixed and left settled until we obtain two phases (about 45 min). The lower phase was collected and dried as described above to obtain the ethyl

*Corresponding author. E-mail: laminesaid@yahoo.fr. Tel: +22997123468.

acetate extract. The alcoholic and ethyl acetatic extracts were stored in labeled sterile bottles and kept at -20°C until further use.

Microorganism's cultures

The tested microorganisms include ten references, twenty height *Staphylococcus* meat isolated strains and three fungal strains (*Penicillium citrinum*, *Aspergillus tamarii* and *Fusarium verticilloides*). The three fugal strains were part of the microorganisms isolated in a Beninese traditional cheese wagashi by Sessou et al. (2012). The reference strains were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilus* A24974, *Micrococcus luteus* ATCC 10240, *Proteus vulgaris* A25015, *Streptococcus oralis*, *Enterococcus foecalis* ATCC 29212 and *Candida albicans* MHMR. The *Staphylococcus* strains used in this study were those isolated from three different meat products in Ivory Coast by Attien et al. (2013) and stored in the Laboratory of Biology and Molecular Typing in Microbiology (University of Abomey-Calavi, Benin).

Antimicrobial activity

Sensitivity test

The agar perforation method inspired of those described by Bauer et al. (1966) was used to screen the antimicrobial activity. For this method, four to five perforations were performed under aseptic conditions, on Mueller Hinton agar Petri dish previously flooded by the appropriate bacterial culture (adjusted to 0.5 McFarland standard). Twenty five microliter of extract solution (20 mg/ml) were aseptically lodged in the hole. These dishes were kept for 15-30 min at room temperature before incubation at 37°C for 24 and 48 h.

After the incubation period, the dishes were examined for inhibitory zones. Each sample was used in triplicate for the determination of antibacterial and antifungal activity.

Determination of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations (MIC) of the plant crude extract was performed by macrodilution method (Saha and Rahman, 2008). First, the extracts were diluted in sterilized distilled water to the highest concentration of 20 000 µg/ml and then nine dilution were performed to obtain successively the concentrations of 10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 µg/ml in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (10^6 UFC/ml) to obtain 2 ml as a final volume. Culture medium without samples and others without micro-organisms were used in the tests as controls. Tubes were incubated at 37°C for 18-24 h and growth was indicated by turbidity. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth.

Determination of Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the tested microorganisms was determined by sub culturing the test dilutions onto a fresh solid medium and incubated further for 18-24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC (Farshori et al., 2013).

Evaluation of the cytotoxicity activity of *G. kola* extracts

The cytotoxic effect of the extracts was evaluated according to an adaptation of the method described by Kawsar et al. (2008). The tests were carried out twice on 72 h larvae of *Artemia salina* (ARTEMIO JBL GmbH D-67141 Neuhofem). Briley, a test was constituted of 16 *A. salina* larvae in a 2 ml solution containing 1 ml of the extract tested concentration and 1 ml of sea water. The number of surviving larvae is counted after incubation (24 h) and the LD_{50} was calculated using the regression line obtained from the surviving larvae according the extracts concentration representation.

Antifungal activity

The *in vitro* antifungal activity of the extracts was evaluated according to the method previously described by Dohou et al. (2004). The assay was performed on the Potato-Dextrose Agar medium. Briefly, the extracts use for the antifungal activity was dissolved with sterilize distillated water or if necessary with a water-ethanol mixture (60:40). One (1) ml of the dissolved extract (20 mg/ml) was thoroughly mixed with 10 ml of the sterilized potato-dextrose agar medium before it was transferred to sterile Petri dishes for solidification. After the medium solidification, a sterile 6 mm disc pretreated with fungal strain was placed in each Petri plate. Plates were incubated at 25 ± 1°C for five days. Each treatment was replicated twice. Fungal radial growth was measured by averaging the two diameters taken from each colony. Percentage growth inhibition of the fungal colonies was calculated using the formula:

$$\text{Inhibition Percentage } (\%) = \frac{\text{Control's growth} - \text{Treatment's growth}}{\text{Control's growth}} \times 100 \quad (1)$$

Antioxidant activity determinations

The antioxidant activity was measured using both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) methods.

The ABTS assay was conducted according to the method described by Re et al. (1999). The working solution of $ABTS^+$ (10 mg of ABTS, 2.6 ml of deionized water and 1.72 mg of potassium persulphate) was left to stand at room temperature for 12 h in the dark before use. This solution was diluted with ethanol until obtaining an absorbance of 0.70 ± 0.02 at 734 nm. Twenty µl of each extract sample (1 mg/ml) was diluted with a fresh prepared ABTS solution to a total volume of 1 ml. All the assays were performed in triplicates, the absorbance was read after 15 min in dark at 734 nm and the reference molecule was ascorbic acid. The concentration of compounds with a capability to reduce $ABTS^+$ radical cation is expressed as µmol equivalent Ascorbic Acid (µmol EqAA) per gram of dry extract using the following formula used by Guenne et al. (2011).

The DPPH method was conducted using an adapted method of Scherer and Godoy (2009). Practically, equal volumes (100 µl) of DPPH (50 µM) and plant extracts (200 µg/ml) were mixed in a 96 well microplate and allowed to stand in darkness for 20-30 min at room temperature. Then, the absorbance was read at 517 nm and the blank was a mixture of methanol and DPPH (v:v). The inhibitory percentage of DPPH radical indicating the antioxidant activity of extracts and BHA, gallic acid was obtain using the formula establish by Schmeda-Hirschmann et al. (2003).

The concentration providing 50% inhibition (IC_{50}) was determined graphically using a calibration curve in the linear range by plotting the extract concentration and the corresponding scavenging effect. Antioxidant Activity Index (AAI) was calculated according to the formula used by Scherer and Godoy (2009).

Statistical analysis

All experiment was done in triplicate and data thus obtained

Table 1. Phytochemical constituents of *G. kola* powdered organs samples.

Group of compounds	Class	Organ		
		Bark	Leaves	Seeds
Nitrogenous compound	Alkaloids	−	−	+
Poly-phenolics compound	Tanins	+	+	+
	Tanins catéchiques	−	+	−
	Tanins galliques	+	+	+
	Flavonoids	−	−	+flavone
	Anthocyans	−	−	−
	Leuco-anthocyanes	−	−	−
	Coumarin	+	−	+
	Quinonics derivate	−	−	−
Terpeniques compound	Triterpenoids	−	+	+
	Steroids	+	−	−
	Cardenolids	−	−	−
Heterosides	Cyanogenics derivate	−	−	−
	Saponosids (IM)	+ (500)	+ (111)	+ (200)
	Reducing compounds	−	+	+
	Free anthracénics	−	−	−
	O-heterosides	−	−	−
	O- heterosides at GR	−	−	−
	C-heterosides	−	−	−
	Mucilags	+	−	+

(+), Presence of secondary metabolite; (-), Absence of secondary metabolite; (IM), Index mouss.

reported as a mean ± standard deviation (SD). The logit model of stata v.12 software is used to qualitative analysis. The data were also analyzed using Graph Pad Prism 5 software for quantitative analysis. Differences of $p < 0.05$ were considered significant.

RESULTS

Phytochemical screening

The phytochemical analysis performed on three organs of *G. kola* revealed the presence of several secondary metabolites (Table 1). It was noted an uneven distribution of these metabolites from one organ to another. Indeed, 40% of the studied secondary metabolites were present in the seed against 28.57% in the leaves as in the bark.

Antimicrobial activity of *G. kola* extracts

Sensitivity test

The ability to inhibit microbial growth of the extracts evaluated on reference strains revealed that the results of extract deferred from an extract to another depending on the organs. Indeed, all bark extracts had no inhibitory effect on the reference strains. It is the same for the aqueous extract of the two others organs of the plant. In contrary, the ethanol extract of the leaves and seeds, as well as ethyl acetate extract of the seeds are active. Thus, the ethanol extract of the leaves and ethyl acetate seeds extract inhibits the growth of 90% (9/10) of the strains while the ethanol extract of the seeds inhibits the totality (100%) of the strains.

Qualitative analysis of the inhibitory capacity of G. kola extracts

The results of the qualitative analysis of data relating to the evolution of the diameter in time (24h and 48h) according to organ, type of extract and the type of microorganism, showed that the inhibition zones diameters of the sensitive strains vary from one species to another. The Husman test conducted for this purpose showed that the logit model premium on the probit model. With a predictive power of 86.11% sensitivity and specificity of 61.11% showing its reliability, the logit model shows in organs level that the probability that the

diameter increases from the leaves to the seed decreased of 0.13. Regarding the type of extract, logit model marginal effects show that the probability that the diameter increases from the ethanol extract with ethyle acetate extract increases of 0.30. Considering the type of microorganism, from Gram + and Gram- bacteria to yeast, the probability that the diameter increases with the time decreases of 0.08. Similarly passing reference strains for food strain this probability decreases by 0.003.

Quantitative analysis of the inhibitory capacity of G. kola extracts

Concerning the reference strains, the inhibition zones diameters of sensitive strains do not differ ($p > 0.05$) in the time (24 and 48 h) with the ethanol extract of the leaves (Figure 1a) and seeds ethyl acetate extract (Figure 1c). On the contrary with the seeds ethanol extract, a change ($p < 0.05$) of diameter is observed between 24 and 48 h for *E. foecalis* (Figure 2b). With the leaves, the largest diameter of inhibition (15 ± 0.00 mm) of the ethanol extract was obtained on *E. foecalis*, while the lowest (7.5 ± 0.70 mm) was recorded with *E. coli* after 48 h of incubation (Figure 1a). With the seeds, the larger diameter (25 ± 0.00 mm) and the lowest (5 ± 0.00 mm) were recorded with the ethanol extract respectively on *S. epidermidis* and *E. coli* (Figure 1b). The compared action with the same type of extract (ethanol) leaves and seeds showed a difference ($p < 0.0001$) of susceptibility according to the strains. During this, the ethanol extract of the seeds generally exhibits inhibition diameters greater than the same extract of the leaves (Figure 1a). The comparative effect of seeds' ethanol extract and ethyl acetate also shows a variation ($p < 0.0001$) of diameters depending on the strains and the type of extract.

On food strains, it is noted with the leaves ethanol extract a remanence effect for the strains *S. equorum*, *S. saprophyticus* ($p < 0.01$) and *S. haemolyticcus* ($p < 0.001$) after 48 h of incubation (Figure 1d). Similarly for the seeds the same phenomenon was observed for *S. simulans* ($p < 0.01$) strain and *S. lentus, S. haemolyticcus* ($p < 0.0001$) after 48 h of incubation with both the ethanol extract (Figure 1e), and ethyl acetate extract. Note that the ethyl acetate extract of the seeds was more active ($p < 0.0001$) with time with *S. lentus* (Figure 1f). The compared effect of ethanol extract of leaves and seeds show that the leaves has a broad spectrum of action by inhibition of 100% food strains while the seeds ethanol extract is active on 44.44% of the strains. Nevertheless the greatest inhibition diameter obtained with the leaves ethanol extract is 22 ± 0.3 mm (*S. lentus*), whereas that obtained with the seeds' ethanol extract was 28 ± 2.3 mm (*S. haemolyticcus*). Passing leaves to seed, the action of this ethanol extract is not different ($p > 0.05$) for *S. lentus* strains, *S. simulans,* and *S. sciuri*. The comparative action of seeds' ethanol and ethyl acetate extracts shows that the ethyl acetate extract is active on 90% of food strains with a greater inhibition diameter of 24 ± 1.3 mm obtained with *S. lentus*. Also we observed for both type of extract a difference of action ($p < 0.01$) using *S. xylosus* strain and ($p < 0.0001$) *S. equorum* and *S. saprophyticus*.

Minimum inhibitory (MIC) and bactericide (MBC) concentrations of G. kola extracts

Our results show that all extracts have a minimum inhibitory concentration below the starting concentration (20 mg/ml), while for the MBC, some extracts have a concentration ≥ 20 mg/ml (Table 2).

Considering the reference strains, the MICs obtained vary depending on the type of extract and organs. With seeds, a greater sensitivity of the strain *C. albicans* (0.039 mg/ml) is observed to the ethyl acetate extract and *E. foecalis* strain (0.078 mg/ml) to the ethanol extract. With the leaves, the smallest minimum inhibitory concentration (0.156 mg/ml) of the ethanol extract was obtained with *P. aeruginosa* strain. MBC are generally higher than the MIC, but in some cases is equal to the MIC, it is the case of the seeds ethyl acetate extract with *C. albicans* strain which has the greatest sensitivity (MBC = 0.039 mg/ml). Otherwise, to the tested dose, the leaves ethanol and seeds ethyl acetate extracts had no bactericidal effect (MBC > 20 mg/ml) on the *S. epidermidis* strain.

Like with the reference strains, the MIC of meat isolated strains vary depending on the type of extract (Table 2). A greater sensitivity of *S. equorum* to the seeds ethyl acetate extract with an MIC of 0.078 mg/ml was observed. On the contrary, the smallest MIC obtained with the leaves ethanol extract was 0.625 mg/ml (*S. saprophyticcus*) while it was 2.5 mg/ml (*S. sciuri* and *S. haemolyticcus*) with the seeds ethanol extract. In addition, the MBC vary from 0.078 mg/ml to a concentration greater than 20 mg/ml. Indeed, the smallest MBC (0.078 mg/ml) was obtained with the seeds ethyl acetate extract on the *S. equorum*. With the ethanol extract, the lower MBC leaves (1.25 mg / ml) was obtained with the *S. saprophyticcus*, while seeds' (10 mg/ml) was recorded with *S. lentus*, *S. . simulans* and *S. haemolyticcus*. The leaves ethanol extract had no bactericidal effect on *S. simulans* and *S. sciuri* at 20 mg/ml.

Evaluation of bactericidal and bacteriostatic effects of G. kola extracts

The ratio MBC/MIC shows the kind of effect exerted by the extracts on the tested strains. The results of this ratio show that both extracts have bactericidal and bacteriostatic effects on reference strains and on meat

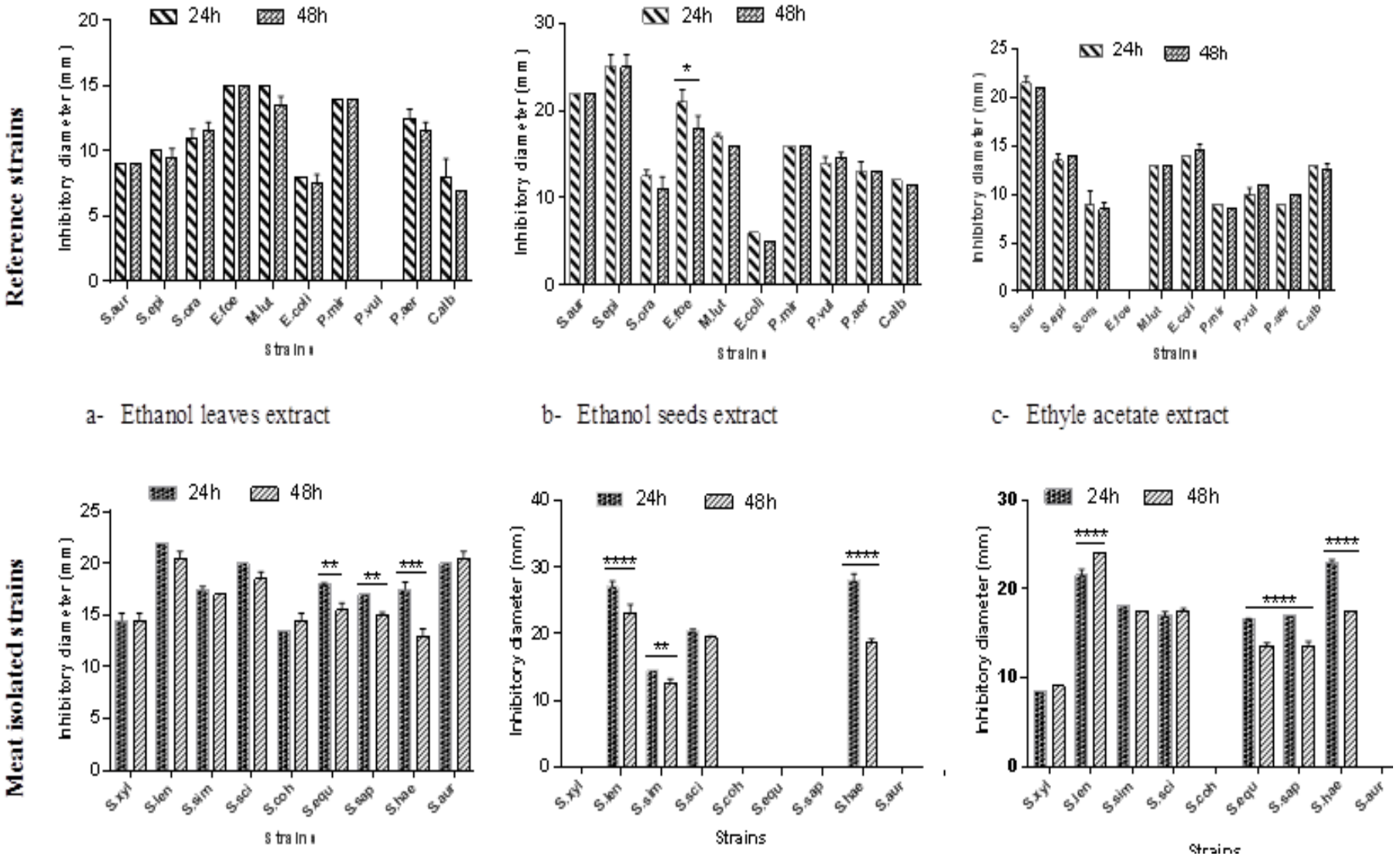


Figure 1. Medium inhibitory diameter zone of *G. kola* extracts on reference and meat isolated *Staphylococcus* strains after 24 and 48 h. For reference strains S. aur, *Staphylococcus aureus*; M. lut, *Miccrococcus luteus*; S. epi, *Staphylococcus epidermidis*; S. ora, *Streptococcus oralis*; P. aer, *Pseudomanas aeruginosa*; E.foe , *Enterococcus foecalis*; P. vul, *Proteus vulgaris*; *E. coli*, *Escherichia coli*; C. alb, *Candida albicans*; P. mir, *Proteus mirabilis ;* For meat isolated strains , S. sci*, S. sciuri*; S. aur*, S. aureus*; S. sim*, S. simulans;* S. xyl*, S. xylosus;* S. coh*, S. cohnii*; S. equ*, S. equorum*; S. sap*, S. saprophyticus*; S. hae*, S. haemolyticus* and S. len*, S. lentus.* *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Table 2. Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations of *G. kola* extracts on reference strains and meat isolated *Staphylococcus* strains.

Strain	MIC (mg/ml)			MBC (mg/ml)		
	$EtOH_{leaves}$	$EtOH_{seeds}$	EAC_{seeds}	$EtOH_{leaves}$	$EtOH_{seeds}$	EAC_{gr}
Reference strains						
S.aur	0.312	0.312	0.152	0.625	1.25	2.5
S.epi	0.625	0.312	0.078	>20	1.25	>20
S.ora	2.5	5	2.5	5	10	10
E.coli	5	5	2.5	5	10	10
E.foe	0.625	0.078	-	1.25	2.5	-
M.lut	1.25	0.156	0.078	2.5	0.156	0.156
P.mir	0.625	0.312	0.625	20	10	2.5
P.vul	-	0.625	0.625	-	5	1.25
P.aer	0.156	2.5	0.312	10	20	5
C.alb	5	0.156	0.039	5	0.156	0.039
Meat isolated *Staphylococcus* strains						
S.xyl	2.5	-	0.625	2.5	-	10
S.len	1.25	5	2.5	2.5	10	20
S.sim	2.5	5	5	>20	10	20
S.sci	2.5	2.5	2.5	>20	20	5
S.coh	1.25	-	-	20	-	-
S.equ	1.25	-	0.078	10	-	0.078
S.sap	0.625	-	0.625	1.25	-	10
S.hae	1.25	2.5	0.625	5	10	10
S.aur	2.5	-	-	10	-	-

EtOH, ethanol extract; EAC, ethyl acetate extract. **For reference strains** , S. aur, *Staphylococcus aureus*; M. lut, *Miccrococcus luteus*; S. epi, *Staphylococcus epidermidis*; S. ora, *Streptococcus oralis*; P. aer, *Pseudomanas aeruginosa*; E.foe , *Enterococcus foecalis*; P. vul, *Proteus vulgaris*; E. coli, *Escherichia coli*; C. alb, *Candida albicans*; P. mir, *Proteus mirabilis ;* **For meat isolated strains** , S. sci*, S. sciuri*; S. aur*, S. aureus*; S. sim*, S. simulans;* S. xyl*, S. xylosus;* S. coh*, S. cohnii*; S. equ*, S. equorum*; S. sap*, S. saprophyticus*; S. hae*, S. haemolyticus* and S. len*, S. lentus.*

isolated *Staphylococcus* (Table 3). With reference strains, the bactericidal effect of extracts decreases as follows: leaves ethanol > seeds ethanol > seeds ethyl acetate. *M. luteus* and *C. albicans* strains were very sensitive to all the tested extracts. With the meat isolated *Staphylococcus*, leaves ethanol extract displays a bactericidal effect on three strains (*S. xylosus, S. lentus* and *S. saprophyticcus*). It can be noted that from these results, the antibacterial activity is more interesting with the leaves.

Antifungal activity of G. kola extracts

The antifungal activity of our extracts was evaluated at mycelial development stage of the three fungal strains. The extracts show an antagonism effect to the growth of the three tested fungal strains. Figure 2 shows the appearance of the plates on *F. verticillioides* after five days of incubation.

Extracts inhibition rate varies depending on the strain and shows that the interaction between the fungal strains and the extracts has a highly significant difference ($p < 0.0001$). The percentage inhibition of the various extracts varies between 38.3 to 78.1% (Figure 3). Considering the susceptibility of the strains toward tested extracts, only the seeds ethanol extract has a difference in action ($p < 0.001$) between *F. verticillioides* and *A. tamari*; the same difference was observed between the strains *F. verticillioides* and *P. citrinium* on the one hand ($p < 0.01$ for leaves ethanol extract and $p < 0.05$ for the seeds ethanol extract) and between *A. tamarii* and *P. citrinium* on the other hand.

Antioxidant activity of *G. kola* extracts

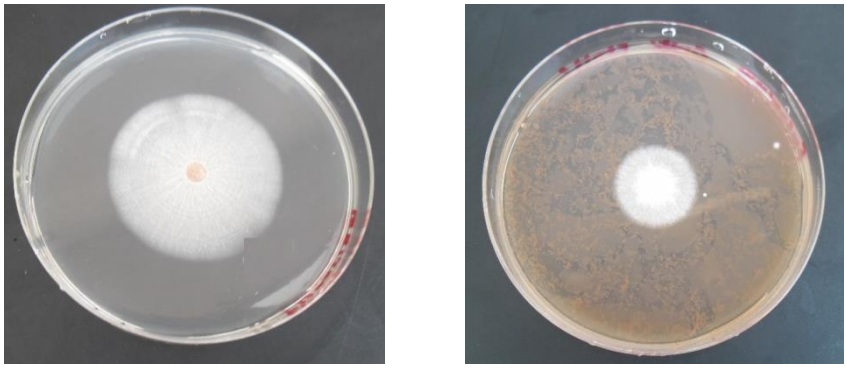
The antioxidant activity profiles using the DPPH radical and $ABTS^{+}$ cation obtained reveal that the extracts possess antioxidant dose-dependent activity (Table 4).

By DPPH method, the results show that seeds' ethyl acetate extract has the lowest concentration (25.03 ±

Table 3. Bactericidal and bactériostatic effect of *G. kola* extracts on reference and meat isolated *Staphylococcus.*

Strain	MBC/MIC		
	Leaves ethanol extract	**Seeds ethanol extract**	**Seeds ethyl acetate extract**
Reference strains			
Staphylococcus aureus	2.003*	4.006	16.44
Staphylococcus épidermidis	>20	4.006	>20
Streptococcus ora	2*	2*	4
Escherichia coli	1**	2*	4
Enterococcus foecalis	2*	32.05	-
Micrococcus luteus	2*	1**	2*
Proteus mirabilis	32	32.05	4
Proteus vulgaris	-	8	2*
Pseudomonas aeruginosa	64.10	8	16.02
Candida albicans	1**	1**	1**
Meat isolated *Staphylococcus* strains			
Staphylococcus xylosus	1*	-	16
Staphylococcus lentus	2*	2*	8
Staphylococcus simulans	-	2*	4
Staphylococcus sciuri	-	8	2*
Staphylococcus cohnii	16	-	-
Staphylococcus equorum	8	-	1*
Staphylococcus saprophyticus	2*	-	16
Staphylococcus haemolyticus	4	4	16
Staphylococcus aureus	4	-	-

With *= Bactericidal effects and without * = Bacteriostatical effects.



a. Culture plate without extract b. Culture plate with extract

Figure 2. Antifungal activity of leaves ethanol extract with *F. verticilloide.*

3.64 µg/µl) to inhibit 50% of DPPH radical, while the leaves ethanol extract displays the largest (lower scavenging activity) IC_{50} value (163.5 ± 14.84 µg/µl). The seeds ethanol extract meanwhile has an intermediate IC_{50} of 84.5 ± 13.43 µg/µl. Compared to reference molecules (gallic acid and the BHA), the antioxidant power of our extracts displays lower values. At the same dose, the antioxidant power of gallic acid is four times higher than BHA. Antioxidant power varies from one molecule to another.

With the ABTS method, the strongest antioxidant activity (22.99 ± 1.34 µmol EqAA/g) of the extracts was obtained with the seeds ethyl acetate extract, while the lowest activity (10.79 ± 2.25 µmol EqAA/g) was recorded with the leaves ethanol extract. The antioxidant ascorbic acid (35.02 ± 0.73 µmol EqAA/g) which is a pure

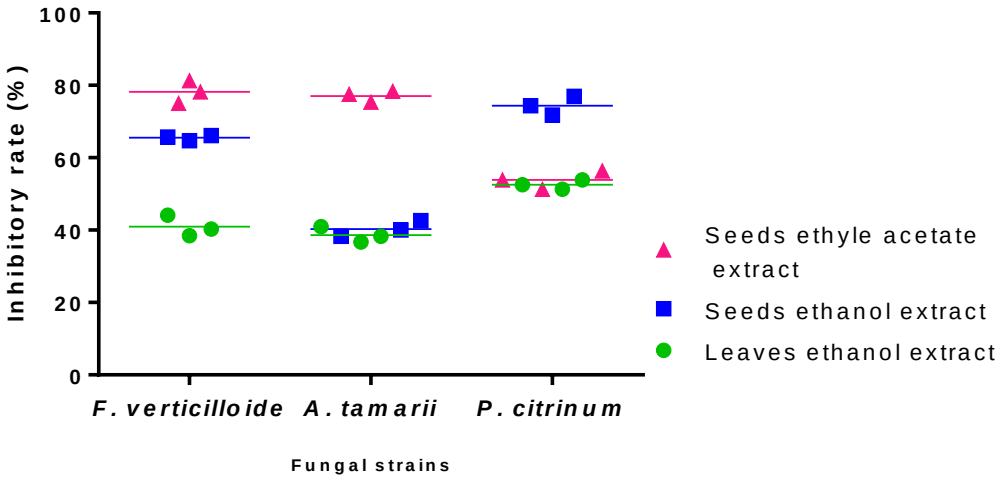


Figure 3. *G. kola* extracts inhibition rate of mycelia development.

Table 4. Free radical scavenging activity results by DPPH radical and $ABTS^+$ cation methods.

Extracts and reference molecules	DPPH		ABTS
	IC_{50} (µg/µl)	IAA	C (µmol EqAA/g)
Seeds ethanol extract	84.5 ± 13.43	0.59 ± 0.09	11.66 ± 0.38
Seeds ethyl acetate extract	25.03 ± 3.64	1.85 ± 0.10	22.99 ± 1.34
Leaves ethanol extract	163.5 ± 14.84	0.30 ± 0.02	10.79 ± 2.25
BHA	4.31 ± 1.10	12.07 ± 2.87	nd
Gallic acid	0.8 ± 0.07	62.74 ± 5.54	nd
Ascorbic acid	nd	nd	35.02 ± 0.73

nd, Not determined.

compound used in this case as a reference molecule is greater than that of our extracts.

However we should note that the antioxidant activity of the extracts follows the same order of effectiveness using the two methods of study and this activity is less high than that observed with the reference molecules.

Artemia salina larvae cytotoxicity test of *G. kola* extracts

The behavior of the larvae introduced in various concentrations of the tested extracts show that the survival of larvae respects a dose-response relation. The dose that induces the death of 50% (LD_{50}) of the larvae was determined from the regression line of each curve. Thus seeds ethyl acetate extract has the lowest LD_{50} of 8.83 mg/ml with a correlation coefficient R^2 = 0.873. This extract is followed by the seeds ethanol extract (LD_{50} = 10.38 mg/ml, R^2 = 0.758). The leaves ethanol extract has the highest concentration (LD_{50} = 10.60 mg/ml, R^2 = 0.655).

DISCUSSION

The phytochemical screening was performed on the powder of three organs (bark, leaves and seeds) in order to highlight the presence of 20 secondary metabolites belonging to four chemical groups. The results reveal, in the nitrogen compounds group, the presence of alkaloids in seeds. This observation is similar to those made in Cameroon by Lacmata et al. (2012). Concerning the phenolic compounds group, our study reveals the presence of tannins with a predominance of gallic tannins (found in the three organs). These results corroborate those of Adesuyi et al. (2012) in Nigeria from the seed of the same species. However, the seeds of *G. kola* were reported, in Nigeria, not to contain tannins (Ghamba et al., 2012). This difference could be explained by the maturity stage of the used fruit before their harvest. Indeed, during a study performed in Congo Brazzaville, Morabandza et al. (2013) demonstrated that the concentration of certain secondary metabolites in the mesocarp of *G. kola* fruit increases with the evolution of maturity. It should be noted that in the three organs, the tannins were

more present in leaves than in seeds and bark. We also note the absence of alkaloids and flavonoids in the leaves. In contrary to those found, Eleazu et al. (2012) showed the presence in a small proportion of alkaloids and flavonoids in the leaves of *G. kola*. This variation can be explained by the difference of those compounds detection method. Beyond tannins, we notice the presence of flavonoid (flovone) and coumarins respectively in the seeds and bark of this plant species. These results corroborate those of Cotterih et al. (1978) when they isolated *G. kola* seeds'; the bioflavonoids called GB1, GB2 and GB1a. Likewise, Okunji et al. (2002) reported that phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybi-flavonols. The cyanogenic derivatives absence is very important because they are the main causes of toxicity due to the production of cyanide ions and manifested by the subsequent mass poisoning from the ingestion of cyanogenic derivatives, acceleration and amplification of breathing, respiratory depression, dizziness, headache, disturbance of consciousness, coma (Bruneton, 1993). From these observations, and from the properties of the flavonoids according to Ortuno et al. (2006), the seeds of *G. kola* could therefore be more advantageously than other organs (bark and leaves) of the plant to be used in the case of inflammations, infections, superficial wound or burn and may allow a reduction in the cholesterol in the body.

Regarding the antimicrobial capacity of the three organs of *G. kola*, the bark extracts (aqueous, ethanol, ethyl acetate), and the aqueous extract of the seeds and leaves had no action on the tested reference microorganisms strains. Our observations are not the same as those made by Indabawa and Arzai (2011) when they showed in their study that the seeds aqueous extract of *G. kola* had antibacterial activity against *S. aureus* at 2000 µg/ml. In the same way, Ghamba et al. (2012) reported that the seeds aqueous extract of *G. kola* had an antimicrobial activity against clinical isolates of *S. aureus*, *P. aeruginosa* and *E. coli* at 50 mg / ml with a mean inhibition diameter of 3.66 ± 0.28 mm. This difference can be explained by the antimicrobial active ingredient concentration in the aqueous extracts of our seeds; these antimicrobial agents in the seeds are not concentrated enough in the aqueous extract, and this may also be due to the origin of the strains. Moreover Burger (1990) reported that no active substance showed its maximum activity in laboratory experiments and the activity can be recorded when a great concentration is used. The organic extracts (ethanol and ethyl acetate) of *G. kola* leaves and seeds have inhibited many microorganisms at 20 mg/ml. The same report has been done on other plants by other authors as Sharmila and Gomathi (2011) in India on *Crossandra infundibuliformis*. Also Bouzid et al. (2011) in Algeria on *Crataegus monogyna* noted that organic extracts (ethanol, methanol dichloromethane and others) were active while the aqueous extract was inactive. These observations can be explained by the solubilization capacity of phyto-molecules in solvents. The activity ratio (CMB/CMI) according to Berche et al. (1991) showed that the ethanol extract of the leaves and seeds has a higher bactericidal activity than the seeds ethyl acetate extract. This difference can be due to the affinity of antimicrobial molecules toward ethanol and ethyl acetate. Indeed during the liquid/liquid extraction, phyomolecules are distributed in the solvents according to their polarity (Cowan, 1999). We can then deduce that ethanol (protic and polar solvent) better dissolves active ingredients contained in the seeds of *G. kola* than ethyl acetate (aprotic and polar solvent).

Through the various extracts, the studied organ of *G. kola* exhibit varied antifungal activity depending on the strains. The comparative effect of the ethanol extract of the two organs (leaves and seeds) showed that the seeds ethanol extract has an inhibition percentage of mycelial growth higher than the leaves one on the three tested fungal strains. The hypothesis that can explains this difference would be the high percentage (50%) of polyphenolic compounds reported, during phytochemical screening, in the seeds in comparison to the leaves (10%). The evaluation of antifungal activity of the same organ extracts shows that the seeds ethyl acetate extract has better anti-fungal activity than the ethanol extract of the same organ on the strains *F. verticilloide* and *A. tamarii*. The large number of polyphenolic compounds extracted with ethyl acetate (Rohman et al., 2010) as well as glycosides (Cowan, 1999) would be a reliable explanation for these observations. Similarly Zareen (2006) showed that the ethyl acetate fraction of *Terminalia glaucescens* rich in polyphenol was more active than other fractions. All these observations come to confirm the previous assumption. Compared to the antifungal specificity, the results testify a difference between the responses of the three fungal strains opposite the extracts studied independently to the extraction organs. Nevertheless, there is a preferential antifungal activity of the extracts according to the strains and plant organs. No conclusions could be drawn from this observation since the difference may be related to the variation of fungi growth.

In this study, the various tested extracts from leaves and seeds showed varying antioxidant activities. The antioxidant activity of *G. kola* seeds has also been proven by Okoko (2009). Likewise Farshori et al. (2013) and Olatunde et al. (2004) report that *G. kola* contains natural antioxidants. Considering the Antioxidant Activity index according to Scherer and Godoy (2009), the results obtained with the extracts showed that any extract possesses a very strong antioxidant activity. Nevertheless of the two organs, the seeds exhibit a more interesting antioxidant activity than leaves. Compared to reference molecules, BHA and gallic acid which showed

potent antioxidant activity (with the respective IC_{50} and AAI of 12.07 ± 2.87 µg/µl and 62.74 ± 5.54 µg/µl); the activity of the extracts is less than that of the reference molecules. It must be remembered that the reference molecules are pure compounds set from the specific active ingredient concentration. In addition, the use of synthetic antioxdants like butylated hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) has been limited due to their toxicity and side effects. Therefore search for the novel sources of natural oxidants will be important for performing such research (Pourmorad et al., 2006). Thus, through its seeds, *G. kola* can be used in the treatment of diseases caused by oxidative stress.

Concerning the extracts cytotoxic test, by referring to the toxicity scale established by Mousseux (1995), extracts LD_{50} is greater than 0.1 mg/ml, value above which the extract is considered exhibiting no toxicity. It appears that all the extracts of the two organs of *G. kola* are not toxic to humans, because there is a correlation between cytotoxicity of shrimp larvae and cytotoxicity in human cells; notably lung cells on one hand and the cells colon on the other hand (José et al., 2002).

Conclusion

The aim of our study was to identify some chemical groups of secondary metabolites present in three organs of *G. kola* and evaluate some of their biological activities including antibacterial, antifungal, antioxidant and cytotoxic activity of the extracts. At the end of this study, it appears that the three organs contain important chemical constituents (flavonoids, tannins, saponins coumarins) and have many biological activities. The different extracts of leaves and seeds presented a broad antibacterial activity on the tested reference strains and some food strains. The ethanol extract of the leaves had the best bactericidal effects while the ethyl acetate extract of the seeds present better antifungal and antioxidant activity. Through their antioxidant property, these organs and particularly seeds may be useful to strengthen the body in oxidative stress situations and prevent various diseases that occur following a radical attack. LD_{50} obtained through the cytotoxic activity allow us to say that the extracts are not toxic.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGMENT

The authors thank UEMOA for the financial support through the project LBTMM-PAES- UEMOA-2012.

REFERENCES

Adefule-Ositelu AO, Adefule AK, Oosa BO, Onyenefa PC (2004). Antifungal activity of *Garcinia kola* nut extract as an ocular bacterial isolates in Lagos. Nig. Q. J. Hosp. Med. 14:112-114.

Adesuyi AO, Elumm IK, Adaramola FB, Nwokocha AGM (2012). Nutritional and phytochemical Screening of *Garcinia kola*. Adv. J. Food. Sci. Technol. 4:9-14.

Ajibola AO, Satake M (1992). Contributions to the phytochemistry of medicinal plants growing in Nigeria as reported in the 1979-1990 literature - A preview. Afr. J. Pharm. Pharm. Sci. 22:172-201.

Akintowa A, Essien A (1990). Protective effects of *Garcinia kola* seed extracts against paracetamol induced hepatotoxicity in rats. J. Ethnopharmacol. 29:207-211.

Akoegninou A, van der Burg WJ, van der Maesen LJG (2006). Flore analytique du Bénin, edited by V. Adjakidj`e, J. P. Essou B. Sinsin, H. Yédomonhan, Wagening University Papers 06.2, Backhus Publishers, pp. 474-475.

Attien P, Sina H, Moussaouie W, Dadié T, Chabi Sika K, Djéni T, Bankole HS, Kotchoni SO, Edoh V, Prévost G, Djè M, Baba-Moussa L (2013). Prevalence and antibiotic resistance of *Staphylococcus* strains isolated from meat products sold in Abidjan streets (Ivory Coast). Afr. J. Microbiol. Res. 7(26):3285-3293.

Bauer AW, Kirby WMM, Sherris TC, Truck M (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45:493-496.

Berche P, Gaillard JL, Simonet M (1991). Bactériologie : Les bactéries des infections humaines, Paris: Flammarion : Médecine & Sciences (ed).

Boumaza A (2009). Effet de l'extrait méthanolique de Zygophyllum cornutum coss contre le stress oxydant associé au diabète sucré et les organes en relation [thèse de Magister], Université Mentouri-Constantine, p. 105.

Bourgeois A (1999). Les MST/SIDA au Cameroun. Diagnostique et traitement. In Biodiagnostic and therapy knowledge for health, Magasine bilingue de santé publique du Cameroun. 4:1-38.

Bouzid W, Yahia M, Abdeddaim M, Aberkane MC, Ayachi A (2011). Evaluation de l'activite antioxydante et antimicrobienne des extraits de l'Aubepine monogyne. Leban. Sci. J. 12(1):59-69.

Braide VB (1991). Pharmacological effect of chronic ingestion of *Garcinia kola* seeds in rats. Phytother. Res. 4:39-41.

Bruneton J(1993). Pharmacognosie, Lavoisier, Paris, Plantes médicinales, 2nd edition. pp. 369-404.

Burger A (1990). The role of natural compounds in Medicinal Chemistry, John Wiley and Sons, 3rd Edition.

Codjia JTC, Assogbadjo AE, Ekué MRM (2009). Diversité et valorisation au niveau local des ressources forestières alimentaires du Bénin. Cahiers Agriculture 32:1-12.

Cotterih P, Scheinmenn F, Stenhuise I (1978). Composition of *Garcinia kola* seeds. J. Chem. Soc. Perkin Trans. 1:532-533.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4):564-582.

Dah-Nouvlessounon D, Adoukonou-Sagbadja H, Diarrassouba N, Sina H, Adjonohoun A, Inoussa M, Akakpo D, Gbenou JD, Kotchoni SO, Dicko MH, Baba-Moussa L (2015). Phytochemical analysis and biological activities of *Cola nitida* bark. Biochem. Res. Int. 2015:1-12.

Dohou N, Yamni K, Badoc A, Douira A (2004). Activité antifongique d'extraits de *Thymelaealythroides*sur Trois champignons pathogènes du riz. Bull. Soc. Pharm. Bordeaux. 143:31-38.

Eleazu CO, Eleazu KC, Awa E, Chukwuma SC (2012). Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. E3 J. Biotechnol. Pharm. Res. 3(2):42-46.

Farshori NN, Al-Oqail MM, Al-Sheddi ES, Siddiqui MA, Rauf A (2013). Antimicrobial potentiality of Polyalthia longifolia seed oil against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Afr. J. Microbiol. Res. 19:1977-1982.

Gangoue-Piéboji J, Baurin S, Galleni M, Ngassam P, Azebaze A, Waktuen P, Pegnyemb DE, Ngameni B, Goffin C, Frere JM (2004). Activité inhibitrice de quelques plantes médicinales camerounaises sur les β-lactamases. Pharm. Méd. Trad. Afr. 13:67-80.

Ghamba PE, Agbo EB, Umar AF, Bukbuk DN, Goje LJ (2012). *In vitro* antibacterial activity of crude ethanol, acetone and aqueous *Garcinia kola* seed extracts on selected clinical isolates. Afr. J. Biotechnol. 11:478-1483.

Guenne S, Ouattara N, Hilou A, Millogo JF, Nacoulma OG (2011).

Antioxidant, enzyme inhibition activities and polyphenol contents of three Asteraceae species used in Burkina Faso traditionnally medicine. Int. J. Pharm. Pharm. Sci. 3(5):524-528.
Houghton PJ, Raman A (1998). Laboratory Hand Book for the Fractionation of Natural Extracts. Chapman and Hall, London, UK.
Indabawa II, Arzai AH (2011). Antibacterial activity of *Garcinia kola* and *Cola nitida* seed extracts. Bayero J. Pure Appl. Sci. Bajopas. 4(1):52-55.
Iwu MM, Igboko O (1982). Flavonoids of *Garcinia kola* seeds. J. Nat. Prod. 45:650-651.
José LC, Zaira L, Hernandez I, Pilar P, Maria D, Garcia G (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BMC Biotechnol. 2:17-35.
Kanmegne G, Ndoumou Omokolo D (2007). Effects of hormones applications on the germination of six populations of *Garcinia kola* Heckel (Guttiferae) seeds. Int. J. Biol. Chem. Sci. 1(3):255-261.
Kawsar SMA, Huq E, Nahar N (2008). Cytotoxicity assessment of the aerial part of Macrotyloma uniflorum Lim. Int. J. Pharm. 4(4):297-300.
Lacmata ST, Kuete V, Dzoyem JP, Tankeo SB, Teke GN, Kuiate JR, Pages JM (2012). Antibacterial Activities of Selected Cameroonian Plants and Their Synergistic Effects with Antibiotics against Bacteria Expressing MDR Phenotypes. Evid. Based Complement. Altern Med. 2012:1-11.
Morabandza CJ, Ongoka RP, Matini L, Epa C, Nkounkou LC, Abena AA (2013). Chemical Composition of the Mesocarp of *Garcinia kola* Heckel (Clusiaceae) Fruit. Res. J. Recent Sci. 2(1):53-58.
Mousseux M (1995). Test de toxicité sur larves de Artemia salina entretien d'un élevage de balanes, [M. DEUST], Université française de Pacifique. Centre universitaire de Nouvelle Calédonie, http://www.documentation.ird.fr/hor/fdi:43863.
N'Guessan JD, Bidie AP, Lenta BN, Weniger B, Andre P, Guede Guina F (2007). *In vitro* assays for bioactivity-guided isolation of antisalmonella and antioxidant compounds in Thonningia sanginea flowers. Afr. J. Biotechnol. 6:1685-1689.
Odeigah PG, Taiwo IA, Akomolafe EO, Durojaiye OO (1999). Hypoglycemic action of medicinal plants with tolbutamide in the albino rats. Diabetes Int. 9:71-73.
Okoko T (2009). Chromatographic characterisation, *in vitro* antioxidant and free radical scavenging activities of *Garcinia kola* seeds. Afr. J. Biotechnol. 8:7133-7137.
Okunji CO, Tantalia AW, Hicks RP, Iwu MM, Skanchy DJ (2002). Capillary electrophoresis determination of biflavonones from *Garcinia kola* in three traditional African medicinal formulations. Plant. Med. 68:440-444.
Olatunde FE, Hansen M, Rain-Haren P, Dragsted LO (2004). Commonly Consumed and Naturally Occuring Dietary Substances affect Bio Makers of Oxidative Stress and DNAdamage in Healthy Rats. Food Chem. Toxicol. 42:1315-1322.
Ortuno A, Baidez A, Gomez P, Acras MC, Porras I, Garcia-Lidon A, Del Rio JA (2006). Citrus paradisi and Citrus sinensis flavonoids. Their influence in the defence mechanism against *Penicillium digitatum*. Food Chem. 98(2):351-358.
Ozsoy N, Can A, Yanardag R, Akev N (2008). Antioxidant activity of Smilax excelsa L. leaf extracts. Food Chem. 110:571-583.
Pietta PG (2000). Flavonoids and antioxidants. J. Nat. Prod. 63:1035-1042.
Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 5:1142-1145.
Razali N, Razab R, Mat Junit S, Abdul Aziz A (2008). Radical scavenging and reducing properties of extracts of cashew shoots (Anacardium occidentale). Food Chem. 111:38-44.
Re R, Pellegrini N, Proteggent A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. 26(10):1231-1237.
Rohman A, Riyanto S, Yuniarti N, Saputra WR, Utami R, Mulatsih W (2010). Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (Pandanus conoideus Lam). Int. Food. Res. J. 17:97-106.
Saha A, Rahman MS (2008). Antimicrobial Activity of Crude Extract from *Calycopteris floribunsa*. Bangladesh J. Microbiol. 25(2):137-139.
Salem HJ (2009). Extraction, identification, caractérisation des activités biologiques des flavonoïdes de Nitraria retusa et synthèse des dérivés acyles de ces molécules par voie enzymatique, [Ph.D, thesis], Institut National Polytechnique de Lorraine, Université de Nancy. pp. 198.
Sanogo R, Diallo D, Diarra S, Ekoumou C, Bougoudogo D (2006). Activité antibactérienne et antalgique de deux recettes traditionnelles utilisées dans le traitement des infections urinaires et la cystite au Mali. Mali Méd. 21:18-24.
Scherer R, Godoy HT (2009). Antioxidant activity index (IAA) by the 2, 2-diphenyl-1- picrylhydrazyl method. Food Chem. 112:654-658.
Schmeda-Hirschmann G, Rodriguez JA, Theoduloz C, Astudillo L, Feresin GE, Tapia A (2003). Free radical scavengers and antioxidants from Peumusboldus Mol. ("Boldo"). Free Radic. Res. 37:447-452.
Sessou P, Farougou S, Azokpota P, Youssao I, Fanou B, Kaneho S, Agniwo B, Yèhouenou B, Ahounou GS, Chabi-Egba M, Sohounhloue D (2012). *In vitro* antifungal activity of essential oil of *Pimenta racemosa* against fungal isolates from wagashi, a traditional cheese produced in Benin. Int. J. Nat. Appl. Sci. 8(1):25-34.
Sharmila N, Gomathi G (2011). Antibacterial, Antioxidant activity and Phytochemical studies of *Crossandra infundibuliformis* leaf extracts. Int. J. Phytomed. 3:151-156.
Zareen S (2006). Phytochemical studies on Terminalia glaucescens, pteleopsis hylodendron and related medicinal plants, [PhD thesis], H.E.J. Research Institute of Chemistry, University of Karachi, p. 204.