EVALUATION OF STAPHYLOCOCCAL ACTIVITY OF *GARCINIA KOLA* ALMONDS

Abstract

The emergence of infectious diseases, particularly staphylococcal infections, treatment failures and the more high cost of treatment of infections caused by resistant staphylococci called to find other care alternatives. This study was initiated to evaluate the antibacterial activity of the aqueous extract from *Garcinia kola* almonds on the *in vitro* growth of *Staphylococcus aureus* strains. The methods of diffusion in agar and liquid media were used for susceptibility testing and MIC and MBC determination. The tests were performed on four strains of *S. aureus* and one reference strain. The minimum inhibitory concentrations of the extracts ranged from 3.12 mg/mL and 12.5 mg/mL and the minimum bactericidal concentrations between 6.25 mg/mL and 25 mg/mL. The lowest value of MIC and MBC was observed with *S. aureus* ATCC 29213 while the greatest value of these same parameters was obtained on *S. aureus* 993C/18 and *S. aureus* 1075C/18. The aqueous almonds extract of *Garcinia kola* had a bactericidal activity on all the strains of *S. aureus* studied. This could justify the use of *Garcinia kola* almonds in the treatment of various diseases in traditional society.

Key words: *Garcinia kola*, aqueous extract, *Staphylococcus aureus*, MIC, MB

INTRODUCTION

Infectious diseases are nowadays the cause of nearly 17 million deaths a year and are a major preoccupation for health workers **(Simporé *et al.*, 2006)**. In developing countries, they account for 45% of deaths, of which 14.2% in Côte d'Ivoire **(Walsh, 2003)**. These infections include severe skin diseases and mucous membranes such as endocarditis and sepsis caused by bacteria of *Staphyloccus* genus **(Duval 1989)**. The prevalence of nosocomial and community-acquired staphylococcal infections is increasing steadily. However, the treatment of these infections has become increasingly difficult because of the emergence of multi-resistant strains **(Mougeot *et al.*, 2001)**. In addition, *Staphylococcus aureus* is currently one of the leading causes of nosocomial infection worldwide because 10-50% of *S. aureus* strains isolated in hospitals are resistant to meticillin, including vancomycin, the glycopeptide used. against methicillin-resistant strains **(Kopp *et al.*, 2004)**. The ability of *S. aureus* to develop multiple resistance to antibiotics increasingly limits therapeutic possibilities and thus poses a serious public health problem **(Accarias, 2014)**. Otherwise, conventional antibiotics used against microbial diseases are expensive, difficult to access by poor people and are still not

effective and appropriate **(Bennet *et al.*, 2000)**. They sometimes have high side effects on human health whose targets are the heart, liver, kidneys, blood **(Odds et al., 2003)**. To fight against pathogenic microorganisms, the search for new natural phytomedicines has become an emergency for ethnoparmacologists, botanists, pharmacists and microbiologists **(Kavitha and Padma, 2008)**. Efforts in this area have focused on plants because of their multiple use by a large portion of the world's population **(Akinnibosun *et al.*, 2008)**. It is within this framework, that we are interested in *Garcinia kola* whose study will contribute to the valorization of the Ivorian medicinal plants. This plant is used by people as an aphrodisiac and also in the traditional treatment of gastritis, stomach upset and many other pathologies **(Odebunmi *et al.*, 2009)**.

I-Material and methods

1.1-Plant material

The plant material used consists of *Garcinia kola* almond powder. These almonds were harvested in August, 2018 on the market of ELIBOU (located on the North Highway about 79 kilometers from Abidjan). The almonds have been grated and dried out of the sun at laboratory temperature (25 to 30 ° C) for 15 days. Once dried, they have been reduced to a fine powder using a GM 300 type Retsch grinder. The powders obtained were stored in sealed flasks. These powder were used for the preparation of plant extracts.

1.2-Bacterial material

Several strains of *S. aureus* were used including one reference strain and four others of different profile provided by the Bio Bank of the Institut Pasteur Côte d'Ivoire (Table I).

Table I: Profile of the bacteria tested.

Strains	**Profile**
S. aureus ATCC 29213	β-lactam reference strains
S. aureus 993C/18	β-lactam resistant strain
S. aureus 1074C/18	Methicellin resistant strain
S. aureus 1075C/18	Wild strain
S. aureus 1076C/18	Wild strain with β-lactam

2-Methods

2.1-Preparation of the aqueous extract

It was carried out according to the method described by **Ackah *et al.* (2008)**. The powder (100 g) of Garcinia kola was dissolved in 1000 ml of distilled water and then homogenized in a Blender at room temperature. The homogenate obtained was first wrung out in a square of white fabric. Then, doubly filtered on hydrophilic cotton and once on whatman paper 3 mm. The filtrate obtained was dried in an oven at 50 ° C. for 48 hours. The mass of extract obtained was stored in sterile, clean, dry flasks then kept out of from heat and moisture. The percentage of the extraction yield was calculated according to the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Mass of dried extract x100}}{\text{Mass of plant powder used}}$$

2.2-Antibacterial tests

2.2.1-Preparation and Seeding of the Concentration Range

The concentration range of the plant extract was prepared in seven test tubes numbered from 1 to 7 by the double dilution method according to a geometric progression of 1/2 reason. In a series of eight hemolysis tubes numbered C1 to C8, 1mL of pure inoculum was introduced. Then, 1mL of plant extract was added to the tubes according to the prepared concentration range. This distribution of plant extract was made so that 1mL of 200 mg/mL plant extract was transferred into the C1 tube. Tube C2 received 1 mL of 100 mg/mL and so on until tube C7 received 1mL of the 3.125 mg/mL solution. The C8 tube received instead of the plant extract, 1 ml of sterile BMH which was used as a growth control. As a result of the volume/volume dilution achieved, the concentration in the tubes was reduced by half. These tubes were incubated at 37°C for 24 hours. **(Toty *et al.*, 2013)**.

2.2.2-Preparation of the bacterial inoculum

The bacterial inoculum was prepared according to the method described by (Toty et al., 2013). The bacterial inoculum was prepared from an isolated 18-hour colony in 10 mL Mueller Hinton broth (MHB) and incubated for 3 to 5 hours at 37°C to obtain a pre-culture. A volume of 0.1

mL was collected and added to 10 mL of BMH twice concentrated. This bacterial suspension is evaluated at about 10^6 cells/mL and constitutes the 10^0 dilution or pure inoculum.

2.2.3-Sensitivity test

The agar diffusion technique was used to study the sensitivity tests. Mueller Hinton medium, poured and dried in a petri dish, was flooded with 3 mL of inoculum. Then, using a sterile die, wells about 6 mm in diameter were drilled into the agar. Each well received 80 µL of the test substance at a concentration of 100 mg/mL. The Petri dishes were incubated at 37°C for 24 hours, after 30 minutes of diffusion at laboratory temperature. The presence or absence of an inhibition zone was observed and the inhibition diameter was measured. Oxacillin was used as a control. The interpretation was made according to **Ponce *et al.* (2003)**.

2.2.4-Antibacterial parameters MIC and MBC.

Minimal Inhibitory Concentration (MIC) was the lowest concentration of the plant extract for which there is no visible growth to the naked eye after 24 hours of incubation. His determination was made by observation of the disorder induced by the growth of the germs present in each tube. From the MIC, the smallest concentration that allows only 0.01% of bacteria in suspension to survive in 24 hours corresponds to CMB. It is determined by spreading on a solid medium of 2 µL of the contents of each tube of concentration greater than or equal to the MIC **(Soussy et al., 2012)**.

2.3-Statistical Analyzes

All results were repeated three times. The data was processed using the Graph Pad Prism 5.0 software (Microsoft, USA). Statistical analysis of the results was performed using Anova One-Way. The value of the averages is accompanied by the standard error on the mean (mean ± SEM).

3-Results

3.1-Extraction

The yield, appearance and color of the aqueous extract of *Garcinia kola* almonds are shown in Table II. This extract in powder form, of brown color obtained a yield of 6.36%.

Table II: Color, appearance and yield of the aqueous extract of *Garcinia kola* (clusiaceae).

	Characteristics		
	Color	Appearance	Yield (%)
aqueous extract	Brown	Powder (not very pasty)	6,36 %

3.2-Antibacterial effects

3.2.1-Sensitivity test

The values of the inhibition diameters of the *G. kola* extract and of the reference molecule are shown in Tables III and IV. The plant extract recorded inhibition diameters ranging from 10.00 ± 0.00 mm to 16.33 ± 0.58 mm. The largest diameter values (16.33 ± 0.58 mm, 15.66 ± 0.58 mm and 15.33 ± 0.58 mm) were obtained with *S. aureus* 993C / 18, *S. aureus* ATCC 29213 and *S. aureus* 1074C / 18 respectively, while *S. aureus* 1076C / 18 and *S. aureus* 1075C / 18 exhibited smaller values (13.33 ± 1.53 mm and 13.00 ± 2.00 mm). These diameters remain lower than those obtained by the reference molecule (oxacillin).

Table III: Inhibition diameters (mm) induced by the aqueous extract

Strains	Concentrations (mg/mL)							
	200	100	50	25	12,5	6,25	3,12	1,56

S. aureus ATCC 29213	15,66 ± 0,58	13,00 ± 0,00	11,67 ± 0,58	10,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 993C/18	16,33 ± 0,58	14,33 ± 0,58	12,00 ± 0,00	11,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 1074C/18	15,33 ± 0,58	12,67 ± 1,15	11,00 ± 1,00	10,00 ± 1,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 1075C/18	13,00 ± 2,00	11,67 ± 1,53	10,67 ±0,53	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 1076C/18	13,33 ±1,53	12,00 ±1,00	11,00 ±1,00	10,00 ±0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00

6,00±0,00: corresponds to the wells diameters

136

137 **Table IV: Inhibition diameters (mm) induced by the antibiotic (Oxacillin)**

Strains	Concentrations (mg/mL)														
	62,50	31,25	15,63	7,81	3,91	1,95	0,98	0,49	0,24	0,12	0,061	0,031	0,016	0,008	0,004
S. aureus ATCC 29213	>52	>52	>52	>52	>52	>52	>52	>52	34 ± 0,00	28 ± 0,00	21 ± 0,00	21 ± 0,00	18 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 993C/18	50 ± 0,00	49 ± 0,00	48 ± 0,00	46 ± 0,00	43 ± 0,00	40 ± 0,00	38 ± 0,00	36 ± 0,00	32 ± 0,00	26 ± 0,00	25 ± 0,00	22 ± 0,00	19 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 1074C/18	39 ± 0,00	39 ± 0,00	35 ± 0,00	35 ± 0,00	33 ± 0,00	32 ± 0,00	30 ± 0,00	28 ± 0,00	28 ± 0,00	26 ± 0,00	22 ± 0,00	18 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 1075C/18	52 ± 0,00	51 ± 0,00	46 ± 0,00	45 ± 0,00	43 ± 0,00	40 ± 0,00	38 ± 0,00	35 ± 0,00	30 ± 0,00	28 ± 0,00	24 ± 0,00	21 ± 0,00	18 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
S. aureus 1076C/18	46 ± 0,00	43 ± 0,00	40 ± 0,00	38 ± 0,00	35 ± 0,00	34 ± 0,00	33 ± 0,00	28 ± 0,00	25 ± 0,00	22 ± 0,00	16 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00

138 6,00±0,00: corresponds to the wells diameters

3.2.2-Determination of antibacterial activity

The results of the antibacterial parameters obtained are mentioned in Tables V and VI. Analysis of the results of the tables revealed that MICs obtained on *S. aureus* strains ranged from 3.12 ± 0.00 mg / mL to 12.50 ± 0.00 mg / mL. The lowest MIC value was observed for *S. aureus* ATCC 29213 (3.12 ± 0.00 mg / mL) and the highest value was obtained with *S. aureus* 993C / 18 and *S. aureus* 1075C / 18 (12.5 ± 0.00 mg / mL). As for MBC, the recorded values ranged from 6.25 ± 00 to 25 ± 00 mg / mL. This made it possible to determine the MBC / MIC ratio. The aqueous extract of *G. kola* obtained MBC / MIC ≤ 2 on all the strains of *S. aureus* (Table V). However, this ratio varied from 2 to 4 with the reference molecule Table VI.

Table V: Antibacterial Parameters of the Aqueous Extract

Strains	Antibacterial parameters (mg/mL)		Ratio efficacy (MBC / MIC)	Effect
	MIC	MBC		
S. aureus ATCC 29213	3,12± 0,00	6,25 ± 0,00	2	Bactericidal
S. aureus 993C/18	12,5± 0,00	25 ± 0,00	2	Bactericidal
S. aureus 1074C/18	6,25± 0,00	12,5 ± 0,00	2	Bactericidal
S. aureus 1075C/18	12,5± 0,00	25 ± 0,00	2	Bactericidal
S. aureus 1076C/18	6,25± 0,00	6,25 ± 0,00	1	Bactericidal

Table VI: Antibacterial parameters of the antibiotic (oxacillin).

Strains	antibactericidal parameters (mg/mL)		Ratio efficacy	Effect
	MIC	MBC	(MBC / MIC)	
S. aureus ATCC 29213	15,63± 0,00	31,25± 0,00	2	Bactericidal
S. aureus 993C/18	7,81± 0,00	31,25± 0,00	4	Bacteriostatic
S. aureus 1074C/18	7,81± 0,00	31,25± 0,00	4	Bacteriostatic
S. aureus 1075C/18	15,63± 0,00	62,5± 0,00	4	Bacteriostatic
S. aureus 1076C/18	3,91± 0,00	7,81± 0,00	2	Bactericidal

4-Discussion

The present study was initiated with the aim of evaluating the antibacterial activity of the aqueous extract of *Garcinia kola* almonds on the *in vitro* growth of *Staphylococcus aureus* strains. During this study, distilled water was used as an extraction solvent. The extraction yield (6.36%) obtained from our study is less than 8.8% recorded by **Yété *et al.* (2015)** during the aqueous extraction of almonds from this same plant. The observed variation in yield could be related to several parameters. Indeed, several authors have reported that the extraction yield may depend on several factors such as the time of harvest of the plant, the plant age, the drying procedure, the solvent, the pH, the temperature, the extraction time and sample composition **(Quy *et al.*, (2014), Stalikas (2007))**. With regard to the antibacterial effects, the results obtained show that the aqueous extract of *G. kola* almonds has an inhibitory activity against the *in vitro* growth of staphylococci with a different degree related to the profile of the strains. All strains of *S. aureus* studied were sensitive to the aqueous *G. kola* extract. These results are similar to those obtained by **Akerele *et al.* (2008)**. These

authors recorded inhibition diameters of 20 ± 2.4 mm on *S. aureus* strains. Overall, *S. aureus* 993C / 18 was more sensitive to the extract studied. However, the inhibition diameters induced by the aqueous extract remain lower than those of the reference antibiotic. Regarding to the measurement of antibacterial activity, it should be recalled that when the MBC / MIC efficacy ratio of an antibacterial substance is less than or equal to two (≤ 2), the latter is described as a bactericidal substance. If the MBC / MIC ratio is greater than two (> 2), then it is called bacteriostatic **(Fauchere and Avril, 2002)**. In view of this principle, the aqueous extract of *G. kola* has a bactericidal effect on all the strains studied. Our results corroborate those of **Morabandza *et al.* (2014)**. These authors have indicated that the aqueous extract of *G. kola* mesocarp has a bactericidal effect on *S. aureus* strains. Similar results were also obtained in the *Harungana madagascariensis* study **(Toty et al., 2013)**. However, the comparison of the performance of our extract with that of the control (oxacillin), indicates that the aqueous extract is bactericidal on all strains of *S. aureus* tested while oxacillin is bactericidal on *S. aureus* ATCC 29213 and *S. aureus* 1076C / 18 but bacteriostatic on the other strains. This indicates that the aqueous extract of *G. kola* has better antibacterial activity than oxacillin. This bactericidal effect of the aqueous extract of *G. kola* could be explained by the presence of secondary metabolites found therein namely alkaloids, anthraquinones, flavonoids, saponosides, tannins, terpenes and steroids **(Morabandza *et al.* 2013)**. Studies have shown that flavonoids are good inhibitors of the sortases, enzymes found in the cytoplasmic membrane of Gram-positive bacteria that catalyze all surface proteins (adhesins and internalins) **(Cushnie and Lamb, 2011)**. According to these authors, epigallocatechin prevents the secretion of coagulase and *S. aureus* α-toxin. Flavonoids also inhibit the release of virulence factors of this bacterium **(Ghedadba *et al.*, 2015)**. The synergistic actions at various levels of the secondary metabolites would be at the base of the antibacterial activity of the extract. The results obtained during this study have justified the use of this plant in traditional medicine.

Conclusion

The *in vitro* study of the aqueous extract of almonds of *G. kola* made it possible to highlight the antibacterial properties of this plant on the growth of the staphylococcal germs studied. The results obtained reveal the presence of antibacterial active principles in the aqueous extract of *G. kola* almonds. The results showed a bactericidal effect of the extract studied on

these strains of *S. aureus*. This bactericidal effect observed is dose dependent. The sensitivity of staphylococcal strains to the aqueous extract of *G. kola* almonds is of great importance in the treatment of pathologies associated with them. The present results justify certain ethnopharmacological uses. They demonstrate that this plant can be used to treat infectious diseases of staphylococcal origin. In view of the results, it would be interesting to undertake studies to evaluate the toxicity and then purify the extract of this plant to consider the development of improved traditional medicines.

Bibliographical references:

Accarias S. Impact du phénotype des macrophages résidents sur la nature de la réponse inflammatoire précoce lors d'une infection par *Staphylococcus aureus*. Thèse de Doctorat, Université de Toulouse, France, **2014**.

Ackah J. A. A. B., Kra A. K. M., Zirihi G. N. & Guédé-Guina F. Evaluation de l'activité antifongique de tekam, un extrait de plante, sur la croissance *in vitro* de *candida albicans*. *Revue Ivoirienne Sciences Technologiques*, **2008;** 11: 119-129.

Akerele J. O., Obasuyi O., Ebomoyi M. I., Oboh I. E. & Uwumarongie O. H. Antimicrobial activity of the ethanol extract and fractions of the seeds of *Garcinia kola* Heckel (Guttiferae). *African Journal of Biotechnology*, **2008;** 7: 169-172.

Akinnibosun H. A., Akinnibosun F. I. & German B. E. Antibacterial activity of aqueous and ethanolic leaf extracts of *Peperomia pellucida* (L.) H. B. & K. (*piperaceae*) on three gram-negative bacteria isolates. *Science World Journal*, **2008;** 3: 33-36.

Bennet D. **M**., **Bhandol P**. **and Leitch I**. **J**. Nuclear DNA amounts in angiosperms and their modern Uses 807 new estimates. *Annals of Botany*, **2000;** 86: 859-909.

Cushnie T.P.T. & Lamb A;J. Recent advances in understanding the antibacterial properties of flavonoïds. Internat J Antimicrob Agents, **2011;** 1–9

Duval J. Evolution des résistances. *In*: LE MINOIR L et VERON M, eds. Bactériologie médicale. Flammarion Paris (France), **1989**.

Fauchere J. L. & Avril J. L. Bactériologie Générale et Médicale, Edition Ellipses, Paris (France), **2002**.

Ghedadba N. Hambaba L. Ayachi A. Aberkane M. C. Bousselsela H. Oueld-Mokhtar S. M. Polyphénols totaux, activités antioxydante et antimicrobienne des extraits des feuilles de Marrubium deserti de Noé. Phytothérapie, **2015;** 1-12.

Kavitha D. & Padma P. R. Biotherapeutic potential of flower and bark extracts of *Couroupita guianensis* Aubl. *Plant Archives*, **2008;** 8: 569-771.

Kopp B. J., Nix D. E. & Armstrong E. P. Clinical and economic analysis of methicillin susceptible and -resistant *S. aureus* infections. *Annals of Pharmacotherapy*, **2004;** 38: 1377-1382.

Morabandza C. J., Okemy A. N., Ongoka R. P., Okiemy-Akieli M. G., Attibayeba A. & Abena A. A. Effets antimicrobiens, anti-inflammatoires et antioxydants du mésocarpe de *Garcinia kola* Heckel (Clusiaceae). *Phytothérapie*, **2014;** 12: 164-169.

Mougeot C., Guillaumat-Taillet .Y. & Libert J. M. *Staphylococcus aureus*: nouvelle détection de la résistance intrinsèque par la methode de diffusion. *Pathologie biologie*, **2001;** 49: 199-204.

Odds F.**C**., **Brown A**.**J**.**P**., **Gow N.A.R**. Antifungal agents: Mechanisms of action. *Trends in Microbiology*, **2003**; 11: 272-279.

Odebunmi E. O., Oluwaniyi O. O., Awolola G. V. & Adediji O. D. Proximate and nutritional composition of kola nut (*Cola nitida*), bitter cola (*Garcinia kola*) and alligator pepper (*Afromomummelegueta*). *African Journal of Biotechnology*, **2009;** 8: 308-310.

Ponce A. G., Fritz R., Del Alle C. & Roura S.I. Antimicrobial activity of essential oil on the native microflora of organic Swiss chard. *Lebensmittel-Wissenschaft und Technologie*, **2003;** 36: 679-684.

Quy Diem Do., Angkawijay A. E., Tran-Nguyen P. L., Huynh L. H., Soetaredjo F. E., Isadj S., & Ju Y-H. Effect of extraction solvent on total phenol content, total flavonoids content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug analysis*, **2014;** 22: 296-302.

Simporé J., Pietra V., Savadogo A., Pignatelli S., Nikiema J. B., Nadembega W. M., Yara J., Zoungrana N., Bakouan D., Colizzi V., Castelli F. & Musumeci S. Reduction of mother in to child transmission of HIV at Saint Camille Medical Centre in Burkina Faso. *Journal of Medical virology*, **2006;** 78: 148-152.

Stalikas C. D. Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science*, **2007;** 30: 3268-3295.

Soussy C. J., Cavallo J. D., Courcol R., Drugeon H., Fabre R. & Jarlier V. Sensibilité aux antibiotiques des souches d'*Escherichia coli* isolées en 1998 et 1999: résultats d'une enquête multicentrique française. *Médecine et Maladies Infectieuse*, **2000;** 30: 650-656.

Toty A. A., Guessennd N., Bahi C., Kra A. M., Otokore D. A. & Dosso M. Évaluation *in-vitro* de l'activité antibactérienne de l'extrait aqueux de l'écorce de tronc de *Harungana madagascariensis* sur la croissance de souches multi-résistantes. *Bulletin de la Société Royale des Sciences de Liège*, **2013;** 82: 12-21

Walsh C. Antibiotics: actions, origins, resistance. *American Society for Microbiology*, Washington, D. C (USA), **2003**.

Yété P., Togbé A., Koudoro Y., Agbangnan P., Ndahischimiye V., Djènontin T. S., Wotto D, Azandégbé E. C. & Sohounhloue D. Etude comparative des Composés phénoliques et activité antiradicalaire des extraits des Graines de *Garcinia kola* (Guttifféraea) et de *Cucumeropsis edulis* (cucurbitacéae) du Bénin. *International Journal of Innovation and Scientific Research*, **2015;** 15: 217-227.