

In Vitro Toxicity Test of *Strychnos johnsonii* (Loganiaceae) on a Strain of *Staphylococcus aureus*

Cecile Okalla Ebongue^{1,2,*}, Fanny Aimee Essombe Malolo³, François Eya Ane Meva³, Lidwine Ngah³, Jean Claude Ndom⁴, Emmanuel Mpondo Mpondo³

¹Clinical biology Laboratory, General Hospital of Douala, Douala, Cameroon

²Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon

³Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon

⁴Department of Biological Sciences, Faculty of Sciences, University of Douala, Douala, Cameroon

Email address:

cecileokalla@yahoo.fr (C. O. Ebongue)

To cite this article:

Cecile Okalla Ebongue, Fanny Aimee Essombe Malolo, François Eya Ane Meva, Lidwine NGAH, Jean Claude Ndom, Emmanuel Mpondo Mpondo. In Vitro Toxicity Test of *Strychnos johnsonii* (Loganiaceae) on a Strain of *Staphylococcus aureus*. *Journal of Diseases and Medicinal Plants*. Vol. 1, No. 3, 2015, pp. 48-52. doi: 10.11648/j.jdmp.20150103.12

Abstract: The aim of this study was to test the in vitro toxicity of an extract of *Strychnos johnsonii* (Loganiaceae) on a strain of *Staphylococcus aureus*. The tests were performed in the bacteriology unit of the Douala General Hospital biology laboratory, dealing with an extract from the bark of stem of *Strychnos johnsonii* (Loganiaceae) harvested at Etome village, South West Cameroon and authenticated by a botanist. The plant extract was obtained by maceration in 300 mL of ethanol for 120 hours. The filtrate obtained was evaporated under vacuum, at 50° C, 250 mbar of pressure and at a speed of 125 rounds per minute. The residual solvent was eliminated in an incubator at 37° C for one week to give dry extract. Selected bacterial strain came from pus collected from an in-patient. By its biochemical and enzymatic characters, this strain showed 90.9% homology with the *Staphylococcus aureus* ATCC 29213-reference strain. No bacterial growth were observed on Mannitol Salt, EMB and Sabouraud-Chloramphenicol agar plates after 48 hours of incubation, evidence that the extract contained no germs before the test. The number of initial colonies for the time t0 averaged 225. The point of intersection between the inhibition curve and the x-axis as the MIC corresponds to 0,04 g/mL. The smallest concentration of the extract for which the growth of *Staphylococcus aureus* is zero on the Mannitol salt agar was 0.04 g/mL. Therefore, the MIC amounts to MBC. The results obtained showed a bactericidal effect, which could be, attributed to the presence of indole alkaloids in the plant.

Keywords: *Strychnos johnsonii*, In Vitro Toxicity, *Staphylococcus aureus*

1. Introduction

Worldwide, the use of traditional medicine is widespread and his health and economic importance is increasing. It is estimated that in Africa, 80% of the populations are using medicinal plants to heal, often because of the lack of access to medicines prescribed by modern medicine but also because these plants are effective [1]. Ethno botany and the Ethno pharmacology are working to identify active deemed plants and that it belongs to the modern research to specify properties and validate uses. Research of new pharmacologic agents undertaken within the plant biodiversity through the screening of natural sources, has enabled the discovery of a large number of drugs that play a major role in the treatment of many human diseases; As examples, the quinine from *Cinchona ledgeriana*, and artemisinin extracted from

Artemisia annua (antimalarial), vinblastine extracted from *Catharanthus roseus* (anti-cancer) [1,2]. Some of these plants are present in Cameroon, a Central African country located in the Gulf of Guinea, in which most of the southern part consists of forest.

Among Cameroonian medicinal plants, the species *Strychnos johnsonii* has interesting pharmacological potential molecules. As revealed in some studies including in central Africa, the *Strychnos* genus is rich in alkaloids, molecules with cytostatic, anti parasitic, antibacterial properties [3-7]. It is part of the large family of Loganiaceae, which has 394 species identified, with around 300 distributed in African's savannas and forests [8,9].

Wood, stem and root bark are the parts of the plant used in

decoction, maceration or infusion for their purgatives, emetic, anti parasitic, antipyretic and analgesics effects [9,10]. In Cameroon, *Strychnos johnsonii* is used as antipyretic. Fever is the major symptom of pathology of viral, parasitic and / or bacterial origin; a decrease in the bacterial load due to the effect of *Strychnos johnsonii* extract may justify its use as a febrifuge [11]. Our study was designed to test the in vitro toxicity of an extract of *Strychnos johnsonii* on a strain of *Staphylococcus aureus*.

2. Materials and Methods

We conducted a study of in vitro toxicity in the bacteriology unit of the Douala General Hospital laboratory, using an extract from the bark of the stem of *Strychnos johnsonii* (*Loganiaceae*) harvested at Etome village, South West Cameroon and authenticated by a botanist.

2.1. Preparation of Plant Extract

The plant extract was obtained by maceration of a quantity equivalent to 100 g of powdered bark of stem in 300 mL of ethanol for 120 hours.

The filtrate obtained was evaporated under vacuum, at 50°C, 250 mbar of pressure and at a speed of 125 revolutions per minute in a rotative evaporator (rotavapor HEIDOLPH G1™) associated with a water bath and a vacuum pump

HEIDOLPH™. The residual solvent was eliminated in an incubator at 37°C for one week to give 1.675 g of dry extract.

2.2. Control of Sterility of the Extract

This extract has undergone scrutiny of sterility by seeding 75 mg, diluted in the Müller Hinton broth and incubated 48 hours at 37°C on Mannitol salt and Eosin Methylene Blue (EMB) agar plates, and at 30°C on Sabouraud-Chloramphenicol agar.

2.3. Selection of the Bacterial Strain

Selected bacterial strain came from pus collected from a hospitalized patient. It had been identified by automatic colorimetric reading of an ID32 STAPH® gallery on Mini API™ (BIOMÉRIEUX SA France) after plating onto Mannitol salt agar and incubation at 37°C for 24 hours. As a result of its biochemical and enzymatic characteristics, this strain showed 90.9% homology with the *Staphylococcus aureus* ATCC 29213-reference strain.

We then performed a susceptibility test of this strain using the ATB STAPH® gallery with automatic turbidimetric reading on Mini API™. Biochemical profile and the results of the susceptibility tests are presented in tables 1 and 2 respectively.

Table 1. biochemical profile of selected *Staphylococcus aureus* stream.

Character	URE	NIT	ADH	VP	ODC	β GAL	ESC	ArgA	GLU	PAL	FRU	PyrA	MNE
Result	+	+	-	+	-	-	-	-	+	+	+	-	+
Character	TUR	MAN	ARA	RAF	β GUR	NOVO	MAL	SAC	LAC	NAG	TRE		
Result	+	+	-	-	-	-	+	+	-	+	+		

URE: urea, NIT: nitrate, ADH: Arginine dehydrogenase, VP: Voges Procaert, ODC: Ornithine decarboxylase, β GAL: β-Galactosidase, ESC: Esculine, ArgA: Arginine, GLU: Glucose, PAL: Naphthylphosphate, FRU: Fructose, PyrA: pyroglutamic β Acid, MNE: Mannose, TUR: Turanose, MAN: Mannitol, ARA: Arabinose, RAF: Raffinose, β GUR: β-glucuronide, NOVO: Novobiocine, MAL: Maltose, SAC: Saccharose, LAC: Lactose, NAG: N - Acetyl - glucosamine, TRE: Trehalose

Table 2. Susceptibility profile of selected *Staphylococcus aureus* stream.

ATB	PEN	OXA	KAN	TOB	GEN	ERY	LIN	CLI	PRI	QDA		
Result	S	S	S	S	S	R	S	S	S	S		
ATB	FUC	FOS	FUR	TSU	VAN	TEC	TET	MIN	LVX	OFL	LNZ	
Result	S	S	S	S	S	S	S	S	S	S	S	

PEN: Penicillin G, OXA: Oxacilline, KAN: Kanamycin, TOB: Tobramycin, GEN: Gentamycin, ERY: Erythromycin, LIN: Lincomycine, CLI: Clindamycin, PRI: Pristinamycine, QDA: Quinupristine – Dalfopristine, TET: Tetracycline, MIN: Minocycline, LVX: Levofloxacin, OFL: Ofloxacin, LNZ: Linezolid, FUC: fucidic Acid, FOS: Fosfomycine, FUR: Nitrofurantoïne, TSU: Cotrimoxazole, VAN: Vancomycine, TEC: Teicoplanine
ATB = antibiotic R = resistant S= sensitive

Table 3. Protocol for the preparation of test samples.

Tubes	T	D ₁	D ₂	D ₃	D ₄	D ₅
NaCl 9‰	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
Inoculum test	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Extract	0 mL	2 mL	1 mL	0,5 mL	0,25 mL	0,125 mL
Mueller Hinton broth	7 mL	5 mL	6 mL	6,5 mL	6,75 mL	6,875 mL

Table 4. Results of the *in vitro* toxicity test of *Strychnos johnsonii* extract on a susceptible to methicillin *Staphylococcus aureus* stream.

Tubes	Concentration of extract (g/mL)	Results of inhibition t ₁ = 24 Hours	Results of inhibition t ₂ = 48 hours
T	0	Uncountable colonies	Uncountable colonies
D ₁	0,08	0 colonies	0 colonies
D ₂	0,04	0 colonies	0 colonies
D ₃	0,02	86 colonies	0 colonies
D ₄	0,01	Uncountable colonies	Uncountable colonies
D ₅	0,005	Uncountable colonies	Uncountable colonies
D ₆	0,0025	Uncountable colonies	Uncountable colonies

2.4. Preparation of the Test Inoculum

Cultures of 24 hours from the strain of *Staphylococcus aureus* diluted in 5ml of saline water were used to prepare a suspension of 2 McF according to the breakpoints of the French Society of Microbiology. This suspension was diluted to 1/10th to obtain a concentration of inoculum test for about 6×10^8 germs / mL [12].

2.5. Testing

In 6 tubes numbered from D1 to D5 and a control T tube, were introduced successively: 2mL of saline solution (9% NaCl), 1 mL of the test inoculum, and with the exception of the control tube T, concentrations of extract of *Strychnos johnsonii* following a dilution of geometric progression of reason $\frac{1}{2}$ adjusted to 10 mL adding Mueller Hinton broth. Ten μ L of each dilution were then inoculated on Mannitol salt agar and incubated at the time t₀ = 0 hours, t₁ = 24 hours and t₂ = 48 hours, at 37°C, at the rate of two plates per dilution [13].

2.6. Determination of MIC and MBC

The MIC (Minimum Inhibitory Concentration) components are defined as the smallest concentration of extract for which no growth is visible to the naked eye. In this study, the growth was not clearly visible because of the opacity of the extract, the MIC was then been defined as the

meeting point of intersection of the bacterial inhibition curve with the x-axis within a period of 24 hours [14]; and the MBC (Minimum Bactericide Concentration), the smallest concentration of extract for which no bacterial growth was observed on Mannitol Salt agar [13]. The count was conducted by the «Standard Plate Count» technique using the following formula:

$$N = \Sigma C / V (n_1 + 0,1n_2) d$$

N: number of colonies

C: sum of colonies counted on all selected plates of two successive dilutions and which contains at least 15 settlements and not more than 150 colonies.

V: volume of the inoculum applied to each plate, in ml;

n₁: number of plates selected at the first dilution;

n₂: number of plates selected at the second dilution;

d: dilution rate corresponding to the first chosen dilution.

3. Results

3.1. Sterility Control of the Extract

No bacterial growth were observed on Mannitol Salt, EMB and Sabouraud-Chloramphenicol agar plates after 48 hours of incubation, evidence that the extract contained no germs before the test.

3.2. Determination of MIC and MBC

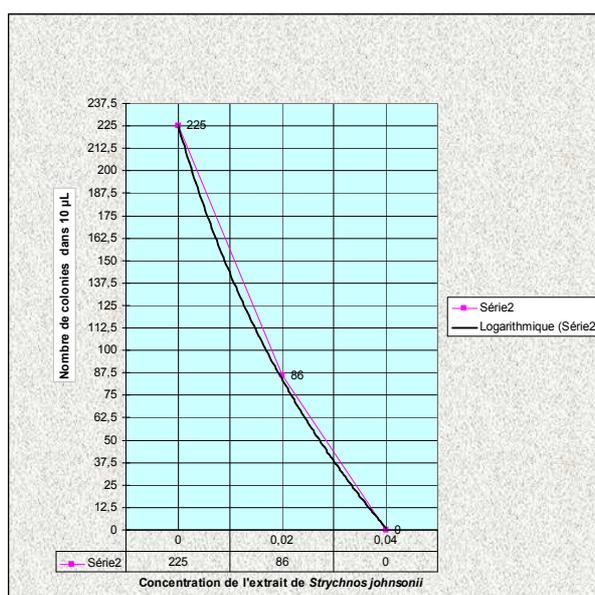


Figure 1. Inhibition curve of *Staphylococcus aureus* according to *Strychnos johnsonii* extract concentration

The number of initial colonies for the time t0 averaged 225. Table 4 presents the results of the in vitro toxicity test of the extract from the bark of *Strychnos johnsonii* on *Staphylococcus aureus*. The point of intersection between the inhibition curve and the x-axis as the MIC corresponds to 0,04 g/mL (Figure 1). The smallest concentration of the extract for which the growth of *Staphylococcus aureus* is zero on the Mannitol salt agar was 0.04 g/mL. Therefore, the MIC amounts to MBC.

4. Discussion

The extract from the bark of *Strychnos johnsonii* stem is active on *Staphylococcus aureus* in vitro. The method used for the determination of the MIC due to an inability to appreciate the absence or the presence of the cloudiness in the tubes, has already been applied for the determination of MIC and MBC of some antibiotics in milk solutions, on bacteria isolated from bovine mastitis [14]. Regardless of the time of exposure of the bacteria to the extract, bacterial growth increases from 0.01 g/mL concentration of the extract is no longer possible from 0.04 g/mL. It decreases at a concentration of 0.02 g/mL and vanish over time.

The MBC/MIC equal to 1 report characterized a bactericidal power [15]. This bactericidal effect seems to depend on the concentration and the time of exposure of the bacteria to extract. It can be attributed to the presence of indole alkaloids. Indeed, some indole alkaloids of the Loganiaceae family are active in bacteria, the example of the Dihydrocorynantheol and the active Tetrahydroalstonine on the Gram + and already isolated bark of stem of *Strychnos johnsonii* [16-19].

Unlike twigs, the extract from the bark of *Strychnos johnsonii* stem is active on *Staphylococcus aureus* [17]. Indeed, some indole alkaloids of the Loganiaceae family are active in bacteria. Their antibacterial action is not conferred to their cycles itself, but rather to oxygen-containing groups (-OH, R-OH, -COOCH₃, -OCH₃) present on these cycles [17].

5. Conclusion

This study was to test the in vitro toxicity of an extract from Cameroonian *Strychnos johnsonii* on a strain of *Staphylococcus aureus* in order to justify its use as febrifuge plant. The results obtained showed a bactericidal effect, which could be, attributed to the presence of indole alkaloids in the plant.

Ethical Considerations

We obtained the authorisation of the Ethics Committee of the General Hospital of Douala.

References

- [1] World Health Organization. (2002). Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005.
- [2] Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, 27(1), 1-93.
- [3] Sapi, J. "G. Massiot in The Alkaloids: Chemistry and Pharmacology Vol. 47." (1995): 173-226.
- [4] Tchinda, A. T., Ngono, A. R., Tamze, V., Jonville, M. C., Cao, M., Angenot, L., & Frédérick, M. (2012). Antiplasmodial alkaloids from the stem bark of *Strychnos malacoclados*. *Planta medica*, 78(4), 377-382.
- [5] Cao, R., Peng, W., Wang, Z., & Xu, A. (2007). β -Carboline alkaloids: biochemical and pharmacological functions. *Current medicinal chemistry*, 14(4), 479-500.
- [6] Rajesh P, Rajesh Kannan V, Latha S, Selvamani P. Phytochemical and Pharmacological Profile of Plants belonging to *Strychnos* Genus. Bioactive Phytochemicals: Perspectives for Modern Medicine. 2014; 1:275 – 321.
- [7] Philippe, G., Angenot, L., De Mol, P., Goffin, E., Hayette, M. P., Tits, M., & Frédérick, M. (2005). In vitro screening of some *Strychnos* species for antiplasmodial activity. *Journal of ethnopharmacology*, 97(3), 535-539.
- [8] Leeuwenberg AJM. Flore du Cameroun. Museum National d'histoire naturelle. 1972; 16(12): 90, 140.
- [9] Bisset, N. G. (1970). The African species of *Strychnos*, Part I. The ethno botany. *Lloydia*, 33, 201-43.
- [10] Denoël A, Jaminet F, Detilleux G, Van Sumsen M, Merveille L. Contribution à l'étude phytochimique des *Strychnos* du Congo Belge. Ministère des colonies, Direction de l'agriculture, Bruxelles: Imifi; 1953. 208p
- [11] Sandberg, F., Lunell, E., & Ryrberg, K. J. (1969). Pharmacological and phytochemical investigations of African *Strychnos* species. *Acta Pharmaceutica Suecica*, 6(1), 79-102.
- [12] Comité de l'Antibiogramme de la Société Française de Microbiologie. Recommandations [en ligne]. 2014 [consulted on 30th may 2014]. Disponible: <http://www.Sfm-microbiologie.org>, CASFM_EUCAST_V1_0_2014.pdf.
- [13] Larcher C, détermination de la sensibilité aux antibiotiques, détermination de la CMI. [On line]. 2008 [consulted on 15th February 2014]. http://christelle.larcher.free.fr/IMG/pdf/determination_CMI-tr.pdf
- [14] GANIERE, J. P., Mangion, C., & Péridy, M. (2004). Détermination des Concentrations Minimales Inhibitrices ET Bactéricides de la cefquinome, la marbofloxacine, la tylosine et la spiramycine en solution dans du lait vis-à-vis de bactéries isolées de mammites bovines. *Revue de médecine vétérinaire*, 155(8-9), 411-416.
- [15] Talbert M, Willoquet G, Gervais R. Le guide de Pharmacoclinique [en ligne]. Reuil-Malmaison: Wolters Kluwer France; 2009. [Consulted on 20th April 2014].

- [16] Verpoorte R, Kodde EW, Doorne H Van, Baerheim Svendsen A. Antimicrobial active Alkaloids from *Strychnos afzelii*. *Planta medica* 1978; 33:237–242
- [17] Verpoorte, R., Van Beek, T. A., Thomassen, P. H. A. M., Aandewiel, J., & Svendsen, A. B. (1983). Screening of antimicrobial activity of some plants belonging to the Apocynaceae and Loganiaceae. *Journal of ethnopharmacology*, 8(3), 287-302.
- [18] Massiot G, Thepenier P, Jacquier MJ, Men-Olivier LL, Verpoorte R, Delaude C. Alkaloids of *Strychnos johnsonii*. *Phytochemistry* 1987; 26(10): 2839-2846.
- [19] Buckingham J, Baggaley KH, Roberts Andrew D, Szabo LF. *Dictionary of Alkaloids*. Second edition. 2009. 2374p.