



Received on 03 November 2014; received in revised form, 09 January, 2015; accepted, 28 February, 2015; published 01 July, 2015

## IN VITRO ANTIBACTERIAL POTENTIAL OF GLYCOSIDIC AND AGLYCONIC CRUDE EXTRACTS OF *CASSIA SIEBERIANA* DC. (CESALPINIACEAE) AND *KHAYA GRANDIFOLIOLA* C.DC. (MELIACEAE): A COMPARATIVE SURVEY

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### Keywords:

Ivorian traditional pharmacopoeia, glycoside, aglycone, antibacterial potential, *in vitro*

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**ABSTRACT:** *Cassia sieberiana* and *Khaya grandifoliola* are two plants from Ivorian traditional pharmacopoeia. In this study, we have compared *in vitro* antibacterial potential of glycosidic and aglyconic crude extracts from both plant species against 3 reference bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* ATCC 25923) and 4 others clinical (*Proteus mirabilis*, *Salmonella sp*, *Shigella sp*, *Vibrio cholerae*) isolated from pathological specimen at hospital. The results have revealed on all tested bacteria, a clear antibacterial potential of aglycones compared to glycosides. Indeed, at same concentrations, total aglycones of each plant have inhibition zones more extensive than their corresponding glycosides, and their MIC are clearly lower against bacterial strains. Moreover, we note that the aglycones are active against *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* 27853 while when the glycosides are inactive. This suggests that in the glycoside, the sugar moiety inhibits the antibacterial action.

**INTRODUCTION:** The discovery and the use of antibiotics are the cause of the greatest success of modern medicine. Today, the emergence and the spread of multi resistance bacteria to antibiotics in human populations have become a very worrying public health<sup>1</sup>. In sub-Saharan Africa, and particularly in Côte d'Ivoire, many cases of multi resistance to antibiotics have been reported<sup>2</sup>. Faced with proven bacterial antibioresistance, it is urgent to explore various potential strategies that could lead to the discovery of new families of more effective and less expensive antibiotics.

Although combinatorial chemistry techniques have succeeded as methods of optimizing structures and have been used very successfully in the optimization of many recently approved agents, the utility of natural products as sources of novel structures, but not necessarily the final drug entity, is still alive and well<sup>3</sup>.

The study of secondary metabolites from plant species in the search for new therapies to counter the bacterial antibioresistance, is an interesting strategy and is topicality. Therefore, we are interested in *Cassia sieberiana* and *Khaya grandifoliola*, two medicinal plants commonly used in traditional therapy in Côte d'Ivoire, which according to some ethno pharmacological informations, collected from healers, treat bacterial diseases. Both plants have been subject of some studies<sup>4-12</sup>. However, for biological as much as

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.6(7).2728-33</p> <hr/> <p>Article can be accessed online on: <a href="http://www.ijpsr.com">www.ijpsr.com</a></p>
<p><b>DOI link:</b> <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.6(7).2728-33">http://dx.doi.org/10.13040/IJPSR.0975-8232.6(7).2728-33</a></p>	

phytochemical works on root bark and trunk bark respectively, *C. sieberiana* and *K. grandifoliola* are poorly documented. Our study reports a comparative investigation of *in vitro* antibacterial activity of glycosides and aglycones crude extracts obtained from *C. sieberiana* and *K. grandifoliola* against bacterial strains.

## MATERIALS AND METHODS:

All chemicals used were of analytical grade.

### Collection of plant materials:

*Cassia sieberiana* root bark and *Khaya grandifoliola* trunk bark were collected from Vavoua, a town in central-western of Côte d'Ivoire. They were authenticated at the Centre National de Floristique (CNF) at the University Félix Houphouët-Boigny (Côte d'Ivoire) by Professor Laurent AKE-ASSI, an emeritus botanist. Fresh drugs were dried in the open air under cover the sun for 30 days, then cut and pounded in a mortar and then sieved. The plant powders obtained were sampled and subjected to extraction to give the crude extracts to be analyzed.

### Preparation of the glycosidic crude extracts:

A decoction of 250 g of plant powder is carried out in 500 ml of distilled water for 30 min. After filtration, the decoction was concentrated in a rotary evaporator under reduced pressure. The concentrate is then dried in a sterilizer (50° C) for 72 hours to give the glycosidic crude extracts encoded CS1 (for *C. sieberiana*) and KG1 (for *K. grandifoliola*).

### Preparation of the aglyconic crude extracts:

150 g of plant powder introduced into 500 ml of HCl (2N) were heated at reflux for 40 min. After cooling and filtration, the aglycones are extracted by ethyl acetate. The organic phase is dried over anhydrous MgSO<sub>4</sub>, and then concentrated to dryness in a rotary evaporator to give the aglyconic crude extracts identified CS2 (for *C. sieberiana*) and KG2 (for *K. grandifoliola*).

### Antibacterial tests:

The tests were performed *in vitro* at the Institut Pasteur (IP) of Côte d'Ivoire, against 3 reference bacterial strains and 4 clinical isolates, which were provided by the laboratory of Bacteriology-Virology of IP. The reference antibiotics (BIOMEDIS) used as positive control, were analytical grade (**Table 1**).

TABLE 1: TESTED BACTERIAL STRAINS

Bacterial strain	profil	origin	Gram	Reference antibiotic
<i>S. aureus</i> ATCC 25923	sensitive	reference	Positive	Oxacillin (OX) Cefoxitin (FOX) Vancomycin (VAN) Ceftazidime (CAZ)
<i>P.aeruginosa</i> ATCC 27853	sensitive	reference	negative	Imipenem (IPM) Tricaricillin+Clavulanic acid (AMC)
<i>E. coli</i> ATCC 25922	sensitive	reference	negative	
<i>P. mirabilis</i> n° 1940Y/13	wild	Pus	negative	
<i>Salmonellasp</i> n° 1585C/13	Wild	Pus	negative	Amoxicillin+Clavulanic acid (AMC)
<i>Shiguelia sp</i> n° 929C/12	Wild	Stools	negative	Cetotaxime (CTX) Aztreonam (ATM)
<i>V. cholerae</i> n° 2029C/12	Wild	Stools	negative	

**Sensitivity tests:** Inhibition of *in vitro* bacterial growth by CS1, CS2 KG1, and KG2 was performed by the agar diffusion method<sup>13</sup>. The Mueller-Hinton (MH) agar plates were seeded by flooding with standardized suspension of the bacterial test. Using a sterile pipette, the wells of 6 mm in diameter, were cut into the agar. Each well

received 80µl of plant extract at various concentrations (200, 100, 50, 25mg/ml). After 30 min of diffusion at room temperature, the plates were incubated at 37°C for 18 to 24 hours. The antibacterial potential was evaluated by measuring with a caliper, the diameter of the bacterial inhibition zone.

### Determination of inhibition parameters of bacterial growth MIC, MBC:

To determine the MIC and MBC, tests were performed according to the method of broth dilution. The range of concentrations of CS1, CS2, KG1, and KG2 was prepared in the 9 test tubes which were seeded volume/volume with the inoculum adjusted to 0, 5 Mc Farland ( $10^6$  cells/ml). The extract concentrations varied from two to one, that is to say, for concentrations of 200 to 0.78 mg/ml, respectively, were obtained from 100 to 0.39 mg/ml. The sterility control received 2 volumes of MH broth. All test tubes are incubated in an incubator (37°C) for 18 hours<sup>14, 15</sup>.

### Minimum Inhibitory Concentration (MIC):

The MIC is the lowest concentration of extract producing a complete inhibition of bacterial growth appreciable to the naked eye after the incubation time.

### Minimum Bactericidal Concentration (MBC):

Agar medium Muller-Hinton cast Petri dish is inoculated by parallel ridges with 10µl inoculum from the tubes showing no visible growth in the MIC determination. The MBC is the lowest concentration of extract which leaves more than 0.01% of surviving germs compared to a control without extract.

### RESULTS AND DISCUSSION:

The *in vitro* antibacterial potential of CS1, CS2, KG1 and KG2 was evaluated by the agar diffusion method. The inhibition effect of these extracts on bacterial growth was tested against Gram (+) and Gram (-) bacteria. The antibacterial activity of each crude plant extract was assessed by measuring the diameter of the bacterial inhibition zone formed around each extract. The obtained results are shown in Fig.1-7.

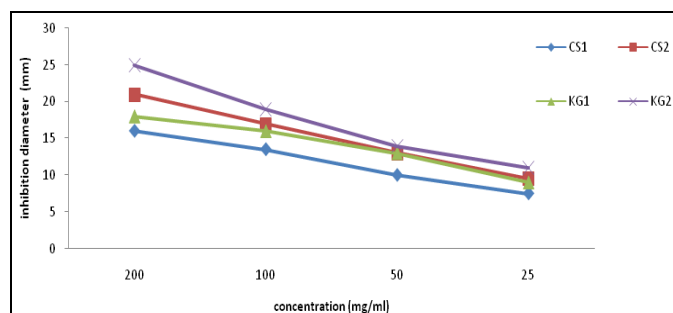


FIG.1: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS AGAINST *S. AUREUS*

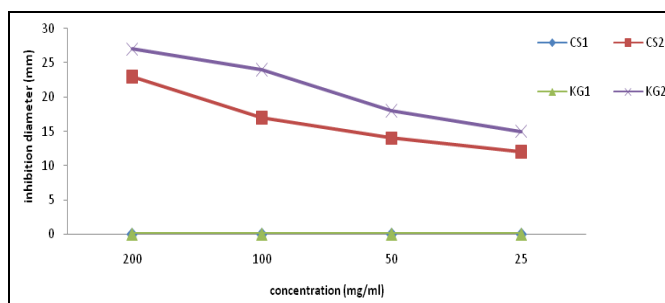


FIG.2: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS AGAINST *E. COLI*

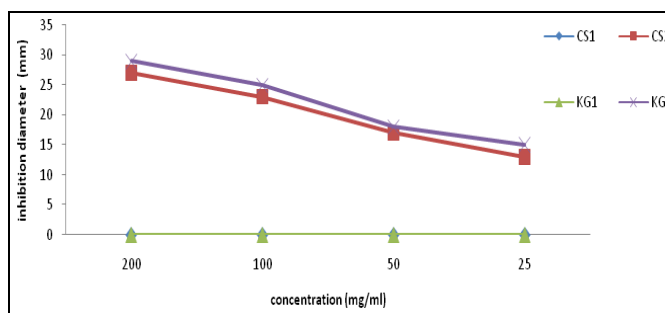


FIG.3: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS AGAINST *P. AERUGINOSA*

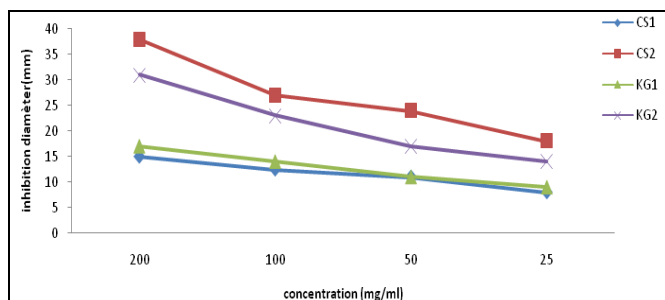


FIG. 4: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACT AGAINST *V. CHOLERA*

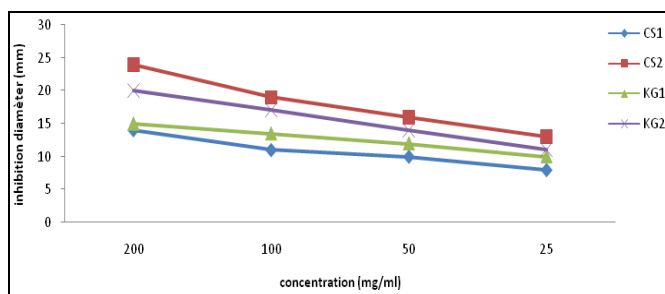


FIG.5: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS AGAINST *P. MIRABILIS*

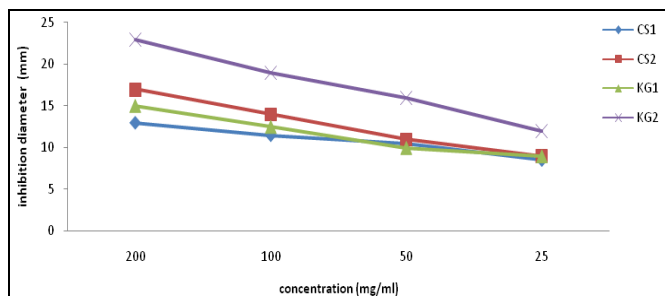
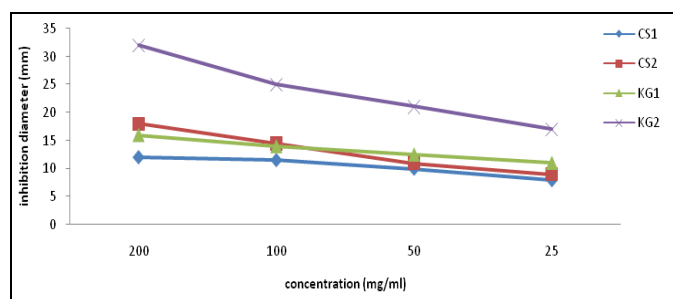


FIG. 6: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS AGAINST *SALMONELLA*



**FIG.7: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACT AGAINST SHIGELLA SP.**

The Fig. 1-7 show that the bacterial inhibition zones increased with the concentration of crude plant extracts. This suggests that our crude extracts inhibit bacterial growth in a dose-response relationship. Furthermore, with the exception of *E. coli* and *P. aeruginosa*, the zones of inhibition generated by CS1 and KG1 on other tested bacteria, demonstrate their effectiveness. The measured diameters of inhibition range from 7.5 (CS1 to 25mg/ml of *S. aureus*) to 38mm (CS2 to 200 mg/ml of *V. cholerae*); which is clearly significant. *E. coli* and *P. aeruginosa* are resistant opposite *K. grandifoliola* while a study conducted in 2013 reported that they are sensitive against methanolic extracts of this plant<sup>16</sup>. Indeed, the method and the solvent of extraction had a big influence on biological activity of plant extracts<sup>17</sup>.

For all tested strains, the Fig.1-7 show that at equal concentrations CS2 and KG2 sign larger zone of inhibition than those of CS1 and KG1. Indeed, at 100mg/ml, CS1, CS2 and KG1, KG2 induce the diameters of bacterial inhibition zones 13.5, 17, 16 and 19 mm respectively against *S. aureus*. We note also that CS1 and KG1 don't sign zone of bacterial inhibition against *E. coli* and *P. aeruginosa*, while their aglycones respectively CS2 and KG2 at 200 mg/ml, induce zones of bacterial inhibition which diameters are 23mm and 27 against *E. coli*, 27 and 29mm on *P. aeruginosa*. The diameter of the inhibition zones of bacterial growth is proportional to the intensity of the antibacterial activity. Thus, we show that crude extracts of aglycones are more active than those of the glycosides.

In aqueous medium, we observed a progressive decrease in the intensity of the disturbance induced by the growth of bacteria with increasing the concentration of the plant extract; illustrating a dose-dependence of the turbidity of the culture

medium. The determined values of MIC and MBC are reported in the **Table 2**.

**TABLE 2: PARAMETERS OF BACTERIAL GROWTH INHIBITION**

Bacterial strain	Extract	MIC	MBC	MBC/MIC
<i>E. coli</i> ATCC 25922	CS1	-	-	-
	CS2	3.12	6.25	2
	KG1	-	-	-
	KG2	3.12	12.5	4
<i>P. aeruginosa</i> ATCC 27853	CS1	-	-	-
	CS2	6.25	12.5	2
	KG1	-	-	-
	KG2	6.25	12.5	2
<i>S. aureus</i> ATCC 25923	CS1	25	100	4
	CS2	1.56	3.12	2
	KG1	6.25	25	4
	KG2	1.56	6.25	4
<i>P. mirabilis</i> n° 1940Y/13	CS1	100	100	1
	CS2	6.25	6.25	1
	KG1	25	50	2
	KG2	3.12	6.25	2
<i>Salmonella</i> sp n° 1585/13	CS1	50	200	4
	CS2	6.25	12.5	2
	KG1	50	200	4
	KG2	1.56	3.12	2
<i>Shigella</i> sp n°929C/12	CS1	12.5	100	8
	CS2	3.12	12.5	4
	KG1	25	100	4
	KG2	3.12	6.25	2
<i>V. cholerae</i> n° 2029C/12	CS1	50	200	4
	CS2	0.39	1.56	2
	KG1	6.25	50	8
	KG2	0.78	1.56	2

These antibacterial parameters allowed one hand to confirm, quantify and compare the activities; and secondly, to characterize the nature of the effect shown by a plant crude extract on a given bacterium. According to Marmonier's method<sup>18</sup> that a substance is bactericidal if CMB/MIC  $\leq 4$ , and bacteriostatic if CMB/MIC  $> 4$ , we have clarified CS1, CS2, KG1 and KG2 action's mode. All tested bacterial strains showed different sensitivities against CS1, CS2, KG1 and KG2 (**Table 2**). By analyzing the **Table 2**, *V. cholerae* is the most sensitive. As for *P. mirabilis*, it is the least sensitive. CS1 gives higher MIC. However, CS2 sign smaller MIC.

The glycosidic crude extract of *K. grandifoliola* gives MIC at 6.25mg/ml against *S. aureus*, 25 mg/ml against *P. mirabilis*, 50mg/ml against *Salmonella* sp., 25mg/ml against *Shigella* sp. and 6.25 mg/ml against *V. cholerae* as its aglyconic extract induced lower CMI (1.56mg/ml, 3.12mg/ml, 1.56mg/ml, 3.12mg/ml, 0.78mg/ml

against the same bacteria respectively) (**Table 2**). All These MIC indicate clearly that crude extracts of aglycones are more effective than crude extracts of glycosides. It is well what we observed in solid medium. Ultimately, the MIC values (**Table 2**) generally coincide with those of the diameters of inhibition of bacterial growth (**Fig. 1-7**). Indeed, the extracts which have induced a large zone of bacterial inhibition, have smaller MIC against the corresponding bacterial strains. Similar reasoning with CMB, led us to the same conclusion. Studies have shown antibacterial action of aglycones<sup>19</sup>.

The expected results let suppose that the weak antibacterial potential of the glycosides compared to the one of the aglycones, is due to the glycone that seems to inhibit the antibacterial activity. Witness, once released by acid hydrolysis, the aglycones find the fullness of their antibacterial activity. We demonstrated it with CS1 and KG1 which are inactive against *E. coli* and *P. aeruginosa* whereas CS2 and KG2 are active against these bacterial strains.

The MBC/MIC ratio of CS1, CS2 and of KG1, KG2 are presented in **Table 2**. The results show that crude extracts of aglycones have a more pronounced antibacterial action compared to crude extracts of glycosides. Indeed, more the values of MIC and MBC are near, more the substance is bactericidal; and more they are far-off, more the substance is bacteriostatic.

Through this study, we contribute to show that in reality, microorganisms are more sensitive to aglycones as glycosides. The aglycones appear to be better indicated for the treatment of bacterial infections. The tested bacteria Gram (+) and Gram (-) indicate that the aglycones don't act on the bacterial wall but rather to an internal mechanism. According to a study, these phytochemicals would be supposed to act on DNA, RNA or protein synthesis of bacteria<sup>20</sup>. Our crude plant extracts possess antibacterial activity against all tested bacterial strains, except *P. aeruginosa* and *E. coli*. This activity is linked to the presence of secondary metabolites in these plants. Indeed, some authors reported that *C. sieberiana* and *K. grandifoliola* contain alkaloids, flavonoids, tannins and saponins<sup>21, 22</sup>.

**CONCLUSION:** In this scientific investigation, we have demonstrated the antibacterial properties of *Cassia sieberiana* and *Khaya grandifoliola*, two Ivorian medicinal plants, comparing the antibacterial potential of their glycosidic and aglyconic crude extracts. The results indicated on the whole, the presence of active antibacterial phytosubstances in each plant. Bactericidal and bacteriostatic effects of such plants have been shown against the tested bacterial strains. What seems to justify their ethnopharmacological use in the treatment of some infectious diseases in Côte d'Ivoire.

The aglyconic crude extracts signed an antibacterial effect more pronounced than the glycosidic crude extracts. This report demonstrates that the sugar moieties inhibit antibacterial activity of the glycosides. Therefore, we have reason to suggest the acid hydrolysis of plant drugs before extraction as a means of improving or activation of their antibacterial properties; and why no general way to their pharmacological properties. The obtained results in this survey are among other additional steps needed to achieve a contribution in the enhancement of *Cassia sieberiana* and *Khaya grandifoliola*.

**ACKNOWLEDGEMENTS:** We express post mortem, our infinite recognition to Professor Laurent AKE-ASSI.

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**How to cite this article:**

Traoré L, Boua BB, Guessennd NK, Kadja BA, Mamyrbékova-Békro JA and Békro YA: *In Vitro* Antibacterial Potential of Glycosidic and Aglyconic Crude Extracts of *Cassia Sieberiana* Dc. (Cesalpiniaceae) and *Khaya Grandifoliola* C.Dc. (Meliaceae): A Comparative Survey. *Int J Pharm Sci Res* 2015; 6(7): 2728-33.doi: 10.13040/IJPSR.0975-8232.6(7).2728-33.

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