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Terminalia glaucescens Planch. Ex Benth. (Combretaceae), a Medicinal Plant of Côte d'Ivoire Pharmacopoeia: Antibacterial Activity on *Staphylococcus* and *Pseudomonas*, Acute Toxicity on Mice and Lethal Effect on Vero E6 Cells

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ABSTRACT

Terminalia glaucescens, a medicinal plant is used in traditional treatment of various pathologies in Côte d'Ivoire. The aim of present study was to evaluate antibacterial activity and the innocuousness of *Terminalia glaucescens*. For that, antibacterial tests and acute toxicity tests by oral and intraperitoneal way have been done on *Swiss* mice. The method of diffusion on gelosed medium has been used for sensitivity tests. The acute toxicity was evaluated by oral and intraperitoneal administration, as well as the toxicological parameters after administration of *T. glaucescens* aqueous extract. Another study based on the aqueous extract cytotoxic effect on African green monkey kidneysVeroE6 cells has been achieved by different concentrations. Sensitivity tests have permitted to obtain inhibition zone which varies from 12 to 35 mm and from 12 to 19 mm respectively on *Staphylococcus* and *Pseudomonas in-vitro* growth. Acute toxicity study by oral route has shown a weak toxicity of *Terminalia glaucescens* with a LD_{50} superior to 5000 mg/kg body weight. By intra-peritoneal route, the LD_{50} has been 500 mg/kg body weight. It has been certified moderately toxic. A cytotoxic effect of *Terminalia glaucescens* aqueous extract on African green monkey kidneys VeroE6 cells has been observed proportionally to concentrations used. Results have proved that *T. glaucescens* aqueous extract have a high inhibitory action on bacteria tested. Finally, it has been proved that *Terminalia glaucescens* can be used to treat illnesses without any fear of toxicity.

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INTRODUCTION

A large proportion of populations in developing countries has resorted to ancestral knowledge focused on medicinal plants for their primary health cares. Their usages for therapeutic issue and their accidental ingestion or by confusion with other comestible plants determine frequent accidents in the world[1]**.** The number of these medicinal plants has been described in African pharmacopoeia[2]. In Côte d'Ivoire, the traditional medicine uses medicinal plants in the treatment of various pathologies of which the bacterial infections[3] and cancer. Indeed, for 58 millions of death recorded in 2005 on the world level, the cancer is responsible for 13%. This proportion is high than the proportion of death caused by VIH/SIDA, tuberculosis and malaria[4]. Among these medicinal plants, *Terminalia glaucescens* Planch. Ex Benth. (Combretaceae) is an important plant of African traditional medicine against several illnesses. Concerning its biological properties, several studies have proven its effects one *Plasmodium falciparum*[5]; its antibacterial properties[3,6] and its leishmanicidal, trypanocide, anti helminthiasis activity and antiscabie[5] have been also proven. Therefore, to recommend the use of *Terminalia glaucescens* as an alternative means in the bacterial infections treatments in Côte d'Ivoire, we had to realize, on one hand, antibacterial activities of *Terminalia glaucescens* aqueous extract on *Staphylococcus* and *Pseudomonas in-vitro* growth and proceed to test acute toxicity among rodents to value its innocuousness on another hand.

MATERIAL AND METHODS

Plant material

The stem bark of *Terminalia glaucescens* Planch. ex Benth. (Combretaceae) have been collected at Ahougnansou-Allahou (Department of Tiebissou, Côte d'Ivoire) situated to 42 km from Yamoussoukro (political capital) and 285 km from Abidjan, the country economic capital. The plant has been identified by the National Floristic Center of the University Felix Houphouet Boigny, Abidjan, Côte d'Ivoire **(Figure 1).**

Figure 1: Bark (**A**) and stem crushed (**B**) of *Terminalia glaucescens* (Combretaceae)

Bacterial material

The bacterial material has been constituted strains of *Staphylococcus aureus* resistant to meticilline isolated from routine exams to the laboratory and a strain of *Staphylococcus aureus* reference ATCC 25923 exits of the biobank of the Pasteur institute of Côte d'Ivoire **(Table I).**

Laboratory animals

Mice that have been used were male and female from *Mus musculus* species, albino and Swiss race. Those animals have been bred in following stalling conditions: ambient temperature (25° ± 2°C); natural lighting of the day between 6 hours and 18 hours and darkness between 18 hours and 6 hours. Six (6) lots of six mice (3 males and 3 females) have been constituted and a control lot of six mice.Their weights have varied from 20g to 29g. All the animals came from the Management Unit of Animal Resources of the Pasteur Institute of Côte d'Ivoire.

Table I: Bacterial strains studied and their references

Code	Bacterial name	Biological Product	Service	Resistance Phenotype
Hospital strains				
1397C/12	*Staphylococcus haemolyticus*	Blood	Ext	BLST04, RCFQ, AMST02, MLST06
1398C/12	*Staphylococcus hominis*	Blood	Ext	BLST04, RCFQ, AMST02, MLST06
264C/12	*Pseudomonas aeruginosa*	Stink	Ext	BLE213
1093C/13	*Pseudomonas aeruginosa*	Stink	Ext	Wild
Reference strains				
ATCC 25923	*Staphylococcus aureus*			Wild
ATCC 27853	*Pseudomonas aeruginosa*			Wild

BLST04 : Phenotype Méti-R ; **RCFQ** : Cross-Resistance to Fluoroquinolones, **AMST02** : Phenotype KTG, **MLST06** : Phenotype M (by active efflux), **BLE213** : probable plasmidic Céphalosporinase ; **Ext** : Extern

Methods

Total aqueous extract preparation

The aqueous extract of *T. glaucescens* bark stem has been prepared according to the method described by Olakunleand al. in 2005[7] with some modifications. 100 g of *T. glaucescens* bark stem powder have been macerated in 1000 ml of distilled water under agitation by blinder (Philips, Boroglass®). Then, extract obtained has been filtered twice successively on the absorbent cotton and on filters paper (Whatman® paper 3 mm). After this operation, the filtrate has been concentrated in the steam room at 50°C for 3 days. The powder obtained has constituted the total aqueous extract[8].

Antibacterial activity evaluation

The antibacterial activity of *T. glaucescens* bark stem aqueous extract has been realized by the method of diffusion on solid medium of Mueller-Hinton as described by Wiegand and et al., (2007)[9] and taken back by Konan (2015)[10]. From young colonies of 24 hours, a bacterial inoculums has been done in physiological water for every bacterial strain and has been diluted at 1/100. This inoculum prepared has served to sow Petri dishes by brush. Then, holes of 6 mm of diameter have been done on inoculated gelose.

Later, 50 µl of vegetal extract have been introduced in holes by doses. After 15 minutes at ambient temperature, Petri dishes have been incubated at 37°C for 24 hours in a steam room.

Diameter of inhibitory zone Reading

The diameter of inhibitory zone is the zone around the hole where bacteria have not grown. A slide ruler has been used for the reading. The diameters are expressed in mm. So, the strain is resistant to the substance when diameter is below 8 mm, sensitive, when it is between 9 and 14 mm, very sensitive, when it is between 15 and 19 mm and extremely sensitive when it is above 20 mm[11].

Acute toxicity

Realization of different total aqueous extracts concentrations

Different doses of total aqueous extracts have been prepared in aseptic conditions taking into account the body weight of mice and the quantity of product to administer. They have been expressed in body weight mg/kg (mg/kg of body weight).

Lethal dose 50 (LD_{50}) determinations

Male and female Swiss race mice have been used for this test. Twelve hours before the experimentation, animals have been deprived of food but water remained available.

Administration by oral way

The doses of *T. glaucescens* bark stem aqueous extract (500; 2000; 3000; 4500 and 5000 mg/kg) have been administered by oral way (o. w.) to five (5) experimental lots of six (6) mice (3 males and 3 females) by reason of 1 ml of solution by mouse. The control lot has received only distilled water. Animals had a free access to food and water 2 hours later.

Intra-peritoneal administration way

The doses of *T. glaucescens* bark stem aqueous extract (100, 500, 1000, 1500 and 2000 mg/kg of Pc) have been administered by injection intra-peritoneal (i.p) to five (5) lots of six (6) mice (3 males and 3 females). The control lot has received 0,15 ml of solution of NaCl 0.9%. Animals had a free access to food and water 2 hours later.

Clinical observation

Animals have been observed individually and regularly during the first 30 min, and the first 24 hours with a particular attention for the first 4 hours. Later on, observations have been done daily for a period of 14 days. During this period, the number of deaths by lot and symptomatic troubles has been noticed.

Cytotoxicity test

Method that has been used was a modification of the method described by Taylor and al. (1996)[12].To Vero E6cells in culture during 48 hours on plates of 96 wells at a concentration of 0, 75.10^6 cells/ml, a quantity of 100 µl of aqueous extract has been added by concentration of 10; 5; 2,5; 1,25; 0,625 and 0,312 µg/ml in the DMEM. Then, control wells containing only Vero E6 cells and the mixture of DMEM added fœtal calf serum has been also done. The plates prepared in duplicate have been incubated at 37°C under 5% of CO_2. The cells cytological changing (lyse and granulation) have been appreciated by microscopic observation after D1, D2, D3, D4 and D5 incubation.

RESULTS

Extraction average yield

The extraction average yield obtained by aqueous maceration was 14,8 ± 0,34%.

Antibacterial activity

Results of antibacterial activity of *T. glaucescens* bark stem aqueous extract are regrouped respectively in tables II and III on *Staphylococcus* and *Pseudomonas*. The figure 2 shows the effect of *T. glaucescens* bark stem aqueous extract on *Staphylococcus haemolyticus* 1397C/12 and *Staphylococcus hominis* 1398C/12growth.

Table II: Sensitivity of *Staphylococcus* strains opposite to tested substances

Code	Bacterial strains	Total aqueous extract concentration (mg/ml)			Antibiotics	
		100	50	25	FOX	GM
1397C/12	*Staphylococcus haemolyticus*	34	32	29	6	6
1398C/12	*Staphylococcus hominis*	35	33	27	19	11
ATCC25923	*Staphylococcus aureus*	16	14	13	26	22

FOX : Cefoxitin (30 µg); **GM**: Gentamycin (10 µg)

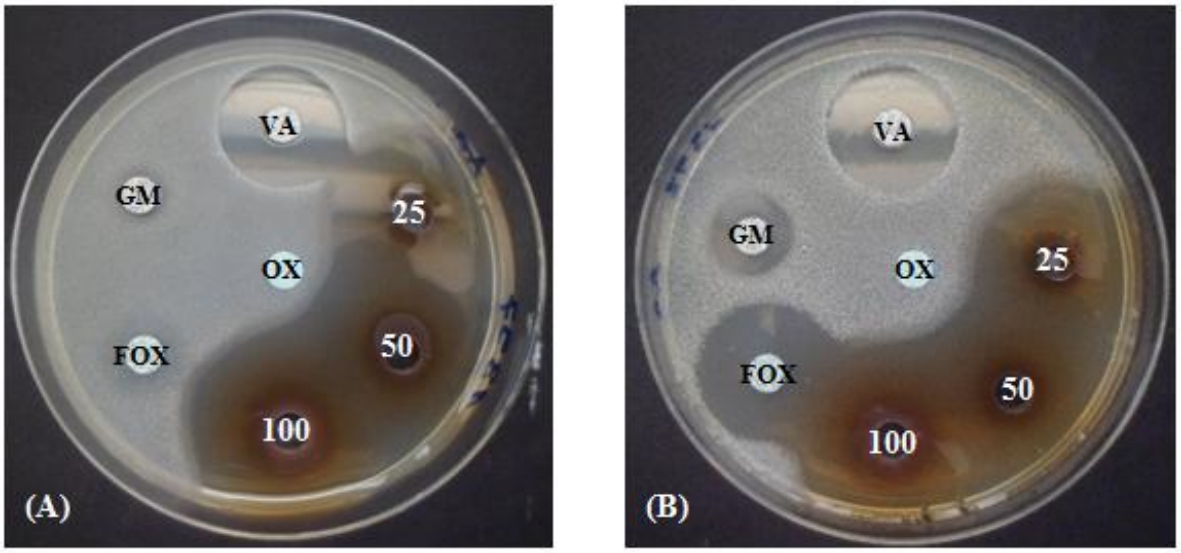
Table III: Sensitivity of *Pseudomonas* strains opposite to tested substances

Code	Bacterial strains	Total aqueous extract concentration (mg/ml)			Antibiotics	
		100	50	25	IPM	CAZ
264C/12	*Pseudomonas aeruginosa*	18	14	12	16	11
1093C/13	*Pseudomonas aeruginosa*	17	15	13	22	21
ATCC 27853	*Pseudomonas aeruginosa*	19	14	12	34	23

IPM : Imipenem (10 µg) ; **CAZ** : Ceftazidime (10 µg)

Results of sensitivity tests obtained have permitted to underline the significant antibacterial activity opposite to the totality of studied strains.

The diameters of inhibitory zone have varied respectively from 13 to 35 mm and from 12 to 18 mm for *Staphylococcus* and *Pseudomonas* according to different aqueous extract concentrations.



OX : Oxacillin ; **FOX** : Cefoxitin ; **GM** : Gentamycin ; **VA** : Vancomycin ; values are expressed in mg/mL

Figure 2: Effect of *T. glaucescens* bark stem aqueous extract on *Staphylococcus haemolyticus* 1397C/12 **(A)** and*Staphylococcus hominis* 1398C/12 **(B)** growth.

Acute toxicity

The plant toxicity evaluation has been only determined by acute toxicity. Mortality observed at the administration time of *T. glaucescens* bark stem aqueous extract by oral and intraperitoneal way is presented in tables IV and V.

The toxicity curve of *T. glaucescens* bark stem total aqueous extract has permitted to determine LD_{50} and LD_{100}.

The lethal dose 100(LD_{100}) that has been determined was the dose which has caused the death of 100% of mice in a lot. The lethal dose 50 (LD_{50}) was the dose which has caused the death of 50% of mice in a lot.

Administration by way

The results obtained by oral administration way are consigned in table IV and are represented in the figure 3. The LD_{50} value determined (DL_{50}> 5000 mg/kg of Pc), has permitted to class *Terminalia glaucescens* in the category of weakly toxic products according to Hodge and Sterner toxicity classification.

Table IV: Mortality rate later force-feeding of *T.glaucescens* aqueous extract by different doses

Lots	Doses (mg/kg Pc)	Log of doses	Mortality	Probability of mortality (%)
Witness	0	0	0	0
Exp 1	500	2,7	1	16,7
Exp 2	2000	3,3	2	33,3
Exp 3	3000	3,5	2	33,3
Exp 4	4500	3,6	3	50
Exp 5	5000	3,7	3	50

Exp: experimental lot

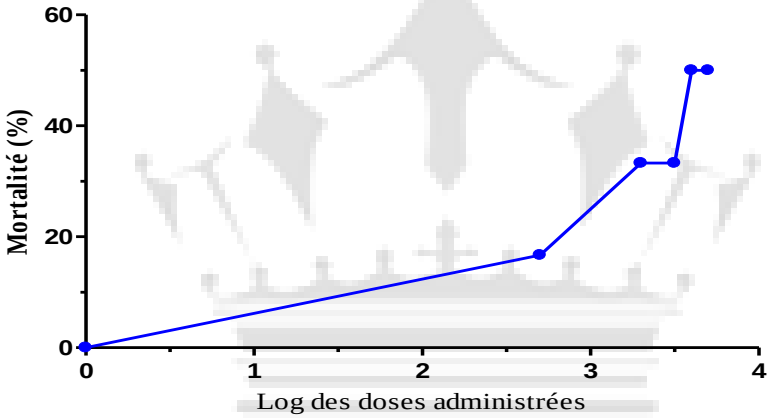


Figure 3: Mortality evolution of mice following *T.glaucescens* aqueous extract by different doses by force-feeding

Intra-peritoneal administration way

The results obtained by Intraperitoneal administration way have been consigned in table V and represented on figure 4.TheLD_{50} value obtained (LD_{50} = 500 mg/kg of Pc) has permitted to class*T. Glaucescens* in the category of moderately toxic products according to Hodge and Sterner toxicity classification.

Table V: Mortality rate later intra-peritoneal administration of *T.glaucescens* aqueous extracts by different doses

Lots	Doses (mg/kg Pc)	Log of doses	Mortality	Probability of mortality (%)
Witness	0	0	0	0
Exp 1	100	2	2	33,3
Exp 2	500	2,7	3	50
Exp 3	1000	3	6	100
Exp 4	1500	3,1	6	100
Exp 5	2000	3,3	6	100

Exp : experimental lot

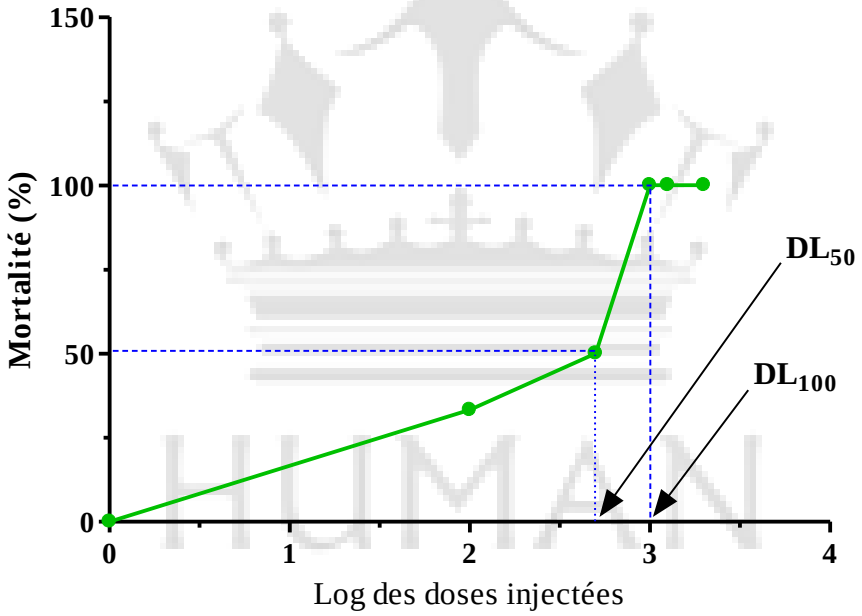


Figure 4: Mortality evolution of mice following *T.glaucescens* aqueous extract by different doses by intra-peritoneal way

Changes in behaviors observed

At the level of animals, there was a commotion, death occurred at the 24th hour. After 72 hours, the surviving animals found themselves normal behavior comparable to that of controls. It was noted a twist of the hind legs, accelerated heart rate and activity of animals was reduced and some they just lay in the belly with the hind legs apart, death occurred after 4 hours by intraperitoneal injection.

Cytotoxicity of *T. glaucescens* aqueous extract

The results of *T. glaucescens* aqueous extract effect by different concentrations on Vero E6 cells viability following the time are presented in table VI and illustrated by figure 5. Results of table VI have permitted to class the aqueous extract noncytotoxic for 0.312 µg/ml. However, it is declared cytotoxic for concentration of 0,625 µg/ml at J4 and J5. It is also cytotoxic for concentration of 1.25 µg/ml at J3 and for concentration of 10 µg/ml at J2. From this study, it is probable to remark that *T. glaucescens* aqueous extract at 10 µg/ml inhibits Vero E6 cells growth from 98.17 to 1.66% at the first to the fifth day of experience (figure 5).

Table VI: *In vitro* cytotoxic profile of *T. glaucescens* aqueous extract

Date and Substances		**Concentration (µg/ml)**					
		10	**5**	**2,5**	**1,25**	**0,625**	**0,312**
Day 1	Control	-	-	-	-	-	-
	AqE	±	-	-	-	-	-
Day 2	Control	-	-	-	-	-	-
	AqE	+	±	±	±	-	-
Day 3	Control	-	-	-	-	-	-
	AqE	+	+	+	+	±	-
Day 4	Control	-	-	-	-	-	-
	AqE	+	+	+	+	+	-
Day 5	Control	-	-	-	-	-	-
	AqE	+	+	+	+	+	-

AqE: aqueous Extract; **-** : no cytotoxic effect ;**+** : cytotoxic effect; **±** : appreciable cytotoxic Effect

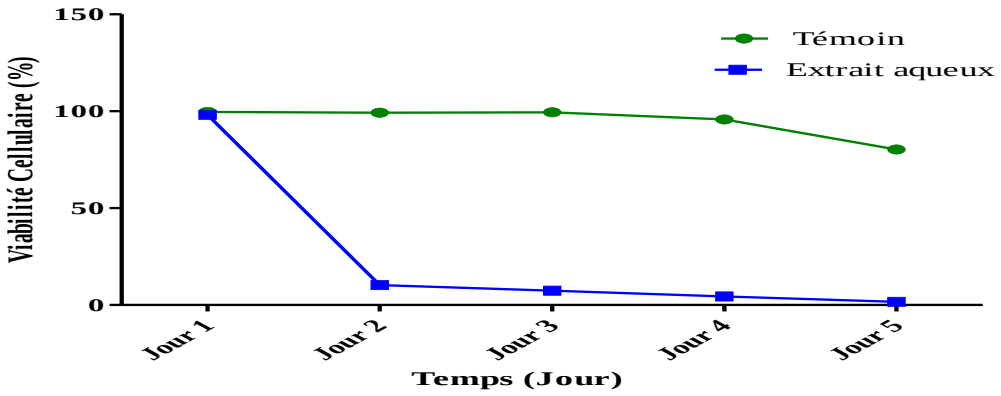


Figure 5: Vero E6 cells viability according to the culture time with the aqueous extract and the control

DISCUSSION

The aqueous maceration average yield has been 14.8 ± 0.34%, this observation has proved that water extracts an important quantity of *T. glaucescens* stem bark constituent. Its usage in traditional medicine would be justified by this property.

Antibacterial activity results have indicated that aqueous extract obtained from *T. glaucescens* stem bark has been active on the growth of all the infectious strains as well on hospitable origin and reference strains.

This study has shown that *T. glaucescens* aqueous extract has produced high inhibitory zones on *Staphylococcus* than on *Pseudomonas.* For 25 mg/ml, the inhibitory zone on *Staphylococcus haemolyticus* 1397C/12 has been 29 mm, whereas, for the same concentration, the inhibitory zone has been 13 mm on *Pseudomonas aeruginosa* 1093C/13. The *Staphylococcus* genus is responsible for cutaneous infections and *Pseudomonas* responsible for nosocomial infections and sores surinfected. For high activity of *T. glaucescens* aqueous extract on these strains, this plant could be used as alternative for the health care in contact with these germs. Gentamycin has not described any inhibition zone opposite to *Pseudomonas* strains however it had a diameter of 11 mm respectively on *Staphylococcus haemolyticus* 1397/12 and *Staphylococcus hominis* 1398C/12 methicillin-resistant with KTG phenotype and phenotype M (active efflux) and across resistance to fluoroquinolone. Whereas inhibition zone of *T. glaucescens* aqueous extracts have varied from 29 and 27 mm to 25 mg/ml for these respective strains. According to Ponce and al. (2003)[11]**,** *Staphylococcus haemolyticus* 1397/12 and *Staphylococcus hominis* 1398C/12 have been extremely sensitive to *T. glaucescens* aqueous extract. This activity indicates that *T. glaucescens* stem bark aqueous extract is more efficient than aminosides (gentamycin) sold on markets. The antibacterial activity of *T. glaucescens* stem bark aqueous extract can be explained by its richness of saponins, tannins, polyphenols, alkaloids and flavonoids[13,5]**.** The aqueous extracts have been effective on *Pseudomonas aeruginosa* strains for 25 mg/ml according to Ponce et al. (2003)[11]**,** however imipenem has been categorized intermediate on *Pseudomonas aeruginosa* 264C/12 and effective on *Pseudomonas aeruginosa* 1093C/13 hospitable origin. The clinical card of male and female mice treated by *T. glaucescens* stem bark aqueous extract was probably characterized by a cardiac rhythm acceleration due to a blockage of M2 muscarinic receptors driving to a deletion of vagal tonus according to Kenneth (2001)[14], a respiratory difficulty, and of the convulsions by central nervous system

attacks (blockage of acetylcholine production in central nervous system synapses) according to Goulle et al. (2004)[15]. Acute toxicity has permitted to situate the tolerance limits of this plant. The LD_{50} determined by oral way (> 5000 mg/kg of pc) and by intra-peritoneal way (500 mg/kg of pc) have permitted to class *T. glaucescens* stem bark aqueous extract respectively weakly and moderately toxic substances according to chemical substances toxicity scale of Hodge and Sterner (1980)[16]**.**The difference of toxicity according to administration method has been also observed with *Pilostigma reticulatum* (Caesalpiniaceae) leaves decoction[17]**,** *Ziziphus mauritiana* (Rhamnaceae) crude extract[18]**,** *Bridelia ferruginea* (Euphorbiaceae) aqueous extract[19] and *Gomphrena celosioides* Mart (Amaranthaceae) aqueous extract[20]**.** The relative cytotoxicity results by concentrations of *T. glaucescens* aqueous extract tested corroborate Idrissa et al. (2012)[21] results**.** They have shown that *C. giganteus* aqueous extract used in icterus treatment presents a raised cytotoxicity on African green monkey kidney Vero E6cells. The results mentioned in this work, which has been observed on cellular scale, can guide to therapeutic doses.

CONCLUSION

This study has brought a contribution for a best knowledge of *Terminalia glaucescens* stem bark aqueous extracts antibacterial activity on *Staphylococcus* and *Pseudomonas* as well as its acute toxicity used in traditional medicine in Côte d'Ivoire. The results set obtained is favorable for the security of *Terminalia glaucescens* employment in bacterial infections. Also, the tests have shown that this plant can be used in the treatment of cancer because of its cytotoxic activity raised.

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