European Journal of Biotechnology and Bioscience
ISSN: 2321-9122
Impact Factor: RJIF 5.44
Received: 07-11-2018; Accepted: 08-12-2018
www.biosciencejournals.com
Volume 6; Issue 6; November 2018; Page No. 24-29

Antibacterial and acute toxicity studies of culinary leaves from *Adansonia digitata* L. (Malvaceae) and *Amaranthus cruentus* L. (Amaranthaceae) growing in Côte d'Ivoire

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Abstract
Adansonia digitata L. (Malvaceae) and *Amaranthus cruentus* L. (Amaranthaceae) leaves are consumed by people of northern of Côte d'Ivoire. Methanol extracts from these leaves have been investigated for their antibacterial activity and acute toxicity in rats. Antibacterial activity was evaluated by using agar diffusion and microdilution in 96 wells methods against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.* The extracts acute toxicity were carried out based on OECD guidelines 423 with high limit dose of 2000 mg/kg body weight of test animal. All extracts had bacteriostatic activities against test bacteria with MICs values greater than 30 mg/ml. The highest dose administered did not produced mortality or changes in general behaviour of test animals. In conclusion consumption of leaves of *Adansonia digitata* and *Amaranthus cruentus* may prevent bacterial infections and is not toxic in experimental animals.

Keywords: Malvaceae, Amaranthaceae, *Adansonia digitata*, *Amaranthus cruentus,* antibacterial, acute toxicity

1. Introduction
The promotion and consumption of indigenous vegetables is very important when considering increase in food insecurity, human population and alleviation of malnutrition in developing countries. This study focuses of *Adansonia digitata* L. (Malvaceae) and *Amaranthus cruentus* L. (Amaranthaceae) leaves which are much consumed by populations in Côte d'Ivoire.

A. digitata is commonly known as baobab tree native to Africa. This species is a multi-purpose tree which offers protection and provides clothing, food and medicine as well as raw material for many useful items [1-9].

The fruit pulp, seeds, leaves, flowers, roots and bark of baobab are edible and they have been studied by scientists for their pharmaceutical properties such as anti-asthma, antiviral, anti-anaemia, anti-oxidant, antimicrobial, anti-malarial, anti-diarrhoea and anti-inflammatory activities [10-18]. The phytochemical analysis revealed presence of alkaloids, saponins, flavonoids, tannins and terpenoids [6].

A. cruentus is an annual pseudo-cereal with broad leaves. This plant is known for its nutritional value and is used as a forage crop and a leafy vegetable [19]. Concerning its chemical composition, pectinic polysaccharides were isolated and characterized from aerial part [20]. Two polyphenols derivatives, (+)-catechin and (-)-epicatechin which recognized biologically important, were isolated from the leaves [19]. The mild-flavoured leaves of this plant are rich in minerals and vitamins (A and C).

To our knowledge, toxicity and antibacterial activity have never been evaluated for *A. cruentus* and *A. digitata growing in Côte d'Ivoire*. We also wanted to verify acute toxicity of these two plants and to know if they have activities that could be useful for this populations.

The purpose of this work is to evaluate antibacterial activity and teste acute toxicity of methanolic extracts from *A. digitata* and *A. cruentus* leaves.

2. Material and Methods
2.1 Plant Material
The leaves from *A. digitata* and *A. cruentus* were collected in February 2017 in Korhogo (north of Côte d'Ivoire) and were identified by Pr. Ipou Ipou Joseph from the Centre National de Floristique of the University Félix Houphouët-Boigny.

2.2 Preparation of Extracts from Leaves
The preparation of the extracts of *A. digitata* and *A. cruentus*

were performed like follow. The fresh plant leaves (150 g) were introduced into glass jar (350 mL), water (120 mL) was added and then closed. The closed jar containing the mixture was placed in boiling bath (100 °C) for 45 minutes. Subsequently, the boiled leaves were dewatered using a strainer before being reduced to pulp using an electric grinder. Then, 10 g of each leaf pulp were extracted with MeOH (100 mL) for 48 hours. The obtained solutions were filtered using whatmann no.1 filter paper and then evaporated under reduced pressure to yield 485.7 mg and 312.9 mg of crude methanolic extracts for *A. digitata* and *A. cruentus*, respectively.

2.3 Biological Activity

For biological tests we used the same ones as those realized by Touré *et al*., 2018b [21].

2.3.1 Antibacterial Assays

The antibacterial activity was evaluated on *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (CIP) 4.83, *Pseudomonas aeruginosa* (CIP) 103467, *Escherichia coli* (CIP) 54127AF and *Staphylococcus aureus* sensitive to penicillin according to the protocol used by Ahoua *et al*., 2015 [22]. These strains from the Institut Pasteur of Côte d'Ivoire and the National Laboratory of Public Health of Côte d'Ivoire were provided by the Microbiology Laboratory of Centre Suisse de Recherches Scientifiques en Côte d'Ivoire.

2.3.1.1 Sensitivity test

Mueller-Hinton agar in Petri dishes with a thickness of 4 mm were soaked with an inoculum equivalent to 0.5 of McFarland. After drying, wells with a diameter of 6 mm were made in the agar using sterile Pasteur pipette. Fifty microliters (50 µL) of extract at 1500 µg/mL in DMSO or antibiotic at 25 µg/mL in distilled water was poured in the wells. Plates were left at ambient laboratory temperature for 15 to 30 min for a pre-diffusion of the solutions, and then incubated at 37 °C for 18 h. After incubation, the diameters (mm) of inhibition zones were measured. The tests were carried out twice.

2.3.1.2 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MICs) were determined by using broth microdilution method in 96-wells microplates. The plant extracts were solubilized in DMSO (30 mg/mL) and serially diluted in Mueller-Hinton medium, from 1500 to 1.5 µg/mL. The final concentrations were 50 to 0.05 µg/mL for antibiotics. All the tested bacteria were used with an initial inoculum of 3×10^6 bacteria/mL. The microplates were incubated at 37 °C for 18 h.

2.3.2 Acute toxicity

The acute oral toxicity test was performed by using the Organization for Economic Co-operation and Development (OECD) guidelines 423 [23].

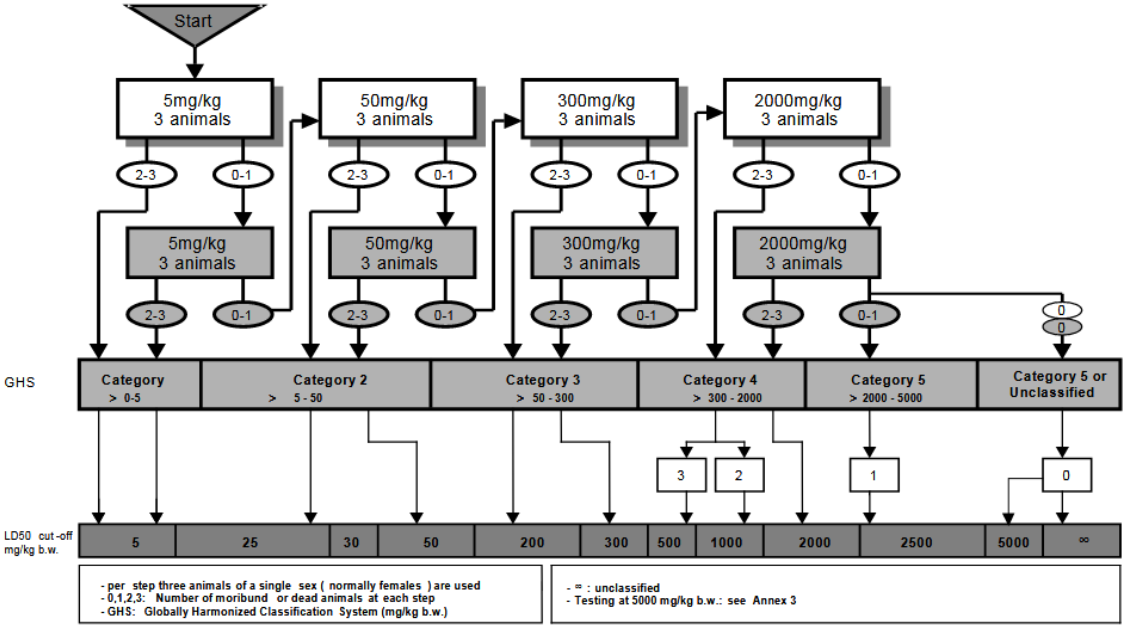

Fig 1: Test procedure with a starting dose of 5 mg/Kg body weight [25].

2.3.2.1 Experimental animals

Experiments were performed using healthy non-pregnant young adult female rat (Wistar) weighing 108-115 g. Female rats were chosen because of their greater sensitivity to treatment.

The animals were randomly divided into thirteen groups each containing three rats. They were identified by the markings using a yellow stain. They were marked on head, body, tail, head and body, body and tail, to ease observation [24, 25]. The animals were housed in polypropylene cages (55 cm x 32.7 cm x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2 ºC). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24 h period. Each cage was identified by a card. This card stated the cage number,

number and weight of the animals it contained, test substance code, administration route and dose level. They have been fed from granules of the company IVOGRAIN with tap water in baby bottles [26, 27].

2.3.2.2 Administration of test substance

The test substance was administered in a single dose by gavage using specially designed rat oral needle. Animals were fasted all night.

Following period of fasting, animals were weighed and test substance was administered orally at a dose of 5, 50, 300 and 2000 mg/kg. After this, food for the rat was withheld for 3 to 4 hours.

Control rats are subjected to intra-gastric gavage of physiological serum (NaCl) at the rate of 10 mL / kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

2.3.2.3 Signs recorded during acute toxicity studies

Animals were observed individually after at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes.

Direct observation parameters include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then these were maintained for a further 14 days with a daily observation.

2.3.3 Statistical analysis

Data were expressed as mean values ± SD (standard deviations). All data were analyzed by one-way ANOVA and differences between means were assessed with Dunnet/Turkey's multiple comparison tests. Differences were considered significant at $p < 0.05$. All analyses were carried out using Graph Pad software (USA).

3. Results and discussion

3.1 Results

3.1.1 Antibacterial activity

In the current investigation, the antibacterial activity of methanolic extracts from *A. digitata* and *A. cruentus* were evaluated against gram-positive and gram-negative bacteria. The diameters of the inhibition zones were measured.

The antibacterial activity carried out on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli strains* showed that all the tested bacteria were sensitive to the methanolic extracts from *A. cruentus* with inhibitory diameters ranged from 9 to 9.50 mm (Table 1). Contrariwise the methanolic extract of the leaves from *A. digitata* were only active on *S. aureus* sensitive to penicillin and *S. aureus* CIP with a diameter of inhibition of 8.50 mm. *Pseudomonas aeruginosa* ATCC (0 mm), *Pseudomonas aeruginosa* CIP (0 mm) and *E. coli* ATCC (7 mm) were not sensitive to this extract

The actives extracts were bacteriostatic with MICs values (table 2) greater than 3000□□g/ml (Table 2). The MIC values of the positive controls ranged from 0.19 to up to 50 µg/ml for tetracycline and from 1.56 to up to 50 µg/ml for gentamicin.

Table 1: Inhibition diameters (mm) of methanolic extracts from *A. digitata* and *A. cruentus* on tested bacteria

		Bacteria				
		***P. aeruginosa* ATCC**	***P. aeruginosa* CIP**	***S. aureus Sensitive***	***S. aureus* CIP**	***E. coli* ATCC**
Extracts of culinary leaves	*A. digitata*	0 ± 0.00^{c}	0 ± 0.00^{c}	8.50 ± 0.29^{d}	8.50 ± 0.29^{b}	7.00 ± 0.00^{d}
	A. cruentus	9 ± 0.00^{b}	9 ± 0.58^{b}	10.50 ± 0.87^{c}	9.50 ± 0.29^{b}	9.00 ± 0.00^{c}
Positive control	Tetracycline	27.00 ± 0.00^{a}	27.00 ± 0.58^{a}	23.00 ± 0.00^{b}	26.00 ± 0.58^{a}	23.00 ± 0.58^{b}
	Gentamycin	26.50 ± 0.87^{a}	28.00 ± 0.00^{a}	25.00 ± 0.00^{a}	27.00 ± 0.58^{a}	26.00 ± 0.00^{a}
	F	952.33	1140.00	342.80	491.60	1115.00
	P	< 0.001				

P.: Pseudomonas; S.: Staphylococcus; E.: Escherichia; F: Fisher statistic; P: Probability; CIP: Collection de l'Institut Pasteur; ATCC: American Type Culture Collection

Table 2: Minimal Inhibitory Concentration (MIC) (µg/mL) values of the active extracts from *A. digitata* and *A. cruentus*

		Bacteria				
		***P. aeruginosa* ATCC**	***P. aeruginosa* CIP**	***S. aureus* Sensitive**	***S. aureus* CIP**	***E. coli* ATCC**
Extracts of culinary leaves	*A. digitata*	>3000	>3000	>3000	>3000	>3000
	A. cruentus	>3000	>3000	3000	>3000	>3000
Positive control	Tetracycline	0.19	3.125	50	0.19	>50
	Gentamycin	3.125	1.56	>50	1.56	>50

3.1.2 Acute toxicity

The present study conducted as per OECD guidelines 423 revealed that all extracts (*A. digitata* and *A. cruentus*) did not produce any mortality throughout study period of 14 days even when the limit dose was maintained at 2000 mg/kg body weight. The oral medium lethal dose (LD_{50}) was indeterminable being in excess of 2000 mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and extracts were practically non-toxic. Table 3 indicates the parameters observed before and after administration of test

substance for the three extracts. All parameters observed were normal even at the highest dosage of 2000 mg/kg body weight of test animal. This clearly indicated that the above extracts do not produce oral toxicity. The LD_{50} of extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect.

Table 3: Effect of methanolic extracts of *A. digitata* and *A. cruentus* on acute oral toxicity test in rats.

Response	Unmarked		Head		Body		Tail	
	Before	After	Before	After	Before	After	Before	After
Alertness	N	N	N	N	N	N	N	N
Grooming	A	A	A	A	A	A	A	A
Pain response	A	A	A	A	A	A	A	A
Torch response	A	A	A	A	A	A	A	A
Tremors	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A
Gripping strength	N	N	N	N	N	N	N	N
Corneal reflux	P	P	P	P	P	P	P	P
Pupils	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Skin colour	N	N	N	N	N	N	N	N
Lacrimation	N	N	N	N	N	N	N	N
Hyper activity	A	A	A	A	A	A	A	A

NB: N: Normal; A: Absent; P: Present

Table 4: Body weight of different animals (control and treated) for methanolic extract from *A. digitata*

Weekly weather	First day	Third day	Sixth day	Eighth day	Twelfth day	Fourteenth day
Weight in (g) of untreated control animals	108± 2	109± 3	111± 2	114± 2	115± 4	117± 3
	111± 3	112± 2	114± 3	116± 4	117± 3	119± 2
	113± 2	114± 3	115± 3	117± 3	119± 2	121± 4
Weight in (g) of animals treated at the dose of 5 mg/kg of body weight	109± 3	112± 2	115± 3	118± 2	121± 2	124± 2
	110± 2	113± 3	116± 4	119± 3	122± 3	125± 3
	111± 3	113± 2	116± 4	119± 2	121± 2	125± 2
Weight in (g) of animals treated at the dose of 50 mg/kg of body weight	108± 2	111± 3	115± 2	118± 3	121± 2	125± 3
	110± 4	113± 3	117± 3	120± 4	123± 3	126± 3
	112± 3	114± 2	115± 2	119± 3	123± 3	128± 2
Weight in (g) of animals treated at the dose of 300 mg/kg of body weight	111± 3	114± 4	117± 3	121± 3	125± 3	127± 2
	112± 2	115± 4	117± 3	122± 3	126± 3	128± 3
	114± 2	119± 4	121± 3	123± 3	126± 3	129± 3
Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	110± 2	112± 3	115± 3	118± 2	123± 2	128± 3
	112± 4	113± 3	117± 2	121± 3	125± 2	129± 4
	115± 3	116± 2	121± 3	125± 4	128± 3	131±2

Table 5: Body weight of different animals (control and treated) for methanolic extract from *A. cruentus*

Weekly weather	First day	Third day	Sixth day	Eighth day	Twelfth day	Fourteenth day
Weight in (g) of untreated control animals	108± 2	109± 3	111± 2	114± 2	115± 4	117± 3
	111± 3	112± 2	114± 3	116± 4	117± 3	119± 2
	113± 2	114± 3	115± 3	117± 3	119± 2	121± 4
Weight in (g) of animals treated at the dose of 5 mg/kg of body weight	110± 3	112± 2	115± 4	118± 2	121± 2	124± 2
	111± 2	112± 4	116± 3	118± 2	122± 3	125± 3
	112± 3	114± 2	117± 2	119± 3	121± 2	126± 2
Weight in (g) of animals treated at the dose of 50 mg/kg of body weight	109± 2	111± 2	113± 2	117± 2	121± 2	123± 2
	110± 3	112± 3	114± 3	117± 3	119± 2	122± 3
	111± 2	114± 2	116± 2	118± 2	121± 3	124± 2
Weight in (g) of animals treated at the dose of 300 mg/kg of body weight	111± 2	113± 3	114± 2	117± 3	120± 2	124± 2
	112± 3	115± 2	116± 3	120± 2	121± 3	125± 4
	113± 3	115± 3	117± 3	121± 3	124± 2	127± 3
Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	111± 2	113± 3	114± 2	118± 4	121± 3	127± 4
	113± 2	115± 2	119± 3	123± 3	126± 4	129± 3
	115± 3	116± 2	120± 4	124± 2	127± 2	132± 2

Table 6: Organ weight (g) of female rats in acute toxicity test

Groups	Treatment and dose	Organs weights (gms)			
		Liver	Heart	Kidney 1	Kidney 2
Control	Weight in (g) of untreated control animals	3.81 ± 0.30 3.82 ± 0.32 3.83 ± 0.29	0.44 ± 0.04 0.45 ± 0.02 0.44 ± 0.04	0.35 ± 0.023 0.35 ± 0.025 0.36 ± 0.024	0.35 ± 0.023 0.36 ± 0.026 0.36 ± 0.023
A. digitata	Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	3.85 ± 0.28 3.86 ± 0.27 3.85 ± 0.29	0.46 ± 0.04 0.44 ± 0.03 0.43 ± 0.02	0.38 ± 0.021 0.41 ± 0.020 0.40 ± 0.021	0.37 ± 0.022 0.40 ± 0.019 0.41 ± 0.022
A. cruentus	Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	3.87 ± 0.30 3.88 ± 0.28 3.90 ± 0.26	0.46 ± 0.04 0.47 ± 0.02 0.47 ± 0.03	0.39 ± 0.023 0.42 ± 0.021 0.43 ± 0.025	0.41 ± 0.023 0.43 ± 0.022 0.43 ± 0.025

3.2 Discussion

Antibacterial activity of leaves from *A. digitata* and *A. cruentus* had not been the subject of preliminary study. These plants extracts possess significantly ($p < 0.05$) antibacterial activity against tested organisms. According to scale of diameter of inhibition of Ponce *et al*. (2013) [28], *A. digitata* and *A. cruentus* extracts presented a small diameter of inhibition with values between 8 and 10 µg/mL. All tested bacterial strains were sensitive to these two extracts. Inhibition zone diameter varied with degree of efficacy and different phytoconstituents of plant on the organism tested. Antibacterial activity of the plants may be due to presence of various active principles in their leaves. Indeed the studies of Toure *et al*. (2018a) [20] on *A. cruentus* showed high levels of polyphenols in this plant. Besides that, these compounds are well known for their antimicrobial properties [29].

The different activities observed are weak compared to that of reference molecule. These plants are bacteriostatic against the strains.

In addition, acute oral toxicity test is used for evaluating any adverse effects appearing within a short time after a single large oral dose of the test substance or after multiple doses given within 24 h. The methanolic extracts from *A. digitata* and *A. cruentus* at a dose of 2000 mg/kg did not cause any observable signs or symptoms of toxicity. The normal behaviour of test animals during a period of 14 days suggests the non-toxic nature of the above extracts. The results showed that leaves from *A. digitata* and *A. cruentus* did not cause death and result in any other signs of toxicity. To our knowledge, this is the first time that study of acute toxicity of these species has been studied. We have shown that these two plants can be consumed by population without risk.

4. Conclusion

Our work was aimed at encouraging consumption of culinary leaves in Côte d'Ivoire in general, and Korhogo in particular. This study has shown that *A. digitata* and *A. cruentus* leaves in addition to being non-toxic, possess antibacterial activities even if they remain relatively weak. A detailed antibacterial activity was carried out on methanol extracts of these leaves. Although, the antibacterial study of leaves extract is found less. The antimicrobial potential of these leaves extracts can be useful to study biocontrol activity. The non-toxic nature of methanolic extracts from *A. digitata* and *A. cruentus* is evident from the acute oral toxicity conducted as per OECD guidelines. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs.

5. Acknowledgement

The authors are grateful to the DELTAS Africa Initiative [Afrique One-ASPIRE /DEL-15-008] which support Dr Ahoua Constant for his postdoctoral study. Afrique One-ASPIRE is funded by a consortium of donor including the African Academy of Sciences (AAS) Alliance for Accelerating Excellence in Science in Africa (AESA), the New Partnership for Africa's Development Planning and Coordinating (NEPAD) Agency, the Welcome Trust [107753/A/15/Z] and the UK government.

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