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Antibacterial activity and acute toxicity studies of culinary leaves from *Corchorus olitorius L.*, *Vigna unguiculata* L.Walp and *Hibiscus sabdariffa* L. used in the North of Côte d'Ivoire.

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ABSTRACT

Methanol extracts of leaves from *Corchorus olitorius, Vigna unguiculata* and *Hibiscus sabdariffa* L. were investigated for their antibacterial activity and their acute toxicity in rats. Antibacterial activities were evaluated against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Antibacterial tests of each extract were evaluated by agar disc-diffusion method with Mueller Hinton Media. All extracts were bacteriostatic on *Staphylococcus aureus*, *Escherichia coli*and *Pseudomonas aeruginosa* at MBC of 1.5 mg/ml with MIC value greater than 30mg/ml. Acute toxicity studies were carried out based on OECD guidelines. Animals were orally administered a single dose of 5, 50, 300 and 2000 mg/kg body weight. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of extract for 14 days. Highest dose administered (2000mg/kg body weight) did not produce mortality or any changes in the general behavior of tested animals. These results indicate the safety of oral administration of methanolic extracts from the leaves of *C. olitorius, V. unguiculata* and *H. sabdariffa*.

Keywords: *Corchorus olitorius, Vigna unguiculata*, *Hibiscus sabdariffa,* antibacterial activity, acute toxicity

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INTRODUCTION

The plant resources of African savannahs such as Korhogo in north of Côte d'Ivoire, constitutes an unvaluable source of plants species used in food and in treatment of several diseases. Good nutrition and an adequate well balanced diet combined with regular physical activity is a cornerstone of good health (Tchouya et *al*., 2015). Poor nutrition can lead to a reduced immunity, an increased susceptibility to disease, an impaired physical and mental development and a reduced productivity (Tchouya et *al*., 2015).

Corchorus olitorius is an annual herbaceous plant. The leaves are alternate and the flowers are yellow and small. The fruit is a cylindrical and elongated capsule. It measures 2 to 8 cm in length. The seeds are very small. This species is cultivated in the Caribbean, Brazil, India, Bangladesh, Japan and China. It is an important leafy vegetable in Benin, Nigeria, Cameroun, Soudan, Uganda, Kenya, Zimbabwe and Côte d'Ivoire. In Côte d'Ivoire, the leaves are boiled to make a sticky, mucilaginous sauce which is served with balls of cassava called "placali". Consumption of the leaves is reported to be demulcent, deobstruent, diuretic, lactagogue, purgative and tonic. It is also a folk remedy for aches and pains, dysentery, enteritis, fever, pectoral pains and tumors (Duke, 1981; List,1979).Ayurvedics use the leaves for ascites, pain, piles and tumors. Elsewhere, the leaves are used for cystitis, dysuria, fever and gonorrhea. The cold infusion is said to restore the appetite and strength (Nyadanu et *al*., 2015). It can act as anti-inflammatory (Handoussa, 2013). It has also gastroprotective properties and can be used as an antifertility agent.

Vigna unguiculata (beans) are one of the staple local foods consumed by the populace of Korhogo (Côte d'Ivoire). A study among healthcare givers in Ilorin (Nigeria) reported that they prescribed bean meals as a major component of daily dietary therapy for persons with diabetes (Olarinoye et *al*., 2007).It is known that the beans and their products have low GI, depending on the mode of preparation (Oboh et *al*., 2010; Ohwovoriole et *al*., 1984).

What appears to be unknown is whether differences exist in the way members (referred to as varieties) of the same species of beans (*V. unguiculata*[Linn] Walp) have differential effects on blood glucose when equal amounts are consumed.

*Hibiscus sabdariffa*L. (Roselle) is an annual dicotyledonous, herbaceous shrub belonging to the Malvaceae family. This plant is widely distributed in tropical and subtropical regions of Africa, Asia, and South America (Hassan et *al*., 2016).*H. sabdariffa* has been shown to exert various biological properties including antimicrobial properties (Da-Costa-Rocha et *al*., 2014; Hopkins et *al*., 2013).The aqueous extracts of this plant are reported to possess direct inhibitory properties against *Helicobacter pylori*(Hassan et *al*., 2016) as well as anti-enzymatic properties against urease (Hassan et *al*., 2017).

The purpose of this work is to evaluate the antibacterial activity of methanolic extracts from *C. olitorius, V. unguiculata* and *H. sabdariffa*. This activity was selected because of its great medicinal relevance (Austin et *al*., 1999; Hamil et *al*., 2003). This study also aims to test the acute toxicity of these extracts.

MATERIAL AND METHODS

Plant material

The leaves from *C. olitorius, V. unguiculata* and *H. sabdariffa* were collected in February 2017 in Korhogo (north of Côte d'Ivoire). They were identified by Pr. Ipou Ipou Joseph from the Centre National de Floristique of the University Félix Houphouët-Boigny.

Preparation of extracts from leaves

For the preparation of the extracts of *C. olitorius* and *H. sabdariffa,* 150 g of each leave were introduced into glass jars with a capacity of 350 mL (H_2O), and then closed. The closed jars were placed in boiling bath (100 ° C) for 45 minutes for cooking the leaves. Subsequently, the boiled leaves were dewatered using a strainer before being reduced to pulp using an electric grinder. Then, 10 g of each leave 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 617.7 mgand412.8 mg of crude methanolic extracts for *C. olitorius* and *H. sabdariffa,* respectively.

For the preparation of the extracts of*V. unguiculata*, 10 g were extracted with MeOH(100 mL) for 48 hours. The obtained solution was filtered using whatmann no.1 filter paper and then evaporated under reduced pressure to yield 317.7 mg of crude methanolic extracts for *V.unguiculata*.

Biological Activity

Antibacterial Assays

The antibacterial activity was assessed according to the protocol used by **Ahoua *et al*., 2015** using the agar diffusion technique. Stock solutions of plant extracts were prepared at 30 mg/ml in dimethyl sulfoxide (DMSO) and at 1 mg/ml (in distilled water) for antibiotics (tetracycline and gentamicin).

Concerned bacteria strains including *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (CIP) 4.83, *Pseudomonas aeruginosa (*CIP) 103467, *Escherichia coli* (CIP) 54127AF and *Staphylococcus aureus* sensitive to penicillin from the Institut Pasteur of Côte d'Ivoire, 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.

Sensitivity test

Mueller-Hinton agar in Petri dishes (thickness = 4 mm) were soaked with an inoculum equivalent to 0.5 of McFarland. After drying, wells (diameter = 6 mm) were made in the agar using sterile Pasteur pipette. Fifty microliters (50 µl) of extract (1500 µg/ml) or antibiotic (25 µg/ml) 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.

Determination of Minimum Inhibitory Concentration (MIC)

The extracts showing an inhibitory diameter of at least 9 mm were selected to determine the minimum inhibitory concentrations (MICs) using broth microdilution method in 96-wells microplates. The MIC is the lowest concentration at which the visible growth of a strain was completely inhibited (no visible turbidity in wells). 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 × 106 bacteria/ml. The microplates were incubated at 37 °C for 18 h.

Determination of Minimum Bactericidal Concentration (MBC)

MBC is the lowest concentration of antibiotic or crude extract in which less than 0.01 % of the initial inoculum survived after 18–24 h. Medium from wells with no visible growth and from the initial inoculum (dilution 10^{-1}, 10^{-2}, 10^{-3}; 10^{-4} and 10^{-5}) was plated on agar, and colonies counted. The value MBC/MIC allowed to determine whether an extract was bacteriostatic (MBC/MIC > 4) or bactericidal (MBC/MIC < 4).

Acute toxicity

The acute oral toxicity test was performed by using the Organization for Economic Co-operation and Development (OECD) guidelines 423 (OECD, 2001).

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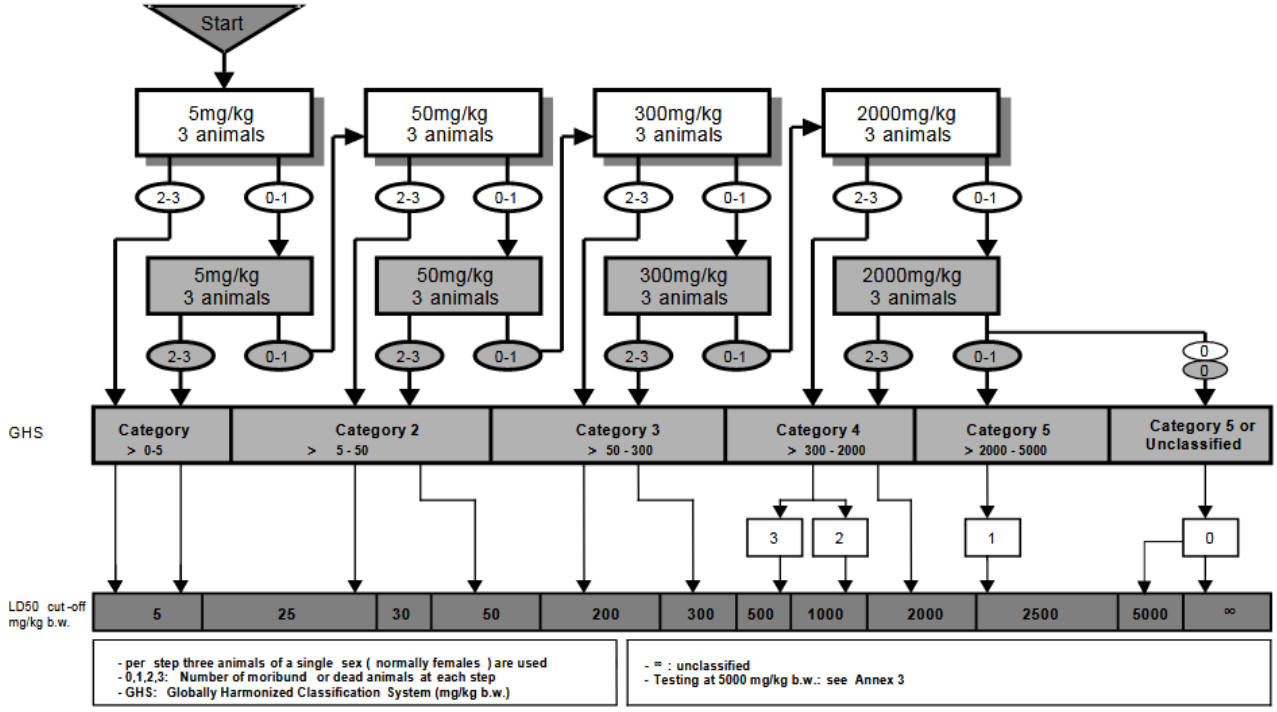

Figure 1: Test procedure with a starting dose of 5 mg/Kg body weight (OECD 2001)

Experimental animals

Experiments were performed using healthy non-pregnant young adult female rat (Wistar)weighing 107-114 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 the observation(Venkatesan et *al.*, 2005; Mitjans et *al*., 2008).

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2ºC). 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 IVOGRAINwith tap water in baby bottles(Awouters et *al*., 1978; Lalitha et *al*., 2012).

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 the 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(Mitjans et *al*., 2008; Lalitha P., et *al*., 2012).

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 (Venkatesan et *al.*, 2005; Lalitha et *al*., 2012).

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 (Lalitha et *al*., 2012).

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 (Venkatesan et *al.*, 2005; Lalitha et *al*., 2012).

Statistical analysis

Data were expressed as mean values ± SD (standard deviations). All the data were analyzed by one-way ANOVA and differences between the 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).

RESULTS AND DISCUSSION

Results

Antibacterial activity

In the current investigation, the antibacterial activity of methanolic extracts from *C. olitorius, V. unguiculata* and *H. sabdariffa* were evaluated against gram-positive and gram-negative bacteria. The diameters of the inhibition zones were measured.

According to**Ponce *et al*., 2003**, based on the diameter of inhibition, the strain is qualified as:
- non-sensitive or resistant if the diameter is less than 8 mm;
- sensitive if the diameter is between 9 and 14 mm;
- very sensitive if the diameter is between 15 and 19 mm;
- extremely sensitive if the diameter is greater than 20 mm.

Table 1 shows the inhibition diameters of the extracts on the tested bacteria. The results showed that the three extracts were active against the tested bacteria. *Pseudomonas aeruginosa*ATCC (22 mm), *Staphylococcus aureus* CIP (22.50 mm) and *Escherichia coli*ATCC (20 mm)were extremely sensitive to the methanolic extract of the leaves from *Corchoruus olitorius* while *P. aeruginosa* CIP (13 mm) and *S. aureus* sensitive to penicillin (17 mm) were sensitive and very sensitive, respectively, to the same extract. All the tested bacteria were sensitive to the methanolic extracts from *Vigna unguiculata* and *Hibiscus sabdariffa* with inhibitory diameters ranged from 9 to 10 mm.

The active extracts were bacteriostatic on all the tested bacteria. The MIC values (table 2) of the active extracts were greater than 3000□□g/ml. for *P. aeruginosa* ATCC, *P. aeruginosa* CIP, *S. aureus mr, S. aureus* CIP and *E. coli* ATCC (**Table 2**). Only *V. unguiculata* has a MIC value of 3000□g/ml on *S. aureus* sensitive to penicillin. The MIC values of the standards ranged from 0.19 to up to 50 µg/ml for tetracycline and from 1.56 to up to 50 µg/ml for gentamicin.

ISSN: 0975-8585

Table 1: Inhibition diameters (mm) of extracts on tested bacteria

		Bacteria				
		P. aeruginosa ATCC	*P. aeruginosa* CIP	*S. aureus Sens.*	*S. aureus* CIP	*E. coli* ATCC
Extracts of culinary leaves	*Corchoruus olitorius*	22.00 ± 0.58^{b}	13.00 ± 0.58^{b}	17.00 ± 1.73^{b}	22.50 ± 0.29^{b}	20.00 ± 0.00^{c}
	Vigna unguiculata	10.00 ± 0.00^{c}	9.00 ± 0.00^{c}	9.50 ± 0.29^{c}	9.00 ± 0.00^{c}	10.00 ± 0.00^{d}
	Hibiscus sabdariffa	10.00 ± 0.00^{c}	9.00 ± 0.58^{c}	9.50 ± 0.29^{c}	9.00 ± 0.00^{c}	9.50 ±0.29^{d}
Positive control	**Tetracycline**	27.00 ± 0.00^{a}	27.00 ± 0.58^{a}	23.00 ± 0.00^{a}	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	366.00	456.00	83.803	541.33	686.40
	P	< 0.001				

Table 2: Minimal Inhibitory Concentration (MIC) (µg/mL) values of the active extracts

		Bacteria				
		P. aeruginosa ATCC	*P. aeruginosa* CIP	*S. aureus Sens*	*S. aureus* CIP	*E. coli* ATCC
Extracts of culinary leaves	*Corchoruus olitorius*	>3000	>3000	>3000	>3000	>3000
	Vigna unguiculata	>3000	>3000	3000	>3000	>3000
	Hibiscus sabdariffa	>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

Table 3: Minimal Bactericidal Concentration (MBC) (µg/mL) values of the active extract

	Bacteria				
Extract of culinary leave	*P. aeruginosa* ATCC	*P. aeruginosa* CIP	*S. aureus Sens*	*S. aureus* CIP	*E. coli* ATCC
Vigna unguiculata	ND	ND	>3000	ND	ND

ND: Not determined

Acute toxicity

The present study conducted as per the OECD guidelines 423 revealed that all extracts (*C. olitorius, V. unguiculata* and *H. sabdariffa*) did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000mg/kg body weight. The oral LD_{50} was indeterminable being in excess of 2000mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-toxic. **Table 4** indicates the parameters observed before and after the administration of the test substance for the three extracts. All parameters observed were normal even at the highest dosage of 2000mg/kg body weight of the test animal. This clearly indicated that the above extracts do not produce oral toxicity. The medium lethal dose (LD_{50}) of the extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect.

ISSN: 0975-8585

Table 4: **Effect of methanolic extracts of *C. olitorius, V. unguiculata* and *H. sabdariffa* on acute oral toxicity test in rats (N: Normal; A: Absent; P: Present)**

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

Table 5: Body weight of different animals (control and treated) for extract from*C. Olitorius*

Weekly weather	Firstday	Third day	Sixth day	Eighth day	Twelfth day	Fourteenth day
Weight in (g) of untreated control animals	107± 2	108± 3	110± 2	113± 2	114± 4	116± 3
	110± 3	111± 2	113± 3	115± 4	116± 3	118± 2
	112± 2	113± 3	114± 3	116± 3	118± 2	120± 4
Weight in (g) of animals treated at the dose of 5 mg/kg of body weight	108± 3	111± 2	114± 3	117± 2	120± 2	123± 2
	109± 2	112± 3	115± 4	118± 3	121± 3	124± 3
	110± 3	112± 2	115± 4	118± 2	120± 2	124± 2
Weight in (g) of animals treated at the dose of 50 mg/kg of body weight	107± 2	110± 3	114± 2	117± 3	120± 2	124± 3
	109± 4	112± 3	116± 3	119± 4	122± 3	125± 3
	111± 3	113± 2	114± 2	118± 3	122± 3	127± 2
Weight in (g) of animals treated at the dose of 300 mg/kg of body weight	110± 3	113± 4	116± 3	120± 3	124± 3	126± 2
	111± 2	114± 4	116± 3	121± 3	125± 3	127± 3
	113± 2	118± 4	120± 3	122± 3	125± 3	128± 3
Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	109± 2	111± 3	114± 3	117± 2	122± 2	127± 3
	111± 4	112± 3	116± 2	120± 3	124± 2	128± 4
	114± 3	115± 2	120± 3	124± 4	127± 3	130±2

Table 6: Body weight of different animals (control and treated) for extract from *V. unguiculata*

Weekly weather	Firstday	Third day	Sixth day	Eighth day	Twelfth day	Fourteenth day
Weight in (g) of untreated control animals	107± 2	108± 3	110± 2	113± 2	114± 4	116± 3
	110± 3	111± 2	113± 3	115± 4	116± 3	118± 2
	112± 2	113± 3	114± 3	116± 3	118± 2	120± 4
Weight in (g) of animals treated at the dose of 5 mg/kg of body weight	109± 3	111± 2	114± 4	117± 2	120± 2	123± 2
	110± 2	111± 4	115± 3	117± 2	121± 3	124± 3
	111± 3	113± 2	116± 2	118± 3	120± 2	125± 2
Weight in (g) of animals treated at the dose of 50 mg/kg of body weight	108± 2	110± 2	112± 2	116± 2	120± 2	122± 2
	109± 3	111± 3	113± 3	116± 3	119± 2	122± 3
	110± 2	113± 2	115± 2	117± 2	120± 3	123± 2
Weight in (g) of animals treated at the dose of 300	110± 2	112± 3	114± 2	116± 3	120± 2	124± 2
	111± 3	114± 2	116± 3	119± 2	120± 3	125± 4

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mg/kg of body weight	113± 3	115± 3	117± 3	120± 3	124± 2	126± 3
Weight in (g) of animals	110± 2	112± 3	114± 2	117± 4	121± 3	126± 4
treated at the dose of 2000	112± 2	115± 2	119± 3	122± 3	126± 4	128± 3
mg/kg of body weight	114± 3	116± 2	120± 4	123± 2	127± 2	131± 2

Table 7: Body weight of different animals (control and treated) for extract from *H. sabdariffa*

Weekly weather	Firstday	Third day	Sixth day	Eighth day	Twelfth day	Fourteenth day
Weight in (g) of untreated	107± 2	108± 3	110± 2	113± 2	114± 4	116± 3
control animals	110± 3	111± 2	113± 3	115± 4	116± 3	118± 2
	112± 2	113± 3	114± 3	116± 3	118± 2	120± 4
Weight in (g) of animals	108± 3	110± 3	112± 2	114± 3	116± 2	118± 2
treated at the dose of 5	110± 2	112± 2	113± 3	115± 3	117± 2	118± 3
mg/kg of body weight	112± 3	113± 3	116± 2	117± 2	118± 3	119± 2
Weight in (g) of animals	109± 4	110± 2	112± 3	114± 3	116± 2	120± 3
treated at the dose of 50	110± 3	113± 3	115± 3	117± 3	120± 3	121± 2
mg/kg of body weight	112± 2	113± 2	115± 3	118± 3	120± 2	122± 3
Weight in (g) of animals	110± 2	112± 4	114± 3	116± 3	117± 4	120± 2
treated at the dose of 300	111± 3	113± 2	115± 2	117± 2	120± 2	122± 3
mg/kg of body weight	113± 2	115± 2	117± 4	120± 3	122± 3	125± 2
Weight in (g) of animals	108± 3	110± 3	113± 2	115± 4	120± 3	124± 3
treated at the dose of 2000	112± 3	114± 2	117± 3	120± 3	124± 4	126± 4
mg/kg of body weight	113± 2	115± 3	117± 4	121± 2	125± 2	129± 2

Table 8: 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	3.80 ± 0.30 3.81 ± 0.32 3.82 ± 0.29	0.43 ± 0.04 0.44 ± 0.02 0.44 ± 0.04	0.34 ± 0.023 0.34 ± 0.025 0.35 ± 0.024	0.34 ± 0.023 0.35 ± 0.026 0.35 ± 0.023
C. olitorius	Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	3.84 ± 0.28 3.85 ± 0.27 3.84 ± 0.29	0.45 ± 0.04 0.44 ± 0.03 0.42 ± 0.02	0.37 ± 0.021 0.40 ± 0.020 0.39 ± 0.021	0.36 ± 0.022 0.39 ± 0.019 0.40 ± 0.022
V. unguiculata	Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	3.86 ± 0.30 3.87 ± 0.28 3.89 ± 0.26	0.45 ± 0.04 0.46 ± 0.02 0.46 ± 0.03	0.38 ± 0.023 0.41 ± 0.021 0.42 ± 0.025	0.40 ± 0.023 0.42 ± 0.022 0.42 ± 0.025
H. sabdariffa	Weight in (g) of animals treated at the dose of 2000 mg/kg of body weight	3.92 ± 0.27 3.94 ± 0.28 3.96 ± 0.26	0.46 ± 0.03 0.47 ± 0.02 0.48 ± 0.04	0.41 ± 0.022 0.43 ± 0.021 0.44 ± 0.025	0.42 ± 0.022 0.44 ± 0.021 0.43 ± 0.025

DISCUSSION

Antibacterial activity of leaves from*C. olitorius, V. unguiculata* and *H. sabdariffa* 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), extract of *C. olitorius* at 1500 and 250 µg/mL gave the high zone varied from 20 to 23 mm for *P. aeruginosa* ATTC, *S. aureus* CIP and *E.*

coli ATTC. These strains were extremely sensitive to extract from *C. olitorius*.*P. aeruginosa* CIP and *S. aureus* MR were sensitive at concentration of 250 µg/mL of this extract (Ponce et *al*., 2013). *V.unguiculata* and *H. sabdariffa* extracts presented a small diameter of inhibition than those of *C. olitorius* with values between 8 and 10 µg/mL. All tested bacterial strains were sensitive to these two extracts. The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents of plant on the target organism. The antibacterial activity of the plants may be due to the presence of various active principles in their leaves. Indeed the studies of Oulaï et *al*. (2014) on *V. unguiculata* and *H. sabdariffa* and Constant et *al*. (2014) on *C. olitorius* showed high levels of polyphenols and tannins in these three leaf varieties. Besides that, these compounds are well known for their antimicrobial properties (Oguede et *al*., 2006; Ogbulie et *al*., 2007).

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

In addition,the 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 *C. olitorius, V. unguiculata* and *H. sabdariffa* at a dose of 2000 mg/kg did not cause any observable signs or symptoms of toxicity. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the above extracts. The results showed that the leaves from *C. olitorius, V. unguiculata* and *H. sabdariffa* did not cause death or result in any other signs of toxicity. To our knowledge, this is the first time that the study of the acute toxicity of these species has been studied. We have shown that these three plants can be consumed by the population without risk.

CONCLUSION

Our work was aimed at encouraging the consumption of culinary leaves in Côte d'Ivoire in general, and Korhogo in particular. This study have shown that these 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 leaves from *C. olitorius, V. unguiculata* and *H. sabdariffa*. 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 the methanolic extracts from *C. olitorius, V. unguiculata* and *H. sabdariffa* 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.

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REFERENCES

[1] Ahoua A. R. C., Konan A. G. Bonfoh B. and Koné M. W. Antimicrobial potential of27 plants consumed by chimpanzees (*Pan troglodytes verus* Blumenbach) in Ivory Coast.*BMC Complementary and Alternative Medicine*, 2015, 15, 383.

[2] Austin D.J., Kristinsson K.G. and Anderson R.M. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proceedings of the National Academy of Sciences of the United States of America, 1999, 96, 1152-1156.

[3] Awouters F., Niemegeers C.J.E., Lenaerts F.M. and Janseen P.A.J. Delay of castor oil diarrhea in rats; a new way to evaluate inhibitors of prostaglandin biosynthesis. *J. Pharmacol.,* 1978, 30: 41-45.

[4] Constant F.A., Lessoy T.Z., Eric E.A., Valery G.Y. and Sebastien L.N. Leafy vegetables consumed in southern Côte d'Ivoire: a source of high value nutrients. *J. An. Plan. Sci.*, 2014, 3,20, 3159-3170.

[5] Da-Costa-Rocha I., Bonnlaender B., Sievers H., Pischel I. and Heinrich M. *Hibiscus sabdariffa* L. Aphytochemical and pharmacological review. *Food Chem*., 2014, 165, 424-443.

[6] Duke J. A. Medicinal Plants of the Bible. Buffalo, New York: Trado-Medic Books, 1981.

[7] Hamil F.A., Apio S., Mubiru N.K., Bukenya- Ziraba R., Mosango M. and Maganyi O.W. Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays. *J. Ethnopharmacol*., 2003, 84, 57-78.

[8] Handoussa H., Hanafi R., Eddiasty I., El-Gendy M., Khatib A.E., Linscheid M., Mahran L. and AyoubN. Anti-inflammatory and cytotoxic activities of dietary phenolics isolated from *Corchorus olitorius* and *Vitis vinifera*. *J. Funct. Foods*, 2013,5, 3, 1204-1216.

[9] Hassan S.T.S., Berchová K. and Šudomová M. Antimicrobial, antiparasitic and anticancer properties of *Hibiscus sabdariffa* (L.) and its phytochemicals: *In vitro* and *in vivo* studies. *Ceska Slov. Farm*., 2016, 65, 10-14.

[10] Hassan S.T.S., Berchová K., Majerová M., Pokorná M. and Švajdlenka E. *In vitro* synergistic effect of *Hibiscus sabdariffa* aqueous extract in combination with standard antibiotics against Helicobacter pylori clinical isolates. *Pharm. Biol*., 2016, 54, 1736-1740.

[11] Hassan S.T.S., Švajdlenka E., Berchová-Bímová K. *Hibiscus sabdariffa* L. and its bioactive constituents exhibit antiviral activity against HSV-2 and anti-enzymatic properties against urease by an ESI-MS based assay.*Molecules*. 2017, 22, 722.

[12] Hopkins A.L., Lamm M.G. and Funk J.L., Ritenbaugh C. *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. *Fitoterapia*, 2013, 85, 84-94.

[13] Jahani S., Saeide S., Javadian F., Akbarizadeh Z. and Sobhanizade A. Investigating the antibacterial effects of plant extracts on *Pseudomonas aeruginosa* and *Escherichia coli. Int. J. Infect.*,2016,3, 2, 34081-34085.

[14] Lalitha P., Shubashini Sripathi K. and Jayanthi P.Acute toxicity study of extracts of *Eichhornia Crassipes* (Mart.) Solms. *Asian J. Pharm. Clin. Res*., 2012, 5, 4, 59-61.

[15] List P.H. Hager's Handbuch der pharmazeutischen Praxis. Berlin, 1979.

[16] Mitjans M., Garcia L., Marrero E. and Vinardell M.P. Study of ligmed-A, an antidiarrheal drug based on liguin, on rat small intestine enzyme activity and morphometry. *J. Vet. Pharmacol. Ther.* 2008, 24, 349-351.

[17] Nyadanu D. and Lowor S. T. Promoting competitiveness of neglected and underutilized crop species: comparative analysis of nutritional composition of indigenous and exotic leafy and fruit vegetables in Ghana. *Genetic Resources and Crop Evolution*. 2015, 62, 1, 131-140.

[18] Oboh H., Osagie A. and Omotosho A. Glycaemic response of some boiled legumes commonly eaten in Nigeria. *Diabetol. Croatica*, 2010, 39, 4, 125-131.

[19] OECD Guidelines for the Testing of Chemicals (No. 423) "Acute Oral Toxicity-Acute Toxic Class Method". 2001.

[20] Ogbulie J.N., Ogueke C.C. and Nwanebu F.C. Antibacterial properties of *Uvaria chamae, Congromena latifolium, Garcinia kola, Vemonia amygdalina* and *Afromomium melegueta*. *Afr. J. Biotechnol.*,2007, 6,13, 1549-1553.

[21] Ogueke C.C., Ogbulie J.N. and Njoku H.O. Antimicrobial properties and preliminary phytochemical analysis of ethanolic extracts of Alstonia bonnie. *Niger. J. Microbiol.*,2006,20, 2, 896-899.

[22] Ohwovoriole A.E. and Johnson T.O. Which Nigerian food for the diabetic? *Nig. J. Nutr. Sci.*,1984, 5, 59–62.

[23] Olarinoye J.K., Ano- Edwards G.H. and Alade I. Knowledge and practice of medical nutrition therapy by diabetes care givers in ilorin, Nigeria. *Nig. Med. Pract*., 2007, 51, 4, 55-58.

[24] Ouattara K., Toure A., Ouattara L., Ouattara A. and Coulibaly A. In vitro evaluation of antibacterial activity of flavonoids of *Thonningia sanguinea* Vahl. (Balanophoraceae). *J. Microbiol. Biotech. Res.*,2014,4,6, 38-41.

[25] Oulaï P., Lessoy Z., Rose-Monde M., Ryta D. and Sébastien N. Proximate composition and nutritive value of leafy vegetables cosumed in northern Côte d'Ivoire. *Eur. Sci. J.*,2014, 6,10, 212-227.

[26] Ponce A. G., Fritz R., Del Alle C. and Roura S. I. Antimicrobial activity of essential oil on the native microflora of organic Swiss chard. *Lebensm Wiss Technol.*,2003,36, 679-684.

[27] Venkatesan N., Thiyagarajan V., Narayanan S., Arul A., Raja S., Kumar S.G.V., Rajarajan T. and Perianayagam J.B. Antidiarrheal potential of Asparagus racemous wild root extracts in laboratoire animals. *J. Pharm. Pharmaceut. Sci.*,2005, 8, 1, 39-45.