International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 6 (3): 173-182, 2010
ISSN 1811-7775

Antibacterial Activity, Bioavailability and Acute Toxicity Evaluation of the Leaf Extract of *Alchornea cordifolia* (Euphorbiaceae)

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Abstract: The antibacterial activity, bioavailability and acute toxicity of the leaf extracts of *Alchornea cordifolia* were evaluated. The phytochemical screening was also done. Results of the *in vitro* antibacterial tests showed that all but the hexane extract exhibited antibacterial activities against *P. aeruginosa* (MIC = 2.5 mg mL^{-1}), *E. coli* (MIC = 3.75 mg mL^{-1}), *S. aureus* (MIC = 5 mg mL^{-1}) and *K. pneumoniae* (MIC = 10 mg mL^{-1}). Results of the serum antimicrobial activity test against *P. aeruginosa* showed that with animals administered the extract, serum antimicrobial activity was observed as from 3 h 45 min for the doses 2.84 and 5.68 g kg^{-1}, independently of the sex of animals. The peaks of serum activity (maximum activity, A_{max}) were obtained at 6 and 4 h (T_{max}) with the doses 2.84 and 5.68 g kg^{-1}, respectively. The area under the curve (AUC) was greater with the dose 2.84 g kg^{-1} than with the dose 5.68 g kg^{-1}. Flavonoids, triterpenes, saponins, anthocyanins, steroids, polyphenols and tannins were found in the leaves of this plant. Results of the acute toxicity study of the aqueous leaf extract of *A. cordifolia* with mice showed that the LD_{50} was >32 g kg^{-1}. The data obtained in this study suggest that the leaves of *A. cordifolia* contain antibacterial principle(s) which are biologically available and which may not be toxic. However, at doses ≥4 g kg^{-1}, this extract may have a depressant or sedative effect on the central nervous system, may reduce the pain perception and may induce loss of body weight.

Key words: *Alchornea cordifolia* leaves, antimicrobial, bioavailability, phytochemical, LD_{50}

INTRODUCTION

Alchornea cordifolia (Schumach and Thonn) Müll. Arg (Euphorbiaceae) is an erect and bushy perennial shrub or small tree, up to 4 m high, reproducing from seeds. The stem is woody, greyish, with lightly granulated bark (Olaleye *et al*., 2006) with many branches and bushy when young. The leaves are simple and alternate and are heart shaped at the base with long petioles. The inflorescence consists of auxiliary panicles and the flowers are greenish white. The male flowers are long spikes (8-36 cm) while the females are simple with short stalks. The fruits are 3-chambered capsules containing red seeds (Olaleye *et al*., 2006). There are many convergences in its traditional use throughout tropical Africa as topical anti-inflammatory substance. The crude aqueous methanolic extract of the leaves of *A. cordifolia* has been found to show anti-inflammatory activity (Osadebe and Okoye, 2003). It is also used for the treatment of chancre, yaws, wounds and ulcers, gum inflammation and conjunctivitis (Neuwinger, 2000), toothache (Akendengue and Louis, 1994), parasite infection (Tona *et al*., 1998). The ethanolic leaf extract of this plant was found to exhibit mild *in vitro* activity against *Plasmodium falciparum*. Fifty percent aqueous ethanol extract of the leaf of this plant has been shown to possess spasmolytic properties (Tona *et al*., 2000) antioxidant properties and glutathione S-transferases inhibitory activity (Olaleye *et al*., 2007). The hepatoprotective activity of the ethanol extract of *A. cordifolia* leaves against paracetamol-induced toxicity has also been reported (Olaleye *et al*., 2006).

Non specific urogenital infections are caused by human commensalist bacteria, which become pathogen either due to a change in their normal behaviour/habitat or due to a failure in the immune system. Among these bacteria are *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* (Cheesbrough, 1991). These infections cause serious health problems especially in developing countries where they are more or less endemic.

In a continuation of this search for therapeutic agents from natural sources with potential for the

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treatment of bacterial infections (Gatsing *et al.*, 2006, 2008, 2009; Gatsing and Adoga, 2007), the antimicrobial activity of *A. cordifolia* leaf extracts was investigated against *E. coli*, *P. aeruginosa*, *K. pneumonia* and *S. aureus*. The phytochemical screening of the various extracts, the bioavailability and acute toxicity studies of the aqueous extract were also done.

MATERIALS AND METHODS

Plant material: The leaves of *Alchornea cordifolia* were collected in Dschang, West province, Cameroon, in April 2005 and the plant was identified at the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N° 9656/SRF CAM) is deposited. The total time duration of the study was 6 months (April-September 2005).

Test bacteria and culture media: The test microorganisms, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from the Medical Bacteriology Laboratory of Pasteur Centre, Yaoundé, Cameroon. The culture media, namely Tryptose Phosphate broth (CONSOLAB, Malaysia) and Mueller Hinton agar (International Diagnostics Group PLC, UK) were used.

Experimental animals: A total of 60 Swiss albino mice (30 males and 30 females, 11-12 weeks old) weighing 23-32 g were used for the acute toxicity study, whereas males (132) and females (132) Wistar albino rats, 3 months old, weighing 170-200 g were used for the bioavailability study. These animals were bred in the Animal House of the University of Dschang, Cameroon.

Preparation of extract

Aqueous extract: The leaves of *A. cordifolia* were allowed to dry at room temperature (24±2°C) and were pulverized. Then 200 g of the powder obtained were boiled in 1000 mL of distilled water for 20 min, as indicated by the traditional healer. After filtration, the filtrate was concentrated in a Gefran-600 oven at 40°C and the aqueous extract was obtained. The yield was 24.65% (w/w).

Organic solvent extracts: The powder of *A. cordifolia* leaves was separately extracted with different organic solvents such as methanol, ethanol, acetone, ethyl acetate and hexane. One hundred gram of the powder were mixed with the appropriate solvent in a tightly closed container and left to stand for about 3 days. The mixture was shaken once daily until it was filtered and concentrated on the third day in a rotary evaporator under vacuum at 40°C. The residue left after solvent evaporation weighed 25, 17.35, 5.7, 5.1 and 2 g for the methanol, ethanol, acetone, ethyl acetate and hexane extracts, respectively.

Antimicrobial assay: The antibacterial activity was determined using agar diffusion, agar dilution and broth dilution techniques, as previously used by Gatsing *et al.* (2006) with some modifications. The use of both dilution techniques was imposed by the color of the extracts.

Agar diffusion susceptibility testing was done using the well method. On each Petri dish (9 cm in diameter) containing Mueller Hinton agar medium already inoculated with the test organism (100 µL of the bacteria suspension in Tryptose Phosphate broth, at the concentration of 10^8 cfu mL^{-1}) equidistant wells (6 mm in diameter) were bored using a cork borer. Methanol, ethanol, acetone, ethyl acetate and hexane extracts were dissolved in 10% DMSO (dimethylsulfoxide), whereas the aqueous extract was dissolved in distilled water. The wells were filled with 150 µL of the solution (50 mg mL^{-1}) of various extracts to be tested. Augmentin was used as the standard drug and was tested at the concentration of 0.1 mg mL^{-1}. The Petri dishes were left at room temperature (24±2°C) for about 45 min to allow the extracts to diffuse from the wells into the medium. They were then incubated at 37°C for 24 h, after which the zones of no growth were noted and their diameters recorded as the zones of inhibition.

For the agar dilution method, the solution (maximum concentration) of each active extract (i.e., the extract that induced a zone of inhibition) was prepared in the appropriate solvent and serially diluted. A volume of 0.5 mL of each extract was mixed with 4.45 mL of Mueller Hinton agar and was introduced in the Petri dishes (5.5 cm in diameter) containing 0.05 mL of bacteria suspension of 10^8 cfu mL^{-1} and the mixture was homogenized. The total volume of the mixture was 5 mL, with the test extract concentration in the plate ranging from 50 to 1 mg mL^{-1} and those of augmentin ranging from 25 to 1.562 µg mL^{-1}. After 24 h of incubation at 37°C, the Minimum Inhibitory Concentration (MIC) was reported as the lowest concentration of antimicrobial that prevented visible bacterial growth. The Minimum Bactericidal Concentration (MBC) was determined by the broth dilution method, i.e., by inducing the above concentrations of extracts in Tryptose Phosphate broth medium (in test tubes), by incubating the mixture at 37°C for 24 h and sub-culturing all the tubes (concentrations) in which there was no growth (as seen in the Mueller Hinton agar medium) on already prepared plates containing Mueller Hinton agar medium. The plates were then incubated at 37°C for 24 h

and the lowest concentration showing no growth was taken as the minimum bactericidal concentration.

Evaluation of the bioavailability of the antibacterial principles: The bioavailability of the antibacterial principles contained in the aqueous leaf extract (the most active extract) of *A. cordifolia* was evaluated in rats using the serum antimicrobial activity test (Youmans *et al*., 1975; Liao *et al*., 2005) against *P. aeruginosa* (the most susceptible bacterial strain to the extract).

Administration of the extract to animals and preparation of serum: Animals of each sex were subjected to 15 h fast prior to the administration of the extract or the standard drug (augmentin). The doses of extract used were 2.84 and 5.68 g kg^{-1} (i.e., 4×0.71 g kg^{-1} and 8×0.71 g kg^{-1}, respectively; 0.71 g kg^{-1} being the dose calculated from the MBC; in a preliminary study, the dose 0.71 g kg^{-1} did not show any activity against *P. aeruginosa.* Also, the dose used by the traditional healer (i.e., 0.072 g kg^{-1}) did not show any activity, in this single administration). For each of the two doses used, animals in each sex were organized in 8 groups of 4 rats each, according to the time interval between oral administration of extract and collection of blood samples. For each sex, one negative control group was formed (made up of 4 rats); animals in this group received distilled water and their blood samples were collected at 4 h after administration. The time intervals were 2 h, 3 h, 3 h 45 min, 4 h, 5 h, 6 h, 7 h and 8 h for the animals receiving the extract. The doses of augmentin used were 0.0018 g kg^{-1} (obtained from the MBC of augmentin) and 0.0036 g kg^{-1} (i.e., twice the dose obtained from the MBC) and for each dose, animals in each sex were subdivided into 8 groups of 4 animals each. The time intervals were 10, 20, 40, 60, 120, 180, 225 and 240 min for augmentin. Blood samples were collected by cardiac puncture and were allowed to clot by standing at room temperature for 1 h and then refrigerated for another 1 h. The resultant liquid part was centrifuged at 3000xg for 10 min and then the serum (supernatant) was isolated (Gatsing *et al*., 2005).

Determination of serum antimicrobial activity: This activity was determined by the diffusion sensitivity test on Mueller Hinton agar, using the well method (Gatsing *et al*., 2006). *Pseudomonas aeruginosa* was used for this test. On each Petri dish containing Mueller Hinton agar already inoculated with the test bacteria, equidistant wells were bored with a sterile cork borer of 6 mm of diameter and filled with 100 µL of serum. After 45 min of pre-diffusion at room temperature, the Petri dishes were incubated at 37°C for 24 h and then the diameters of the zones of inhibition were measured and recorded as the serum antimicrobial activity.

Phytochemical screening: The presence of some major antimicrobial substances such as alkaloids, flavonoids, triterpenes, saponins, cardiac glycosides, anthocyanins, polyphenols, anthraquinones, steroids, tannins and phlobatannins in the various leaf extracts of *A. cordifolia* was verified using the methods described by Bruneton (1999).

Evaluation of the acute toxicity: For acute toxicity studies, 60 albino mice (30 males and 30 females) were used. The mice in each sex group were divided into 6 groups of 5 animals each. All animals were subjected to 15 h fast prior to administration of the aqueous leaf extract of *A. cordifolia*. Mice in group 1 (controls) were given distilled water (1 mL per 30 g of body weight), while mice in groups 2, 3, 4, 5 and 6 were treated with graded doses of the plant extract, that is, 2, 4, 8, 16 and 32 g kg^{-1} of body weight. The animals in all groups were observed during the first 3 h after a single oral administration of the extract for behavioural changes, i.e., locomotion, reaction to noise, reaction to pinch, the state of the tail, the state of the excrement. The mice are said to be in activity (i.e., locomotion) when they are roaming in the cage; normal reaction to noise is when the mice are unsettled on hearing a noise; the cries of mice when pinched on their tail is an indicator of normal reaction pinch; the tail is normal when it is flexible (i.e., not rigid); a rigid tail is a sign of anger. After the first 3 h of observation, all the animals had free access to food and water. The deaths were counted within the first 48 h and the Lethal Dose 50 (LD_{50}) was determined. The surviving animals were further observed for two weeks; their weight was recorded everyday, whereas food and water intakes were measured at the end of each week.

Statistical analysis: Data obtained were expressed as Mean±SD and were analyzed using One-way ANOVA. Waller-Duncan test was used to compare means of different groups. A p-value of <0.05 was considered statistically significant.

Ethics: This study was carried out with respect for the welfare of animals, as recommended by Mosihuzzaman and Choudhary (2008).

RESULTS

Antimicrobial activity: Diameters of inhibition of the test microorganisms by the various extracts of the

Table 1: Diameter of inhibition zones of *A. cordifolia* leaf extracts and augmentin on the tested bacteria

		Bacteria and mean diameter of inhibition zone (mm)			
Extracts	Concentrations (mg mL^{-1})	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Aqueous	50	22	20	26	21
Methanol	50	22	16	17	21
Ethanol	50	14	13	15	16
Acetone	50	22	16	19	23
Ethyl acetate	50	13	-	13	14
Hexane	50	-	-	-	-
Augmentin (standard)	0.1	26	25	16	14

Tabulated diameter values are means of two determinations; -: No inhibition

Table 2: MIC and MBC of *A. cordifolia* leaf extracts and augmentin on the tested bacteria

		Bacteria and inhibition parameters			
Extracts	Parameters	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
Aqueous	MIC (mg mL^{-1})	3.75	10	2.5	5
	MBC (mg mL^{-1})	15	40	10	20
	MBC/MIC	4	4	4	4
Methanol	MIC (mg mL^{-1})	15	20	15	15
	MBC (mg mL^{-1})	25	35	30	30
	MBC/MIC	2	2	2	2
Ethanol	MIC (mg mL^{-1})	>50	20	15	15
	MBC (mg mL^{-1})	ND	>50	30	30
	MBC/MIC	ND	ND	2	2
Acetone	MIC (mg mL^{-1})	7.5	>50	5	3.75
	MBC (mg mL^{-1})	>50	ND	>50	>50
	MBC/MIC	ND	ND	ND	ND
Ethyl acetate	MIC (mg mL^{-1})	>50	ND	>50	20
	MBC (mg mL^{-1})	ND	ND	ND	30
	MBC/MIC	ND	ND	ND	2
Augmentin (standard)	MIC (μg mL^{-1})	6.25	12.5	3.125	3.125
	MBC (μg mL^{-1})	12.5	25	12.5	12.5
	MBC/MIC	2	2	4	4

ND: Not determined; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

A. cordifolia leaves are presented in Table 1. Aqueous, methanol, ethanol and acetone extract were active against the microorganisms used, with inhibition diameters varying from 13 to 26 mm; aqueous extract exhibited the highest antibacterial activity. Ethyl acetate extract was active against *E. coli* (13 mm), *P. aeruginosa* (13 mm) and *S. aureus* (14 mm) only; this extract did not show any activity against *K. pneumoniae*. Hexane extract did not show any activity against the tested bacteria. Augmentin (standard antibiotic) presented the inhibition diameters varying from 16 to 26 mm against the microorganisms used.

The results of the dilution sensitivity test (MICs, MBCs) are presented in Table 2. Aqueous leaf extract of *A. cordifolia* was the most active extract with MIC values of 2.5, 3.75, 5 and 10 mg mL^{-1} against *P. aeruginosa*, *E. coli*, *S. aureus* and *K. pneumoniae*, respectively. The MBC values for this extract were 10 mg mL^{-1} (*P. aeruginosa*), 15 mg mL^{-1} (*E. coli*), 20 mg mL^{-1} (*S. aureus*) and 40 mg mL^{-1} (*K. pneumoniae*). *P. aeruginosa* was the most sensitive strain among the bacteria tested. The MIC values of the methanol extract varied from 15 to 20 mg mL^{-1}, whereas its MBC values varied between 25 and 35 mg mL^{-1}. The MIC values of augmentin (standard) were 3.125 μg mL^{-1} against *P. aeruginosa* and *S. aureus*, 6.25 μg mL^{-1} against *E. coli* and 12.5 μg mL^{-1} against *K. pneumoniae*; its MBC values varied between 12.5 and 25 μg mL^{-1}.

Bioavailability: Antibacterial activities of serum against *P. aeruginosa* as a function of time after administration of the extract at the doses 2.84 and 5.68 g kg^{-1} to male and female rats are presented in Fig. 1 and 2, whereas those obtained after administration of augmentin at the doses 0.0018 and 0.0036 g kg^{-1} are presented in Fig. 3 and 4. The sera of animals receiving distilled water (negative control) did not show any antibacterial activity against *P. aeruginosa*. No serum was active at 2, 3 and 8 h after administration of the extract, independently of the doses and sex of animals. For animals receiving augmentin and independently of the sex, serum antimicrobial activity was obtained at 40 min (T_i, initial activity time) for the dose 0.0018 g kg^{-1} and 20 min (T_i) for the dose 0.0036 g kg^{-1}, whereas for those receiving the extract, this activity was obtained at 3 h 45 min (T_i) for the doses 2.84 and 5.68 g kg^{-1}.

The peak of serum activity (i.e., maximum activity, A_{max}) was obtained at 2 and 6 h (T_{max}), respectively with

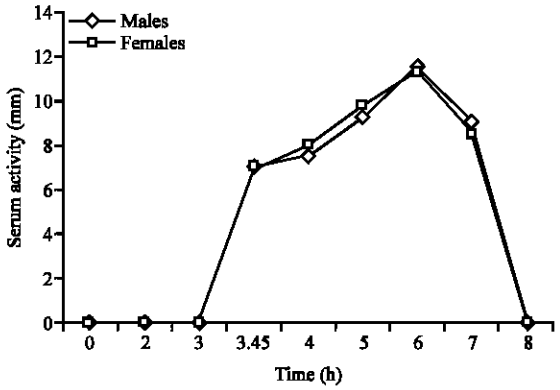

Fig. 1: Serum antibacterial activity against *P. aeruginosa* as affected by time after administration of the dose 2.84 g kg^{-1} of the aqueous leaf extract of *A. cordifolia* to rats. Values of the figure are means of four determinations

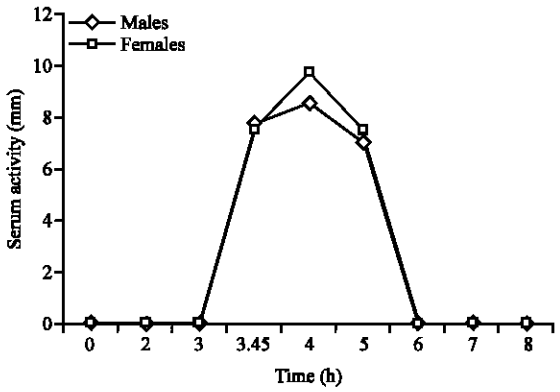

Fig. 2: Serum antibacterial activity against *P. aeruginosa* as affected by time after administration of the dose 5.68 g kg^{-1} of the aqueous leaf extract of *A. cordifolia* to rats. Values of the figure are means of four determinations

the doses of 0.0018 g kg^{-1} (augmentin) and 2.84 g kg^{-1} (extract). With the dose 5.68 g kg^{-1}, the A_{max} was obtained at 4 h (T_{max}), whereas with the dose 0.0036 g kg^{-1}, the A_{max} was obtained at 1 h. This activity of the serum was an indication of the concentration of antimicrobial in the blood. We can say that the maximum concentration (C_{max}) in the blood was reached at these different times (T_{max}), depending on the dose administered. Within the same dose, the activity significantly ($p<0.05$) varied with time. For the dose 2.84 g kg^{-1}, the serum antimicrobial activity was seen from 3 h 45 min to 7 h, whereas for the dose 5.68 g kg^{-1}, the serum activity was observed between 3 h 45 min and 5 h in both sexes, after administration of the extract. Also, for each sex, A_{max} at the dose of 2.84 g kg^{-1} was higher than that obtained at the dose

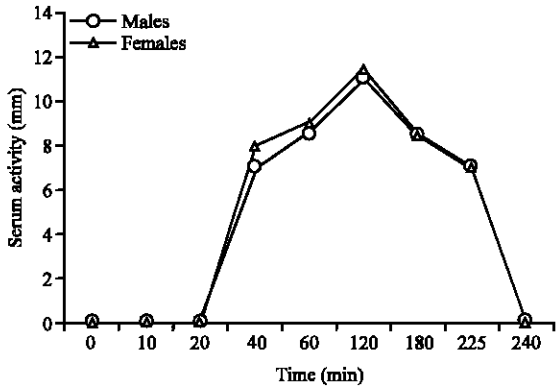

Fig. 3: Serum antibacterial activity against *P. aeruginosa* as affected by time after administration of the dose 0.0018 g kg^{-1} of augmentin to rats. Values of the figure are means of four determinations

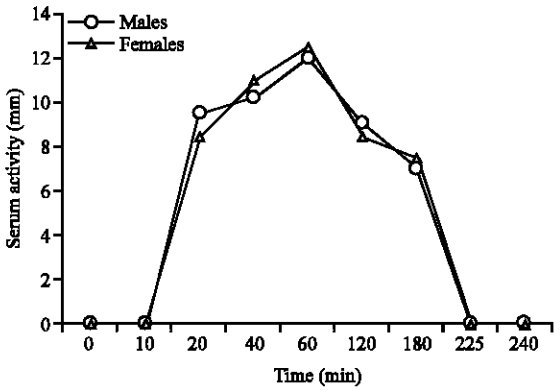

Fig. 4: Serum antibacterial activity against *P. aeruginosa* as affected by time after administration of the dose 0.0036 g kg^{-1} of augmentin to rats. Values of the figure are means of four determinations

5.68 g kg^{-1}. The Area Under the Curve (AUC) was greater with the dose 2.84 g kg^{-1} (Fig. 1) than with the dose 5.68 g kg^{-1} (Fig. 2), independently of sex. However, the half-life ($t_{1/2}$) of elimination of the antimicrobial principle was smaller with the dose 5.68 g kg^{-1} than with the dose 2.84 g kg^{-1}.

Phytochemical composition: The results of phytochemical screening, presented in Table 3 showed the presence of flavonoids, triterpenes, saponins, anthocyanins, polyphenol, steroids and tannins and the complete absence of alkaloids, cardiac glycosides, anthraquinones and phlobatannins in the leaf extracts of *A. cordifolia*. These compounds were differently distributed in the various extracts and varied from abundance to complete absence.

Table 3: Classes of chemical compounds found in the leaf extracts of *A. cordifolia*

	Chemical compounds										
Extracts	Alk	Fla	Tri	Sap	Gly	Ant_1	Pol	Ant_2	Ste	Tan	Phl
Aqueous	-	+	+++	++	-	+	+++	-	++	+++	-
Methanol	-	+	++	++	-	+	++	-	+	++	-
Ethanol	-	+	+	++	-	+	++	-	+	++	-
Acetone	-	+	+	++	-	+	+	-	-	++	-
Ethyl acetate	-	-	+	++	-	-	-	-	+	-	-
Hexane	-	-	+	++	-	-	-	-	-	-	-

Alk : Alkaloids; Fla : Flavonoids; Tri : Triterpenes; Sap: Saponins; Gly: Cardiac glycosides; Ant_1: Anthocyanins; Pol: Polyphenols; Ant_2: Anthraquinones; Ste: Steroids; Tan: Taunins; Phl: Phlobatannins; +: Trace; ++: Relatively abundant; +++: Very abundant; -: Absent

Table 4: Behaviour of males and females mice during the first 3 h of observation in acute toxicity study with *A. cordifolia* aqueous leaf extract

	Doses (g kg^{-1}) and behaviour of mice					
Parameters	0	2	4	8	16	32
Locomotion	+	+	-	-	--	--
Reaction to pinch	+	+	-	-	--	--
Reaction to noise	+	+	+	+	-	--
State of tail	+	+	+	+	+	+
State of excrement (males)	g	g	g	g	g	g
State of excrement (females)	g	g	g	g	g	P
Mortality	N	N	N	N	N	N

+: Normal; ++: Increase; -: Reduce; --: Profoundly reduce; g : Granular; p : Pasty; N : No mortality

Table 5: Food intake as affected by doses of *A. cordifolia* aqueous leaf extract during acute toxicity study

		Doses of extract (g kg^{-1}) and food intake (g)					
Sex	Time (days)	0 (control)	2	4	8	16	32
Males	1-7	27.88 ± 1.17^{a}	21.64 ± 0.17^{b}	20.72 ± 0.019^{bc}	17.28 ± 0.19^{d}	19.08 ± 0.17^{cd}	18.72 ± 0.24^{cd}
	8-14	24.68 ± 0.75^{a}	17.24 ± 0.61^{c}	18.88 ± 0.2^{b}	19.48 ± 0.15^{b}	18.72 ± 0.24^{bc}	18.96 ± 0.01^{b}
Females	1-7	22.04 ± 0.11^{a}	20.48 ± 0.27^{ab}	15.72 ± 0.57^{c}	20.04 ± 0.49^{ab}	18.72 ± 0.01^{c}	15.16 ± 0.16^{c}
	8-14	18.84 ± 0.63^{a}	17.72 ± 0.36^{a}	17.24 ± 0.64^{a}	17.56 ± 0.06^{a}	16.60 ± 0.02^{a}	17.96 ± 0.87

Tabulated values are Means±SD of five determinations. Values on the same line with the same letters are not significantly different ($p>0.05$)

Table 6: Water intake as affected by doses of *A. cordifolia* aqueous leaf extract during acute toxicity study

		Doses of extract (g kg^{-1}) and water intake (mL)					
Sex	Time (days)	0 (control)	2	4	8	16	32
Males	1-7	20.64 ± 1.13^{a}	15.88 ± 0.62^{b}	12.80 ± 0.64^{b}	14.00 ± 1.17^{b}	10.84 ± 0.44^{c}	7.92 ± 0.33^{c}
	8-14	17.36 ± 0.83^{a}	13.96 ± 0.64^{b}	12.24 ± 0.51^{b}	13.04 ± 0.55^{b}	9.96 ± 0.83^{c}	7.40 ± 0.39^{c}
Females	1-7	19.48 ± 0.51^{a}	9.36 ± 0.51^{c}	8.20 ± 0.44^{c}	12.92 ± 0.51^{b}	12.56 ± 0.78^{b}	10.96 ± 0.89^{b}
	8-14	17.36 ± 0.51^{a}	12.44 ± 0.82^{b}	11.96 ± 0.33^{b}	10.52 ± 0.51^{b}	10.28 ± 0.31^{b}	13.04 ± 0.38^{b}

Tabulated values are Means±SD of five determinations. Values on the same line with the same letters are not significantly different ($p>0.05$)

Acute toxicity: The behavioural changes observed during acute treatment are summarized in Table 4. The mice were observed for activity (locomotion), reaction to noise, reaction to pinch, state of excrement and for mortality (within 48 h). After administration of the various doses of extract of *A. cordifolia*, it was noted that mice receiving 4, 8, 16 and 32 g kg^{-1} showed a dose dependent reduction in activity (locomotion) and in the reaction to pinch, compared to the controls. The reaction to noise was also reduced in the mice receiving 16 g kg^{-1} and above. The mice in all groups had normal tail (flexible) and granular excrement, except the female mice in group 6 (32 g kg^{-1}) whose excrement was pasty. However, no mortality was observed within 48 h after administration of the extract. The Lethal Dose 50 (LD_{50}) of this extract was greater than 32 g kg^{-1}, since, up to this dose no death was recorded.

Food and water consumptions recorded two weeks after the administration of plant extract are presented in Table 5 and 6, respectively. From Table 5, significant ($p<0.05$) reductions in food consumption were observed in the treated males during the first and second weeks after administration of the extract. Significant ($p<0.05$) reductions in food intake were also observed in the treated female mice during the first week of observation. From Table 6, significant ($p<0.05$) reductions in water intake are observed in all treated groups (both males and females), compared to the controls.

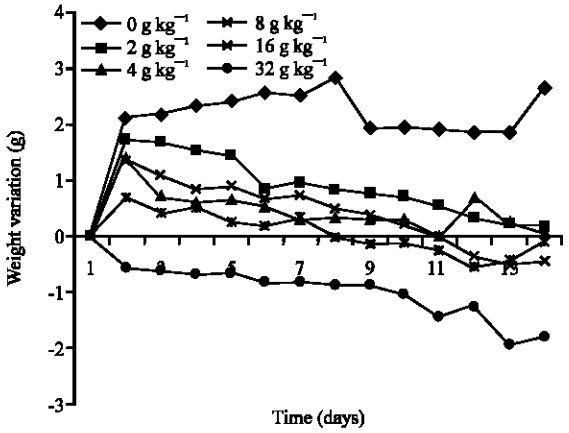

Fig. 5: Weight variation of male mice as affected by doses of *A. cordifolia* aqueous leaf extract during acute toxicity study. Values of the figure are means of five determinations

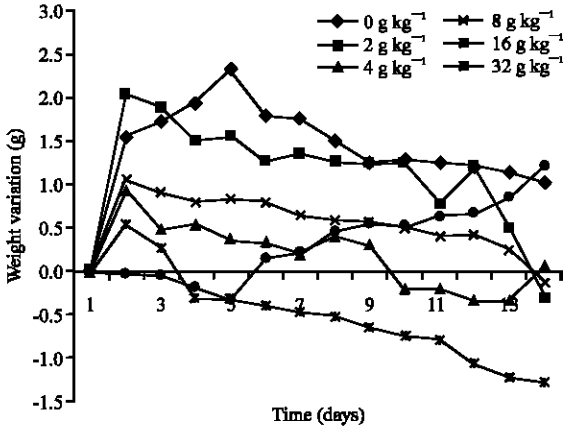

Fig. 6: Weight variation of female mice as affected by doses of *A. cordifolia* aqueous leaf extract during acute toxicity study. Values of the figure are means of five determinations

The weight variation of the surviving mice recorded for two weeks, after the administration of the plant extract, are presented in Fig. 5 and 6 for males and females, respectively. From these figures, in general, mice administered the extract at the doses of 4, 8, 16 and 32 g kg^{-1} showed a dose dependent reduction in body weight, whereas mice that received the extract at the dose 2 g kg^{-1} (male and female) gained weight progressively as from the first day after administration extract; however, their weight gain remained inferior to that of the control.

DISCUSSION

The microorganisms used in this study were found to be susceptible at least to one extract of *A. cordifolia* leaves, suggesting that the antimicrobial principle contained in the leaves of this plant may be of broad spectrum since, it was able to inhibit both Gram-positive and Gram-negative bacteria. These observations corroborate those of Okeke *et al.* (1999) confirming the use of this plant in the treatment of bacterial infections.

The values of the diameters of inhibition, MICs and MBCs show that the degree of activity varied with the germs and the extracts. This variation of the activity could be due to the difference of solubility of the active ingredient in each solvent on the one hand and to the constitutional or structural variability of the tested germs on the other hand. Also, it could be due to the capacity of the microorganisms to modify the structure of the active principle (Brooks *et al.*, 1991). The active principle could be very polar in nature, taking into account the fact that the aqueous extract showed the highest activity against the microorganisms used, compared to the others extracts. The results obtained from the diffusion sensitivity tests showed that the extracts of *A. Cordifolia* might be 500 times less active than the standard antibiotic used (augmentin). This difference in activity could be due to the fact that extracts of plant are mixtures of compounds, while the standard antibiotics are pure compounds. Antimicrobial substances are considered as bactericidal agents when the ratio MBC/MIC ≤4 and bacteriostatic agent when the ratio MBC/MIC >4 (Carbonnelle *et al.*, 1987; Gatsing *et al.*, 2009). For most of the extracts tested (e.g., aqueous, methanol and ethanol extracts), the ratio MBC/MIC was ≤4 against the bacteria strains used, suggesting that these extracts may be classified as bactericidal agents.

The data obtained from the bioavailability study show that the aqueous extract of the leaves of *A. cordifolia* was effectively absorbed into the blood stream and that the active principle contained in that extract was not denatured during its transit in the gastrointestinal tract. However, the serum activity with the extract was observed as from 3 h 45 min, whereas that with augmentin was observed as from 20 min after administration. Moreover, the maximum serum activity with the extract (at 2.84 g kg^{-1}) was seen at 6 h after administration, while that with augmentin (at 0.0018 g kg^{-1}) was observed at 2 h after administration. This may be explained by the fact that the extract is a mixture of compounds, whereas augmentin is a pure compound; the absorption of pure compound being easier and faster than that of an extract.

At the dose of 0.71 g kg^{-1} (dose corresponding to the MBC), the extract did not show any serum antimicrobial activity (preliminary study; data not shown), whereas at the dose of 2.84 g kg^{-1} the serum antimicrobial activity was observed. This result suggests that the

extract may be absorbed at about 25%. The serum activity is a reflection of the blood concentration of the active principle.

The curves expressing the serum antimicrobial activity as a function of time (Fig. 1, 2) show that the peak of activity [A_{max}; corresponding to the maximum concentration (C_{max}) of the active principle in the blood] was reached after 6 h (T_{max}) with the dose 2.84 g kg^{-1} and after 4 h (T_{max}) with the dose 5.68 g kg^{-1}, in both male and female rats. This result suggests that the rate of absorption was greater with the dose 5.68 g kg^{-1} than with the dose 2.84 g kg^{-1}, indicating that the grater the concentration of the substance in the gastrointestinal tract (GIT), the higher the rate of absorption (Craig, 1998). Therefore, the rate of absorption was influenced by the concentration gradient of the extract. However, serum A_{max} with the dose 5.68 g kg^{-1} was lower than that with the dose 2.84 g kg^{-1}, despite the fact that the initial rate of absorption seemed to be higher with 5.68 g kg^{-1} than with 2.84 g kg^{-1}. These results corroborate those of Biber *et al.* (1998) who, in a bioavailability study with rats and humans, observed that at high doses, T_{max} and C_{max} (i.e., maximum concentration) values significantly reduced, compared to those obtained with moderated doses. The data obtained in our study suggest that absorption might have happened in two stages, i.e., rapid at the beginning and slowed down afterwards. At high doses, the extract might have caused some damages to the enterocytes, therefore slowing down the absorption of the active principle(s) contained in the extract.

It is also observed that the time interval under the curve was greater with the dose 2.84 g kg^{-1} (i.e., about 4 h 15 min) than with the dose 5.68 g kg^{-1} (i.e., about 2 h 15 min), indicating that the half-life ($t_{1/2}$) of elimination of the antimicrobial principle from the blood was longer with the dose 2.84 g kg^{-1} than with the dose 5.68 g kg^{-1}. The greater the $t_{1/2}$ value, the longer the drug will stay in the body. This increases the time of exposure of the microorganism to the antimicrobial. This also implies that the area under the cure (AUC) was greater with the dose 2.84 g kg^{-1} than with the dose 5.68 g kg^{-1}. The AUC value is very useful for determining the relative efficiency of different drug products (Bourne, 2008). The values of T_{max} obtained in this study indicate that for a repeated administration, the second administration may be done around 6 h (with the dose 2.84 g kg^{-1}) and 4 h (with the dose 5.68 g kg^{-1}) after the fist administration; since, the extract will take some times to reach the general circulation.

The phytochemical analysis revealed the presence of many classes of chemical compounds such as flavonoids, triterpenes, saponins, anthocyanins, polyphenols, steroids and tannins. The presence of flavonoids and steroids corroborate the work done by Osadebe and Okoye (2003). Some of these bioactive compounds could explain the antibacterial activity observed in this study; the highest activity was observed with the aqueous extract in which tannins, triterpenes and polyphenols were more abundant, in contrast to the others extracts. In fact, tannins have been reported to precipitate bacterial proteins and proteins necessary for their nutrition, therefore, preventing bacteria development (Gatsing *et al.*, 2008). Triterpenes have also been reported to inhibit the membrane structure of microorganisms due to their lipophilic properties (Cowan, 1999).

In general, acute toxicity study did not reveal any negative behavioural changes at low doses (<4 g kg^{-1}) of extract as compared to the control in both male and female mice. However, reduce locomotion and reaction to noise were observed at doses ≥4 g kg^{-1} and ≥16 g kg^{-1}, respectively, in both sexes, suggesting that the aqueous leaf extract of *A. cordifolia* may have a depressant or sedative effect on the central nervous system (Gatsing *et al.*, 2009) at high doses. The extract may act as myorelaxant or tranquilliser on the nervous centres or on the motor fibres (Gatsing *et al.*, 2008). Plants containing chemical constituents like coumarin, flavonoids, monoterpenes, proanthocyanidines and glycolipids, have been reported to possess Central Nervous System (CNS) depressant activity (Abid *et al.*, 2006). In a study involving *Pachyrrhizus erosus* seeds, it has also been reported that the sedative, muscle relaxant and anxiolytic effects of those seeds were due to the interaction of isoflavonoids with the GABA/benzodiazepine receptor complex in the brain (Trofimiuk *et al.*, 2005; Gatsing *et al.*, 2008). The depressant effect of the aqueous extract of the leaf of *A. cordifolia* may therefore, be due to the presence of flavonoids in the extract.

A reduction of reaction to pinch was observed at doses ≥4 g kg^{-1} in both sexes. The effect of the extract on the perception of pain may be due to its action on the nociceptors or to the inhibition of the production of algogenic substances (e.g., prostaglandins, histamines), or to the inhibition of the painful message transmission, at the central level (Nguelefack *et al.*, 2004). As mentioned earlier, preliminary phytochemical screening of the aqueous extract of the leaves of *A. cordifolia* revealed the presence of flavonoids. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception (Chakraborty *et al.*, 2004). Hence, the presence of flavonoids may supplement the action of this extract on pain perception.

In general, all the treated groups showed a reduction in food and water intakes throughout the period of study, compared to the control group. This result suggests that the extract may induce loss of appetite. This may be supported by the fact that, in female mice, the stool was pasty at 32 g kg^{-1}, indicating slight diarrhoea. These results suggest that at such dose, the extract may have an irritating action on the intestinal mucosa inducing an increase in the permeability of the mucosal cells and changes in electrolyte transport (Teke *et al*., 2007). Also, mice treated with the extract showed losses in body weight during the study, compared to the controls. This reduction in weight may be due to the decrease in food and water intakes, as observed during the study.

No mortality was seen over 48 h after a single oral administration of the aqueous leaf extract of *A. cordifolia* to mice, up to the dose 32 g kg^{-1}, suggesting that the LD_{50} was greater than 32 g kg^{-1}. Based on the Hodge and Steiner criteria (Delongeas *et al*., 1983) this extract can be considered practically non-toxic.

CONCLUSION

In the light of the foregoing, it is clear that the leaves of *A. cordifolia* contain antibacterial principles which are active against *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus*. Moreover, the aqueous extract of the leaves (the most active extract) may not be toxic (LD_{50}>32 g kg^{-1}) and is biologically available; therefore, it may be used in the treatment of some urogenital infections. However, subchronic toxicity study should be done to further ascertain the safety of the users of this extract.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Joseph Tchoumboué, Faculty of Agronomy and Agricultural Sciences, University of Dschang and Dr (Mrs) Fonkoua Marie-Christine, Medical Bacteriology Laboratory, Pasteur Centre, Yaoundé, Cameroon, for their co-operation.

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