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Original Article

In Vitro Antibacterial Activity of *Crinum Purpurascens* Herb. Leaf Extract Against the *Salmonella* Species Causing Typhoid Fever and Its Toxicological Evaluation

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Abstract

Background: *Crinum purpurascens* is a herbaceous plant belonging to the Amaryllidaceae family. We aimed to evaluate the antisalmonellal properties of the leaf extracts and fractions of *C. purpurascens*, and the toxicity of the most active extract.

Methods: Three extracts and three fractions were prepared from the leaves of *Crinum purpurascens* Herb. (Amaryllidaceae) and tested for their antisalmonellal activities and toxicity profile. The antibacterial activity was determined using agar diffusion, agar dilution, and broth dilution techniques. Phytochemical screening of the various extracts and fractions was performed. The toxicity profile of the CH_2Cl_2/MeOH (1:1) extract was studied.

Results: All the extracts and fractions, except hexane fraction, showed antimicrobial activity against *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B. The CH_2Cl_2/MeOH (1:1) extract showed the highest activity. The minimum inhibitory concentration values were 2.50 mg/ml against *S. typhi*, and 1.88 mg/ml against *S. paratyphi* A and *S. paratyphi* B. The minimum bactericidal concentration values were 7.50 mg/ml against *S. typhi* and 3.75 mg/ml against *S. paratyphi* A and *S. paratyphi* B. Mice administered high doses of extract exhibited reduced reaction to noise, locomotion, reactivity and reaction to pinch, and losses in body weight. Additionally, the rats that received high doses of the extract showed increase in liver, spleen and kidney to body weight ratios, and decrease in total protein concentrations of the liver and lung, and in hematocrit value.

Conclusion: *C. purpurascens* leaf extract contains antisalmonellal principle(s) and at high doses, may have a depressant or sedative effect on the central nervous system and analgesic activity. Also, it may be anorexiant, hepatotoxic, and nephrotoxic.
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Keywords ● Antibacterial ● phytochemicals ● toxicity

Introduction

Crinum purpurascens is a herbaceous plant belonging to the Amaryllidaceae family. It occurs as a bulb fleshy scale leaves on the lower part of the stem, and

is distributed throughout Asia, America, and Africa.[1] It is often grown for ornamental purposes. However, in Cameroon folk medicine, *C. purpurascens* is used to treat epilepsy, sexual asthenia, and abdominal pain.[2] Extracts and compounds obtained from some species of *Crinum* have been reported to exhibit antitumor, antifungal, antiparasitic, and insecticidal activities.[2]

Typhoid fever is caused by *Salmonella typhi,* whereas paratyphoid fevers are caused by *S. paratyphi* A and *S. paratyphi* B.[3] Typhoid fever continues to be a marked public health problem in developing countries in general and in Sub-Saharan Africa in particular, where it is endemic.[4] Moreover, the typhoid causative organism, *Salmonella typhi*, has rapidly gained resistance to all three first-line antimicrobials (ampicillin, chloramphenicol, and co-trimoxazole).[5,6]

In a continuation of our search for pharmacologically active agents from natural sources with potential for the treatment of typhoid and paratyphoid fevers,[7-13] the antimicrobial activity of aqueous, methanol, and mixture of methylene chloride/methanol (1:1) leaf extracts, hexane, methylene chloride and ethyl acetate fractions of *C. purpurascens* was investigated against *S. typhi*, *S. paratyphi* A and *S. paratyphi* B. Phytochemical screening of the various extracts and fractions, and toxicological study of the most active extract were also performed.

Materials and Methods

Plant Material

The leaves of *Crinum purpurascens* Herb. (Amaryllidaceae) were collected from Maha, NDE Division, West Province of Cameroon, in July 2005. Authentication was carried out by Mr. Nana, a botanist of the Cameroon National Herbarium, Yaounde, where a voucher specimen (N° 0976/SRF/CAM) is deposited.

Test Bacteria and Culture Media

The test microorganisms including *Salmonella typhi, Salmonella paratyphi* A and *Salmonella paratyphi* B, were obtained from the Medical Bacteriology Laboratory of the Pasteur Centre, Yaounde, Cameroon. The culture media used, namely Mueller Hinton agar, Salmonella-Shigella Agar (SS Agar) and Selenite Broth were manufactured by CONSOLAB (Malaysia). SS agar was used for isolation of the *Salmonella* species and for the screening of contaminants from the inoculum. Mueller Hinton agar and Selenite Broth were used for antibacterial tests.

Experimental Animals

In the present study, 60 Swiss albino mice (30 males and 30 females, 11-12 weeks old) weighing 19-22 g, and 50 Wistar albino rats (25 males and 25 females, 8-9 weeks old) weighing 100-120 g were used. These animals were bred in the animal house of the University of Dschang, Cameroon.

Preparation of Extracts and Fractions

Aqueous Extract

After collection, the leaves of *C. purpurascens* were air-dried at room temperature (24 ± 1 °C) and pulverized; 500 g of the powder were infused in 2.5 L of boiled water for 10 min. After cooling, the mixture was filtered using Wattman paper n° 3 and the filtrate was concentrated in a drying oven at 45 °C to obtain 20.7 g of aqueous crude extract.

Organic Solvent Extracts and Fractions

The air-dried and pulverized leaves of *C. purpurascens* (500 g) were macerated three times at room temperature in methanol (MeOH) for 72 hours, and the mixture was filtered. The filtrate was concentrated to obtain 27.5 g of crude methanol extract.

The air-dried and pulverized leaves of *C. purpurascens* (1500 g) were macerated three times at room temperature in a mixture of CH_2Cl_2/MeOH (1:1) for 72 hours. The filtrate was concentrated to obtain 122 g of crude CH_2Cl_2/MeOH (1:1) extract.

Part of the CH_2Cl_2/MeOH (1:1) extract (100 g) was dissolved in water and extracted successively with n-hexane, methylene chloride (CH_2Cl_2) and ethyl acetate (EtOAc) to obtain the various fractions (2.04 g, 8.19 g and 12.78 g, respectively).

Antimicrobial Assay

The antibacterial activity was determined using agar diffusion, agar dilution, and broth dilution techniques, as previously used by Gatsing and co-workers,[4] with some modifications. The use of both dilution techniques was imposed by the color of the extracts/fractions.

Agar diffusion susceptibility testing was performed using the well method.[4] 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 selenite broth, at the concentration of 10^6 cfu/ml) equidistant wells (6 mm in diameter) were bored using a cork borer. Hexane, CH_2Cl_2 and EtOAc fractions, MeOH and CH_2Cl_2/MeOH (1:1) 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) of various extracts and fractions to be tested. Ciprofloxacin was used as

the standard drug, and was tested at the concentration of 0.1 mg/ml. The Petri dishes were left at room temperature (24 ± 1 °C) for about 45 min to allow the extracts and fractions to diffuse from the wells into the medium. The test media were then incubated at 37 °C for 24 h, after which the zones of no growth were noted and their diameters recorded as the zone of inhibition.

For the agar dilution method, the solution (maximum concentration) of each active extract/fraction (i.e. the extract/fraction that induced a zone of inhibition) was prepared in the appropriate solvent and serially diluted. A volume of 0.5 ml of each extract/fraction was mixed with 4.45 ml of Mueller Hinton agar and was introduced to the Petri dishes (5.5 cm in diameter) containing 0.05 ml of bacteria suspension of 10^6 CFU/ml and the mixture was homogenized. The total volume of the mixture was 5 ml, with the test extract/fraction concentration in the plate ranging from 25 to 1 mg/ml and those of ciprofloxacin ranging from 10 to 1 µg/ml. 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/fractions in Selenite 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.

Phytochemical Screening

The phytochemical screening was performed using standard methods described by Harborne,[14] Odebiyi and Sofowora.[15] The plant sample was screened for the following classes of compounds: alkaloids, flavonoids, cardiac glycosides, anthraquinones, anthocyanins, polyphenols, triterpenes, steroids, saponins, tannins and phlobatannins.

Toxicity Profiling

The CH_2Cl_2/MeOH (1:1) extract, which showed the highest antimicrobial activity, was used for the toxicological studies.

For acute toxicity studies, 60 Swiss albino mice (30 males and 30 females) were used. Animal treatment was performed according to the method previously used by Gatsing and others,[16] The deaths were counted within the first 48 hours and the lethal dose 50 (LD_{50}) was determined using the method of Behrens and Karber.[17] The surviving animals were further observed for two weeks, during which their weight, food, and water consumption were recorded.

Subchronic toxicity studies were performed according to the method previously used by Gatsing and colleagues,[16] with the following modifications. The doses of extract used were 0.536, 1.072, 2.144, and 4.288 g/kg, calculated from the minimum bactericidal concentration (7.50 mg/ml) of *Crinum purpurascens* leaf extract. The rats were acclimatized for one week before the start of the treatment. The administration of various doses of the extract and distilled water were done by gastric intubations once a day, for four consecutive weeks. The food and water intakes were evaluated every day and the animals were also weighed.

Collection of Blood and Isolation of Organs during Subchronic Toxicity Study

At the end of treatment period, the animals were anesthetized with chloroform vapor prior to dissection. Blood samples were collected by cardiac puncture into heparinized and non-heparinized centrifuge tubes.

The heparinized blood was used to estimate hematocrit values, while the non-heparinized blood was allowed to coagulate, and then centrifuged and the serum was separated. Serum was assayed for proteins, total cholesterol, creatinine, and transaminases (ALT and AST).

Immediately after blood collection, the animals were killed for tissue study. Liver, lungs, heart, kidneys, and spleen were isolated, blotted freed of blood, weighed (using an electronic balance, Mettler PE 160 (Mettler Balance, France) and stored at -30 °C for measurement of protein concentration.

Preparation of Serum Sample

The blood was allowed to clot by standing at room temperature for 1 hour and then refrigerated for another 1 hour. The resultant liquid part was removed and centrifuged at 3000 x g for 5 minutes, and then the serum (supernatant) was isolated and stored at -30 °C for analysis.

Preparation of Tissue Homogenate

The homogenate of each organ was prepared in 0.9% NaCl solution at the concentration of 15% (i.e. 15 g organ in 100 ml of solution).

Some Indices of Tissue Damage

Possible damage to the liver, kidneys, heart, lungs, spleen, and red blood cells of the animals as a result of repeated administration of CH_2Cl_2/MeOH (1:1) extract of *C. purpurascens* was studied using some biochemical indices of

tissue damage. Total protein concentrations of the above-mentioned organs were determined by the Biuret method, as described by Gornall et al.[18] Serum total cholesterol and creatinine were determined by colorimetric method using commercial kits of DIALAB (Austria); serum transaminases (ALT and AST) activities were determined by the kinetic method using the commercial kits of CHRONOLAB (Switzerland); hematocrit values were determined as described by Benson and Cales.[19]

Statistical Analysis

Statistical analyses were performed using SPSS for Window software version10.0. Group comparisons were done using One Way ANOVA and the Waller-Duncan test.

P value < 0.05 was considered statistically significant.

Ethics

This work was carried out with respect for the welfare of animals, as recommended by WHO.[20]

Results

Antimicrobial Assay

The crude extracts (aqueous, MeOH and CH_2Cl_2/MeOH (1:1)) and fractions (hexane, CH_2Cl_2 and EtOAc) obtained from the leaves of *Crinum purpurascens* were tested against three *Salmonella* species, namely *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B at the concentration of 50 mg/ml, using agar diffusion technique. The data obtained (table 1) showed that hexane fraction was not active against the above bacteria strains, whereas aqueous, MeOH and CH_2Cl_2/MeOH (1:1) extracts, EtOAc and CH_2Cl_2 fractions showed antibacterial activity against these three bacteria species. Among the extracts/fractions, CH_2Cl_2/MeOH (1:1) extract showed the highest antibacterial activity against the three bacteria strains used. The activity of ciprofloxacin against these bacteria was much greater than that of CH_2Cl_2/MeOH (1:1) extract.

Aqueous, MeOH and CH_2Cl_2/MeOH (1:1) extracts, EtOAc and CH_2Cl_2 fractions, which showed antibacterial activity against all the three bacteria stains used, were further studied using dilution technique in agar and broth media, and their MIC and MBC values were obtained (table 2). CH_2Cl_2/MeOH (1:1) extract showed the lowest MIC and MBC values against the bacteria strains tested. For ciprofloxacin

Table 1: Inhibition diameter of various extracts and fractions from the leaves of *Crinum purpurescens* against *S. typhi, S. paratyphi* A and *S. paratyphi* B

Extract/Fraction	Concentration (mg/ml)	Bacteria Strains and Inhibition Diameters (mm)		
		S. typhi	***S. paratyphi* A**	***S. paratyphi* B**
Aqueous Extract	50.0	20.0	20.0	20.0
MeOH Extract	50.0	22.0	22.0	22.0
CH_2Cl_2/MeOH (1:1) Extract	50.0	24.0	26.0	26.0
C_6H_{12} Fraction	50.0	NA	NA	NA
CH_2Cl_2 Fraction	50.0	19.0	20.0	21.0
EtOAc Fraction	50.0	21.0	20.5	19.5
Ciprofloxacin (Standard)	0.1	38.0	36.0	38.0

NA: No activity

Table 2: Inhibition parameters (MIC, MBC) of various extracts and fractions from the leaves of *Crinum purpurescens,* against *S. typhi, S. paratyphi* A and *S. paratyphi* B

Extract/Fraction	Inhibition parameters	Bacteria Strains		
		S. typhi	***S. paratyphi* A**	***S. paratyphi* B**
Aqueous extract	MIC (mg/ml)	5.00	5.00	5.00
	MBC (mg/ml)	25.00	25.00	25.00
	MBC/MIC	5.00	5.00	5.00
MeOH extract	MIC (mg/ml)	2.50	2.50	7.50
	MBC (mg/ml)	15.00	12.50	15.00
	MBC/MIC	6.00	5.00	2.00
CH_2Cl_2/MeOH (1:1) extract	MIC (mg/ml)	2.50	1.88	1.88
	MBC (mg/ml)	7.50	3.75	3.75
	MBC/MIC	3.00	2.00	2.00
EtOAc fraction	MIC (mg/ml)	10.00	5.00	5.00
	MBC (mg/ml)	25.00	25.00	25.00
	MBC/MIC	2.50	5.00	5.00
CH_2Cl_2 fraction	MIC (mg/ml)	5.00	5.00	7.50
	MBC (mg/ml)	>25.00	20.00	25.00
	MBC/MIC	>5.00	4.00	3.33
Ciprofloxacin (standard)	MIC (µg/ml)	2.00	2.00	2.00
	MBC (µg/ml)	8.00	5.00	5.00
	MBC/MIC	4.00	2.50	2.50

MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration

floxacin, the MIC and MBC values were about 1000 times lower than those of the CH_2Cl_2/MeOH (1:1) extract, which was the most active extract.

Phytochemical screening of the various extracts and fractions revealed the presence of different classes of chemical compounds, namely alkaloids, flavonoids, saponins, steroids, cardiac glycosides, and anthocyanins (table 3). The quantities of the chemical compounds vary with the nature of the extract/fraction. The difference between hexane fraction and the other fractions/extracts was at the level of alkaloids and steroids. Only traces of alkaloids were present in hexane fraction, whereas steroids were completely absent in this fraction.

Acute Toxicity

The behavioral changes of animals observed during acute treatment with the extract are summarized in tables 4 and 5, for male and female mice respectively. The mice were observed for activity (locomotion), reaction to noise, reaction to pinch, reactivity state of excrement, and for mortality (within 48 hours) after administration of the various doses of CH_2Cl_2/MeOH (1:1) extract. Male mice in groups 4 (8 g/kg) and 5 (16 g/kg) showed a reduction in activity, whereas mice in groups 1 (control, i.e. 0 g/kg), 2 (2 g/kg), and 3 (4 g/kg) were still roaming in the cage. The reaction to noise was reduced in group 5 and profoundly reduced in group 6 (26 g/kg). Also, the reaction to pinch was reduced in group 5 and profoundly reduced in group 6. Reactivity was reduced in male and female mice at the doses greater than or equal to 8 g/kg and 4 g/kg respectively. The mice in all groups had granular excrement. Eight cases of death were observed within 48 hours after administration of extract, and the lethal dose 50 (LD_{50}) in male mice was calculated to be 15.2 g/kg. For female mice, locomotion was reduced in groups 5 (14 g/kg) and 6 (26 g/kg). The reaction to noise and the reaction to pinch were also reduced in group 4 (8 g/kg) and profoundly reduced in groups 5 and 6. The excrement was granular for all treated groups and control ani-

Table 3: Principal classes of natural compounds present in various extracts and fractions from the leaves of *Crinum purpurescens*

Classes of compounds	Aqueous extract	MeOH extract	CH_2Cl_2/MeOH (1:1) extract	Hexane fraction	EtOAc fraction	CH_2Cl_2 fraction
Alkaloids	+ +	+ ++	+ ++	+	+ ++	+ ++
Flavonoids	+ +	+ +	+ +	+ +	+ +	+ +
Cardiac glycosides	+ ++	+ + +	+ ++	+ ++	+ ++	+ + +
Anthraquinones	-	-	-	-	-	-
Polyphenols	-	-	-	-	-	-
Triterpenes	-	+ ++	+ +	+ ++	-	+ +
Steroids	+ ++	+ ++	+ ++	-	+ ++	+ ++
Saponins	+ +	+ +	+ +	+ +	+ +	+ +
Tannins	-	-	-	-	-	-
Anthocyanins	+	+	+	+	+	+

+ + +: Abundant; + +: Less abundant; + : Traces; - : Absent

Table 4: Behavioral changes observed during the study of acute toxicity of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract with male mice

Study parameters	Doses (g/kg)					
	0	2	4	8	1 6	2 6
Locomotion	N	N	N	D $^{-}$	D $^{-}$	D $^{- -}$
Reaction to pinch	N	N	N	N	D $^{-}$	D $^{- -}$
Reaction to noise	N	N	N	N	D $^{-}$	D $^{- -}$
Reactivity	N	N	N	D $^{-}$	D $^{- -}$	D $^{- -}$
State of excrement	G	G	G	G	G	G
Mortality within 48 hrs	0	0	0	0	3	5

N: Normal; D $^{-}$: Slightly decreased; D $^{- -}$: Profoundly decreased; G: Granular.

Table 5: Behavioral changes observed during acute toxicity study of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract with female mice

Study parameters	Dose (g/kg)					
	0	2	4	8	1 4	2 6
Locomotion	N	N	N	D $^{-}$	D $^{- -}$	D $^{- -}$
Reaction to pinch	N	N	N	D $^{-}$	D $^{- -}$	D $^{- -}$
Reaction to noise	N	N	N	D $^{-}$	D $^{- -}$	D $^{- -}$
Reactivity	N	N	D $^{-}$	D $^{-}$	D $^{- -}$	D $^{- -}$
State of excrement	G	G	G	G	G	G
Mortality within 48 hrs	0	0	0	0	5	5

N: Normal; D $^{-}$: Slightly decreased; D $^{- -}$: Profoundly decrease; G: Granular.

mals. Ten cases of death were observed within 48 hours after administration of extract, and the lethal dose 50 (LD_{50}) of this extract in female mice was calculated to be 11.0 g/kg.

Food and water consumptions of the surviving mice recorded during the two weeks of observation after the administration of the extract are presented in table 6. A general dose-dependent reduction in food consumption was noticed for both male and female mice. This reduction was found to be significant (P=0.036) for practically all treated male and female mice, compared with the control animals. A significant (P=0.021) reduction in water intake was also noticed for all treated groups (for both males and females) compared with the control animals. However, food and water intakes in the second week were greater than those in the first week.

The weights of the surviving mice recorded during the two weeks of observation after the administration of plant extract are presented in figures 1 and 2 for male and female animals, respectively. Mice that received the extract at doses of 8 and 16 g/kg (males) and 4 and 8 g/kg (females) showed a reduction in body weight for the first seven days after administration, while from day 8, the mice gained weight progressively. On the other hand, mice that received the extract at the dose 2 g/kg (male and female) gained weight progressively from the first day after administration. However, their total weight gain remained inferior to that of the control animals.

Subchronic Toxicity

No death occurred during the treatment period either in the controls or in the CH_2Cl_2/MeOH (1:1) extract-treated groups.

The weight gain during the four weeks of subchronic toxicity studies is presented in table 7. In both male and female groups, weight gain decreased with the increase in the dose of extract. These decreases were particularly significant (P=0.025) at the doses of 2.144 and 4.288 g/kg. This was observed almost throughout the four weeks of treatment.

The results of the effects of *C. purpurascens* CH_2Cl_2/MeOH (1:1) extract on organ to body weight ratios of both male and female rats are summarized in table 8. The organs studied were heart, liver, lungs, kidneys, and spleen. For male and female rats treated with this extract, the liver, spleen, and kidneys to body weight ratios significantly (P=0.017) increased, while heart and lungs to body weight ratios significantly (P=0.014) decreased at the doses of 2.144 and 4.288 g/kg, as compared with the control group.

The results of the effect of *C. purpurascens* extract on the total protein concentration of organs for both male and female rats are

Table 6: Food and water intakes by mice as affected by doses of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract during acute toxicity study

		Food intake		Water intake	
Sex of animal	**Dose (g/kg)**	**Week 1**	**Week 2**	**Week 1**	**Week 2**
Males	0	23.57 ± 0.07^{e}	24.92 ± 0.26^{e}	18.53 ± 0.03^{e}	22.51 ± 0.04^{e}
	2	21.46 ± 0.14^{d}	23.67 ± 0.12^{d}	18.19 ± 0.06^{d}	21.70 ± 0.10^{d}
	4	18.22 ± 0.22^{c}	21.43 ± 0.16^{c}	17.03 ± 0.11^{c}	19.03 ± 0.18^{c}
	8	15.62 ± 0.14^{b}	18.71 ± 0.17^{b}	14.90 ± 0.11^{b}	16.91 ± 0.16^{b}
	16	8.34 ± 0.37^{a}	12.94 ± 0.57^{a}	10.49 ± 0.21^{a}	13.21 ± 0.03^{a}
Females	0	22.10 ± 0.18^{c}	24.88 ± 0.05^{d}	19.35 ± 0.11^{d}	22.87 ± 0.13^{d}
	2	21.85 ± 0.09^{c}	23.92 ± 0.09^{c}	18.53 ± 0.16^{c}	21.30 ± 0.25^{c}
	4	18.95 ± 0.33^{b}	21.19 ± 0.22^{b}	16.63 ± 0.15^{b}	18.79 ± 0.18^{b}
	8	16.82 ± 0.19^{a}	19.51 ± 0.40^{a}	14.76 ± 0.09^{a}	16.95 ± 0.15^{a}

Tabulated values are mean ± SEM of five trials. a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.

Table 7: Weight gain as affected by doses of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract during four weeks of subchronic toxicity study in rats

Sex of animal	**Dose (g/kg)**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Males	0	14.66 ± 2.72^{a}	18.29 ± 1.17^{b}	21.60 ± 4.34^{b}	21.92 ± 5.30^{b}
	0.536	14.56 ± 1.54^{a}	18.15 ± 0.56^{b}	19.12 ± 2.13^{b}	18.15 ± 3.73^{b}
	1.072	13.57 ± 1.22^{a}	17.16 ± 1.02^{b}	16.65 ± 2.47^{b}	15.48 ± 4.17^{a}
	2.144	13.27 ± 1.13^{a}	16.92 ± 1.68^{a}	16.47 ± 2.21^{a}	13.52 ± 3.74^{a}
	4.288	13.47 ± 2.61^{a}	16.78 ± 1.26^{a}	15.26 ± 2.95^{a}	12.21 ± 3.57^{a}
Females	0	11.02 ± 2.13^{c}	13.53 ± 0.87^{c}	15.17 ± 1.87^{c}	16.21 ± 2.16^{c}
	0.536	10.16 ± 1.02^{c}	13.02 ± 0.87^{c}	14.21 ± 1.55^{c}	16.07 ± 1.70^{c}
	1.072	7.74 ± 1.22^{b}	11.25 ± 0.56^{b}	9.38 ± 0.89^{b}	11.35 ± 1.17^{b}
	2.144	6.90 ± 1.54^{b}	8.11 ± 1.62^{a}	6.71 ± 1.02^{a}	7.25 ± 3.15^{a}
	4.288	5.07 ± 2.13^{a}	8.07 ± 1.05^{a}	5.93 ± 1.67^{a}	6.15 ± 2.96^{a}

Tabulated values are mean ± SEM of five trials. a, b, c: values on the same column with different letters are significantly different at $P < 0.05$.

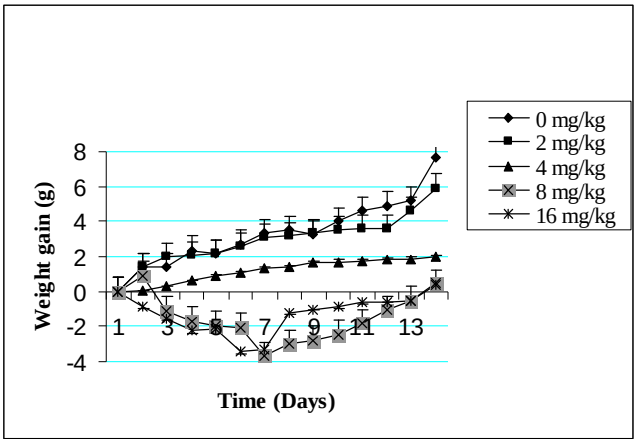

Figure 1: Variation of body weight of male mice as a function of time and dose of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract

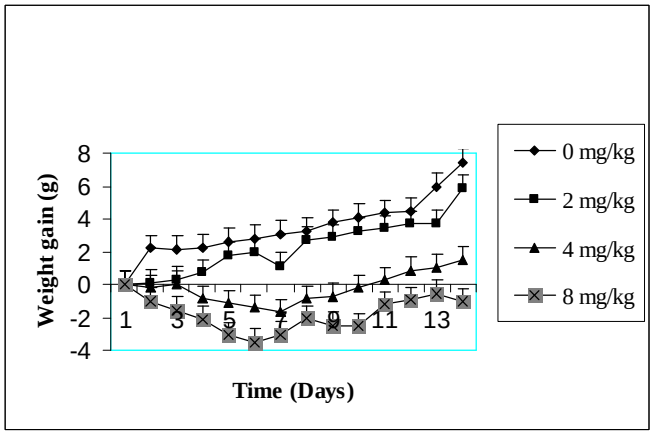

Figure 2: Variation of body weight of female mice as a function of time and dose of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract

Table 8: Organ to body weight ratios as affected by doses of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract after four weeks of administration to rats

Sex of animal	Doses (g/kg)	Heart	Liver	Lungs	Spleen	kidneys
Males	0	0.302 ± 0.003^{b}	3.266 ± 0.052^{a}	0.867 ± 0.006^{e}	0.290 ± 0.001^{b}	0.290 ± 0.020^{a}
	0.536	0.293 ± 0.007^{a,b}	3.261 ± 0.080^{a}	0.861 ± 0.019^{d}	0.280 ± 0.009^{a}	0.292 ± 0.010^{a}
	1.072	0.288 ± 0.001^{a}	3.311 ± 0. 134^{a}	0.848 ± 0.004^{c}	0.308 ± 0.014^{c}	0.294 ± 0.002^{a}
	2.144	0.289 ± 0.001^{a}	3.427 ± 0.158$^{a\,b}$	0.816 ± 0.008^{b}	0.330 ± 0.001^{d}	0.338 ± 0.001^{b}
	4.288	0.290 ± 0.006^{a}	3.786 ± 0.228^{b}	0.814 ± 0.005^{a}	0.346 ± 0.016^{e}	0.358 ± 0.008^{c}
Females	0	0.345 ± 0.001^{d}	3.098 ± 0.002^{a}	0.645 ± 0.011^{e}	0.293 ± 0.011^{a}	0.310 ± 0.015^{a}
	0.536	0.342 ± 0.003^{d}	3.087 ± 0.003^{b}	0.640 ± 0.099^{d}	0.297 ± 0.012^{b}	0.311 ± 0.015^{a}
	1.072	0.335 ± 0.001^{c}	3.118 ± 0.001^{c}	0.616 ± 0.030^{a}	0.320 ± 0.011^{c}	0.328 ± 0.019^{b}
	2.144	0.325 ± 0.001^{b}	3.352 ± 0.018^{d}	0.634 ± 0.015^{c}	0.315 ± 0.012^{d}	0.340 ± 0.019^{c}
	4.288	0.317 ± 0.002^{a}	3.600 ± 0.019^{e}	0.625 ± 0.006^{b}	0.356 ± .012^{e}	0.366 ± 0.009^{d}

Tabulated values are mean ± SEM of five trials. a, b, c, d, e: values on the same column with different letters are significantly different at $P < 0.05$.

presented in table 9. For the male and female rats treated with the extract at doses greater than or equal to 1.072 g/kg, significant decreases (P=0.043)were observed in the total protein concentration of the liver, as compared with the control. For male rats treated with the extract at dose of 1.072 g/kg, significant (P=0.039) increases were observed in the total protein concentration of the heart and kidneys, as compared with the control, while for the female rats treated with the extract at dose of 2.144 g/kg and greater, significant (P=0.032) increases were observed in total protein concentration of the heart and kidneys as compared with the control.

The results of the effects of the extract on the

hematocrit values, serum total cholesterol and serum creatinine are summarized in table 10. For the male rats, significant (P=0.018) decreases in hematocrit values were observed in the groups treated with the extract at dose of 2.144 g/kg and 4.288 g/kg, as compared with the control, whereas for the female rats, this parameter had no significant (P=0.223) change. Serum total cholesterol concentration significantly (P=0.031) increased in male and female rats received the extract at dose of 0.536 g/kg and greater. Serum creatinine concentration significantly (P=0.011) increased in both male and female rats treated with the extract at dose of 1.072 g/kg and greater, as compared with the control.

The results of the effects of the extract on the serum total proteins and serum transaminases (ALT, AST) are summarized in table 11. For male rats, significant increases (P=0.0458) in serum total protein concentrations were observed in the group treated with the extract at dose of 0.536 g/kg and greater, as compared with the control. This increase was also observed in female rats that received extract at dose of 1.072 g/kg, as compared with the control. The serum activities of ALT and AST showed significant (P=0.02) increases in male rats treated with the extract at dose of 2.144 g/kg and 4.288 g/kg, as compared with the control. Also, the serum activities of ALT

Table 9: Effects of doses of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract on tissue total proteins after 4 weeks of administration to rats

Sex of animal	Dose (g/kg)	Tissue total proteins (mg/g)				
		Heart	Liver	Lungs	Spleen	Kidneys
Males	0	94.892 ± 2.246^{a}	241.042 ± 2.246^{d}	213.810 ± .062^{c}	193.388 ± 2.246^{d}	217.412 ± 1.202^{a}
	0.536	96.312 ± 3.983^{a}	239.006 ± 2.237^{d}	215.012 ± 2.246^{c}	191.584 ± 1.472^{d}	219.41 ± 2.246^{a,b}
	1.072	115.312 ± 2.246^{b}	217.414 ± 3.500^{c}	222.230 ± 4.250^{c}	178.974 ± 3.502^{c}	227.022 ± 3.502^{b}
	2.144	133.328 ± 1.884^{c}	209.006 ± 2.247^{b}	200.598 ± 4.074^{b}	165.780 ± 3.076^{b}	230.628 ± 3.603^{c}
	4.288	131.806 ± 2.423^{c}	190.986 ± 3.500^{a}	184.982 ± 3.502^{a}	154.952 ± 2.246^{a}	248.644 ± 1.473^{d}
Females	0	82.878 ± 2.248^{a}	261.258 ± 3.003^{c}	203.000 ± 3.502^{b}	195.478 ± 2.857^{a}	203.598 ± 2.403^{a}
	0.536	104.980 ± 2.620^{b}	259.846 ± 3.061^{c}	204.200 ± 2.687^{b}	192.786 ± 2.402^{a}	205.612 ± 1.991^{a}
	1.072	113.390 ± 2.032^{c}	241.438 ± 3.502^{b}	218.014 ± 2.617^{c}	206.892 ± 2.043^{b}	224.618 ± 1.750^{b}
	2.144	116.994 ± 2.754^{c,d}	220.416 ± 3.233^{a}	190.988 ± 2.246^{a}	204.176 ± 2.471^{b}	225.220 ± 2.512^{b,c}
	4.288	121.318 ± 2.246^{d}	212.610 ± 3.062^{a}	186.176 ± 3.295^{a}	224.018 ± 2.246^{c}	230.620 ± 1.751^{c}

Tabulated values are mean ± Standard error of mean of five trials. a, b, c, d: values on the same column with different letters are significantly different at P < 0.05.

Table 10: Effects of doses of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract on hematocrit values, serum total cholesterol and serum creatinine after 4 weeks of administration to rats

Sex of animal	Dose (g/kg)	Hematocrit (%)	Serum total cholesterol (mg/dl)	Serum creatinine (mg/dl)
Males	0	43.40 ± 0.600^{c}	31.600 ± 0.748^{a}	0.518 ± 0.009^{a}
	0.536	42.600 ± 0.600^{c,b}	39.600 ± 0.748^{b}	0.522 ± 0.008^{a}
	1.072	43.000 ± 0.632^{c,b}	40.800 ± 1.019^{b}	0.580 ± 0.020^{b}
	2.144	40.100 ± 0.321^{a}	58.200 ± 0.080^{c}	0.850 ± 0.060^{c}
	4.288	41.800 ± 0.374^{b}	69.600 ± 0.748^{d}	1.400 ± 0.060^{d}
Females	0	47.100 ± 0.748^{a,b}	37.600 ± 1.469^{a}	0.544 ± 0.010^{a}
	0.536	45.400 ± 1.426^{a,b}	43.200 ± 1.019^{b}	0.582 ± 0.007^{a}
	1.072	47.600 ± 0.660^{b}	52.000 ± 0.894^{c}	0.850 ± 0.060^{b}
	2.144	47.200 ± 0.374^{b}	68.800 ± 0.489^{d}	1.100 ± 0.061^{c}
	4.288	46.800 ± 1.328^{b}	90.400 ± 1.469^{e}	1.570 ± 0.056^{d}

Tabulated values are mean ± Standard error of mean of five trials. a, b, c, d, e: values on the same column with different letters are significantly different at P < 0.05.

Table 11: Effects of doses of *C. purpurescens* CH_2Cl_2/MeOH (1:1) leaf extract on serum total proteins and serum transaminases after 4 weeks of administration to rats

Sex of animal	Dose (g/kg)	Serum total proteins (mg/ml)	Serum ALT (IU/L)	Serum AST (UI/L)
Males	0	46.748 ± 1.324^{a}	14.552 ± 1.137^{a}	11.106 ± 1.130^{a}
	0.536	51.884 ± 1.324^{b}	15.106 ± 0.961^{a}	11.986 ± 1.425^{a}
	1.072	66.478 ± 1.832^{c}	16.770 ± 0.609^{a}	14.083 ± 0.779^{b}
	2.144	75.186 ± 0.908^{d}	18.332 ± 0.746^{b}	20.332 ± 1.820^{c}
	4.288	105.384 ± 1.209^{e}	21.776 ± 0.607^{c}	25.630 ± 1.823^{d}
Females	0	44.320 ± 0.661^{a}	14.128 ± 0.427^{a}	12.510 ± 1.067^{a}
	0.536	47.560 ± 0.915^{a}	16.108 ± 0.677^{b}	13.553 ± 0.673^{a}
	1.072	64.314 ± 0.917^{b}	17.326 ± 0.607^{c}	15.917 ± 1.070^{b}
	2.144	66.480 ± 0.661^{c}	21.332 ± 0.928^{d}	24.594 ± 1.762^{c}
	4.288	71.340 ± 0.661^{d}	23.106 ± 0.607^{e}	28.565 ± 1.539^{d}

Tabulated values are mean ± Standard error of mean of five trials. a, b, c, d, e: values on the same column with different letters are significantly different at P < 0.05.

and AST showed significant (P=0.02) increases in female rats treated with the extract at dose of 0.536 g/kg and greater, as compared with the control values.

Discussion

obtained inhibition diameters and inhibition parameters (MIC, MBC) showed that the leaves of *Crinum purpurascens* contain antisalmonellal substances. Hexane fraction did not show any antibacterial activity against the bacteria strains, whereas CH_2Cl_2 and EtOAc fractions, aqueous, MeOH, and CH_2Cl_2/MeOH (1:1) extracts were active against the bacteria strains. Moreover, the CH_2Cl_2/MeOH (1:1) extract showed the highest antibacterial activity against the three bacteria strains used. These data suggest that the leaves of *C. purpurascens* may contain several antibacterial active principles with different polarities, acting in a synergistic way. Ciprofloxacin was about 1000-fold more active than the CH_2Cl_2/MeOH (1:1) extract. This may result from the fact that ciprofloxacin is a pure compound, unlike the extract, which is a mixture of compounds that tend to dilute the activity of the active principle(s). A bioassay-guided fractionation of this extract will give an insight into the activity of the active principle(s).

Antimicrobial substances are considered as bacteriostatic agents when the ratio MBC/MIC > 4, and bactericidal agents when the ratio MBC/MIC ≤ 4.[4,9-11,21] In the present study, MeOH extract and CH_2Cl_2 fraction, in general showed the ratio MBC/MIC > 4, suggesting that they may be classified as bacteriostatic agents. Whereas CH_2Cl_2/MeOH (1:1) extract rather showed the ratio MBC/MIC ≤ 4, suggesting that it may be classified as bactericidal agent.

Phytochemical analysis revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, anthocyanins and steroids. Some members of these classes of compounds have been found to exhibit antimicrobial activity.[22] Alkaloids were abundant in CH_2Cl_2/MeOH (1:1) and MeOH extracts, EtOAc and CH_2Cl_2 fractions; less abundant in aqueous extract and in traces in hexane fraction. Moreover, steroids were completely absent in hexane fraction. The lack of steroids, coupled with the extremely low concentration of alkaloids (traces) in hexane fraction may be the reason why this fraction did not present any antibacterial activity against the three bacteria strains used. The negative antimicrobial action of hexane fraction could be partly attributable to low penetration of this fraction to the medium. Some alkaloids isolated from the *Crinum* genus have been found to be strong antibacterial agents.[23] The results of the phytochemical screening suggest that the antisalmonellal agents contained in the leaves of *C. purpurascens* may belong to the classes of steroids and/or alkaloids. These classes of compounds may have a synergistic action against the bacteria. The results of the phytochemical screening gave evidence of the presence of compounds of microbiological interest in the leaves of *Crinum purpurascens*. Further tests using standard sample(s) including well defined herbal medicine(s) would enable the confirmation of these preliminary phytochemical findings.

Acute toxicity profiling, in general, did not reveal any negative behavioral changes at doses less than or equal to 4 g/kg, as compared with the control. However, the reduced locomotion, reactivity and reaction to noise observed in mice treated with high doses of extract suggest that the CH_2Cl_2/MeOH (1:1) extract of *C. purpurascens* leaves may have a depressant or sedative effect on the central nervous system at high doses.[16] The effect of the extract on the perception of pain (i.e. reduced reaction to pinch) may be due to its action on the nociceptors, inhibition of the production of algogenic substances (e.g. prostaglandins, histamines) or inhibition of pain transmission at the central level.[24] As mentioned above, on preliminary phytochemical screening, the extract of *C. purpurascens* was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception.[25,26] Hence, the presence of flavonoids may contribute to the action of this extract on pain perception. The LD_{50} values, calculated for both male and female mice, were greater than 5 g/kg and the extract can be considered, in accordance with the Hodge and Sterner scale, as almost non-toxic.[27] It was also observed during the present study that *C. purpurascens* extract caused significant losses in body weight of mice receiving the various doses of the extract at the first week of experimentation. This reduction in weight may result from less food and water intake. The extract may inhibit the centre of hunger or stimulate the centre of fullness.[28] At the second week, a gain of body weight was observed and this increase may result from the progressive disappearance (or excretion) of the extract from the body.

In the subchronic toxicity study, the results of the effects of the extract on the organ to body weight ratios suggest that the extract may induce hypertrophy of the liver, spleen, and kidneys on the one hand, and hypotrophy of the heart and lungs on the other hand.

Hematocrit values obtained in both male

and female rats were within the normal range for rodents (30 – 55%),[19] suggesting that the extract had no significant effect on the red blood cells.

In the present study, significant increases in serum total cholesterol were observed, suggesting that users of the extract may be exposed to the risk of cardiovascular diseases.[29] However, this can only be confirmed by further studies on the HDL and LDL cholesterol. The increase in serum creatinine observed in both male and female rats indicates that the extract, at high dose, may cause injury to the kidneys. In fact, creatinine is a substance produced in the muscle from creatinine and in the normal conditions is excreted in the urine. Therefore, when the kidneys are damaged, the accumulation of creatinine in the serum is notable.[30] Increase of creatinine in the serum may also result from its increased production at the level of the muscle.

Rats that received the extract at high doses exhibited increases in serum total proteins concentration on the one hand, and decreases in total protein concentration of the liver on the other hand, suggesting liver injury. In fact, it has been reported that enhancement in the level of serum proteins is an indication of tissue injury and significant decrease in protein contents of the liver is a reflection of hepatic toxicity.[30] These results confirm the cytotoxic effects of *Crinum* species as reported by some authors.[23]

Transaminases (ALT and AST) are concerned with amino acids metabolism. Large amount of AST are present in the liver, kidneys, cardiac and skeletal muscles, while ALT is found principally in the liver.[31] Small amount of AST are present in the brain, pancreas, and lungs. The serum or plasma levels of both AST and ALT rise whenever there is liver cell damage. The higher activities of both enzymes reflect the greater degree of liver damage.[31] Increased serum activities of these enzymes in the present study indicates that the extract may have significant cytotoxic effect on the liver. The extract could affect the permeability of the cell membrane causing the membrane to become leaky. This would then induce the release of these enzymes from the cells into the blood stream, thereby causing the subsequent plasma or serum elevation of the enzymes.[16,30]

In conclusion, the data obtained in the present study suggest that the leaves of *Crinum purpurascens* Herb. contain antisalmonellal principle(s) and the methylene chloride/methanol (1:1) leaf extract, the most active among the extracts/fractions tested, may exert depressant or sedative effect on the central nervous system and analgesic activity at high doses. Moreover, the extract may be anorexiant, hepatotoxic and nephrotoxic.

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