Journal of Pharmacy and Pharmacology 2 (2014) 393-403

Evaluation of the Anticonvulsant Activity of the Leaf Methanol Extract of *Crassula arborescens* (Mill.) Willd. (Crassulaceae) in Mice

George Jimboyeka Amabeoku, Oluchi Nneka Mbamalu, Tasneem Davids, Samukelisiwe Fakude, Anda Gqwaka, Fiona Mbai, Reighman Pieterse and Aneesa Shaik

Discipline of Pharmacology, University of the Western Cape, Cape Town 7535, Western Cape, South Africa

Abstract: *Crassula arborescens* (Mill.) Willd. subsp. *Arborescens* is widely used for the treatment of various ailments including diarrhoea, corns, epilepsy and as a purgative. However, no information exists in any literature to verify the acclaimed effectiveness of *C. arborescens* in the treatment of the various ailments. The study, therefore, intended to investigate the anticonvulsant activity of the leaf methanol extract of *C. arborescens* in mice. Acute toxicity study and phytochemical qualitative analysis of the plant extracts were also carried out. Chemically-induced convulsion methods were used to assess the anticonvulsant activity of *C. arborescens*. Standard methods were used for the acute toxicity study and phytochemical analysis of the chemical components of the plant extract. PTZ (pentylenetetrazole), bicuculline, picrotoxin, NMDLA (N-methyl-DL-aspartic acid) or strychnine produced tonic convulsions in all the mice used. Leaf methanol extract of *Crassula arborescens*, muscimol, phenobarbitone or diazepam significantly antagonised PTZ, bicuculline or picrotoxin-induced convulsion. *C. arborescens* or LY233053 significantly antagonised NMDLA-induced tonic convulsion. *C. arborescens* or phenobarbitone significantly antagonised strychnine-elicited tonic convulsion. Phenytoin or DMSO (dimethylsulfoxide) did not significantly affect the tonic convulsion produced by PTZ, bicuculline, picrotoxin, NMDLA or strychnine. The LD_{50} value obtained from intraperitoneal administration of *C. arborescens* was 781.6 mg/kg while that following oral administration of the plant extract was over 4,000 mg/kg. The phytochemical qualitative analysis done showed the presence of flavonoids, tannins, reducing sugar, saponins and triterpene steroids. The data obtained in the study show that the leaf methanol extract of *Crassula arborescens* has anticonvulsant activity which may be underpinned by GABAergic, glutaminergic and glycinergic mechanisms. The high LD_{50} value obtained following the oral administration of the plant extract shows that the leaf methanol extract is non-toxic to animals.

Key words: *Crassula arborescens*, Crassulaceae, anticonvulsant activity, GABAergic, glutamatergic and glycinergic mechanisms, mice.

1. Introduction

The use of medicinal plants in South Africa in the treatment and/or management of diseases is an age long tradition. *Crassula arborescens* (Mill.) Willd. subsp. *Arborescens* (Crassulaceae) is one of the medicinal plants used in the country to treat certain ailments. The plant species belongs to the family of Crassulaceae. It is a succulent plant with a soft bark, which is smooth and grey-green in colour, and soft wood. The plant grows up to a height of between 1.2 m to 4 m. The leaves are round to broadly ovate and are approximately 3 × 3 cm in size. They are thick and fleshy with a grey waxy bloom. The apex is round, obscure and sharp, and the margins are reddish rimmed. The flowers are whitish-pink in colour [1]. *Crassula arborescens* is indigenous to South Africa and Western Cape Province in particular. It is locally known in Afrikaans as “beestebal”. It is distributed in the Western Cape Province from the Little Karroo to the Hex River Valley. It is also found in KwaZulu Natal and Swaziland where it is known as

Corresponding author: George Jimboyeka Amabeoku, Ph.D., professor, research fields: pharmacology and ethnopharmacology. E-mail: gamabeoku@uwc.ac.za.

"umchobozovithi" [1]. Infusion of the leaves has been used in the cape to treat epilepsy and corns [1, 2]. Despite the claim of therapeutic success of the plant species by traditional medicine practitioners in the treatment and management of epilepsy, no scientific evidence has been found in any literature to verify the claim. Aim of the present study was to evaluate the anticonvulsant activity of *Crassula arborescens in vivo*, and analyse the chemical composition by colorimetric assays and HPLC analysis. Acute toxicity study of the plant species was also carried out.

2. Materials and Methods

2.1 Plant Material

Fresh leaves of *Crassula arborescens* were collected from Kirstenbosch National Botanical Gardens, Cape Town, South Africa. They were authenticated by Mr F. Weitz, a taxonomist in the Department of Biodiversity and Conservation Biology, UWC (University of the Western Cape). A voucher specimen (CR 22) was then deposited in the Herbarium at the University of the Western Cape.

2.2 Preparation of the Methanol Extract and Stock Solutions

Fresh leaves were separated from the branches, weighed (1 kg), and washed with distilled water. After, the leaves were air dried for an hour and dried in an oven at 35 °C for 2 days. Leaves dried were ground using a commercial blender affording a fine powder (442.5 g), and was extracted (60 g) in a Soxhlet extractor with 500 mL of methanol for 5 h. The filtrate was evaporated to dryness using a Buchi RE11 rotavapor and Buchi 461 water bath. A yield of 18.2 g crude methanol extract of *C. arborescens* was obtained. This was preserved in a refrigerator for further use. Fresh solution of the methanol extract was prepared on each day of the experiment by dissolving a weighed quantity of the methanol extract in a small volume of DMSO (dimethylsulfoxide) and made up to the appropriate volume with physiological saline. The solutions were administered intraperitoneally (i.p.) to mice in a volume of 1 mL/100 g of body weight of animals.

2.3 Animals

Male albino mice bred in the Animal House of the Discipline of Pharmacology, School of Pharmacy, University of the Western Cape, South Africa were used throughout the experiments. Animals weighing between 18-30 g were used in groups of eight per dose of plant extract or drug. They had access to food and water ad libitum. All animals were fasted for 16 h during which they had access to water before the experiments commenced. A 12 h light/12 h dark cycle was maintained during the experiment. An ambient room temperature of 22 ± 1 °C was also maintained. All animals were used for one experiment only.

2.4 Drugs and Chemicals

PTZ (pentylenetetrazole, Sigma Chemical Co.), picrotoxin (Sigma Chemical Co.), NMDLA (N-methyl-DL-aspartic acid, Sigma Chemical Co.), strychnine (Sigma Chemical Co.), phenobarbitone sodium (BDH Chemicals Ltd), 5,5 diphenylhydantoin sodium salt (phenytoin, Sigma Chemical Co.), muscimol (Sigma Chemical Co.) and LY233053 (Sigma Chemical Co.) were all dissolved in physiological saline to appropriate volumes. (+) Bicuculline (Sigma Chemical Co.) was suspended in a small volume of Tween 80 and adjusted to the appropriate volume with physiological saline. Diazepam (Valium, Roche, South Africa) was dissolved in a minimum amount of propylene glycol and made up to the appropriate volume with physiological saline. DMSO (dimethylsulfoxide, Sigma Chemical Co.) solution was prepared by dissolving equal volume, used to dilute the plant extract, in an appropriate volume of physiological saline. All drugs were injected intraperitoneally (i.p.) in a volume of 1 mL/100 g of animal. Control animals received equal volume injections of the appropriate

vehicles which include physiological saline and DMSO. Fresh plant extract and drug solutions were prepared on each day of the experiment. The doses and pre-treatment times of the leaf methanol extract of *C. arborescens* and the standard antiepileptic drugs used were obtained from preliminary studies in our laboratory. The pre-treatment times following the administration of PTZ, bicuculline, picrotoxin, NMDLA or strychnine were 15 min (plant extract), 10 min (phenobarbitone), 20 min (diazepam), 20 min (phenytoin), 30 min (LY233053), 1 h (muscimol) and 15 min (DMSO solution).

2.5 Phytochemical Qualitative Analysis

The methods of Ikhiri et al. [3] and Harborne [4] were used for the phytochemical qualitative analysis of the dried powdered leaf of *C. arborescens* for various chemical compounds (Table 1).

2.6 Assessment of Anticonvulsant Activity

Modified method of Vellucci and Webster [5] by Amabeoku and Chikuni [6] was used to assess the anticonvulsant effect of the leaf methanol extract of *Crassula arborescens*. Each mouse was housed in a transparent perspex mouse cage 30 min before the commencement of the experiment to acclimatize to their new environment. PTZ (95 mg/kg), bicuculline (40 mg/kg), picrotoxin (15 mg/kg), NMDLA (400 mg/kg) and strychnine (2 mg/kg), all standard convulsant drugs, were each administered intraperitoneally to induce tonic administration of each convulsant drug for tonic convulsions which manifested as tonic hind-limb extensions. The animals were observed for 30 min following the administration of each convulsant drug for tonic convulsions which manifested as tonic hind-limb extensions. The time of the onset of tonic convulsions and the number of animals convulsing or not convulsing were obtained during the 30 min period of observation. Experiments were repeated with other groups of animals pre-treated with either the leaf methanol extract of the plant species (25-200 mg/kg, i.p.), phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.), phenytoin (30 mg/kg, i.p.), muscimol (2 mg/kg, i.p), LY233053 (5 mg/kg, i.p.) or DMSO (0.25 mL, i.p.) before the administration of any of the convulsant agents. The ability of the plant extract to prevent or delay the onset of the tonic hind limb extensions was taken as an indication of anticonvulsant activity [6]. Animals that did not convulse during the 30 min period of observation were considered as not having convulsed.

2.7 HPLC Analysis

The leaf methanol extract of *Crassula arborescens* was analysed using HPLC in order to characterise the plant species. Chromatographic system: Agilent 1200 system consisting of degassing system, quaternary pump, auto loading sampler, thermostatted column compartment, diode array detector, fluorescence detector, analyte fraction collector and Agilent ChemStation software; column: Phenomenex Luna (C18) 5 μm and dimensions (250 cm × 4.6 mm). Chromatographic conditions: mobile phase degassed with helium; solvent: water containing 0.01% formic acid; solvent B: acetonitrile containing 0.01% formic acid; mode: flow rate, 0.8 mL/min; injection volume: 50 μL, detector, UV at 370 nm. The analysis was monitored at several wavelengths ranging from 210 nm to 370 nm, the specific wavelength of interest being 350 nm. The HPLC operating conditions were programmed to give the following: 0 min, solvent B: 18%; 15 min, solvent B: 25%; 20 min, solvent B: 35%; 30 min, solvent B: 90%. The run rate was 30 min.

Table 1 Phytochemical screening of dry powder of *C. arborescens* leaf.

Compounds	Tests
Saponins	Frothing test
Tannins	Ferric chloride test
Reducing sugar	Fehling's test
Flavonoids	Shinoda's test
Triterpene steroids	Salkowski test
Alkaloids	Dragendorff's test
Cardiac glycosides	Kedde's test
Quinones	Hydrochloric acid/ether test

2.8 Acute Toxicity Study

The method described by Lorke [7] and modified by Ojewole [8] and that of Hilaly et al. [9] were used to determine the median lethal dose (LD_{50}) of the leaf methanol extract of *Crassula arborescens*. Mice were fasted for 16 h and then randomly divided into groups of eight mice per cage. Graded doses of the plant extract (100, 200, 400, 800, 1,200, 1,600, 2,000, 2,400, 2,800, 3,200, 3,600 and 4,000 mg/kg) were separately administered orally by means of a bulbed steel needle to mice in each test group. The control group received 0.25 mL (p.o.) of physiological saline by means of a bulbed steel needle. The acute toxicity experiment was repeated by administering the graded doses of the leaf methanol extract of the plant species or control vehicle to other groups of animals intraperitoneally. The mice in both the test and control groups were then allowed free access to food and water, and observed for over 5 days for signs of acute toxicity including death. Log dose-response curves were then constructed for the plant extract from which the median lethal dose was calculated where applicable.

2.9 Statistical Analysis

The data on the onset of tonic convulsions were analysed using one-way ANOVA (analysis of variance) followed by Dunnett's multiple comparison test (GraphPad Prism, version 5.0, Graph Pad software, Inc., San Diego Cap 2130, USA). The number of animals convulsing was analysed using the Chi-squared test [10]. Data obtained were expressed as mean (± S.E.M). *P*-values less than 5% ($P < 0.05$) were considered statistically significant.

2.10 Ethical Consideration

The experimental protocol used in this study was approved (07/04/31) by the University of the Western Cape Ethics Committee, Bellville 7535, South Africa and conforms to the University's Regulations Act concerning animal experiments.

3. Results

3.1 Phytochemical Analysis

The phytochemical qualitative analysis of the dried powdered leaf of *Crassula arborescens* revealed the presence of the following chemical constituents: triterpene steroids, saponins, tannins, reducing sugar and flavonoids.

3.2 Anticonvulsant Activity Assessment

3.2.1 Effect of Leaf Methanol Extract of *Crassula arborescens* on PTZ (Pentylenetetrazole)-Induced Tonic Convulsion

PTZ (pentylenetetrazole, 95 mg/kg, i.p.) induced tonic convulsion in the eight mice used. Leaf methanol extract of *Crassula arborescens* (25 mg/kg, i.p.) did not significantly affect the onset or incidence of the tonic convulsion elicited by PTZ (95 mg/kg, i.p.). The plant extract (50 mg/kg, i.p.) did not significantly affect the incidence but significantly delayed the onset of the tonic convulsion produced by PTZ (95 mg/kg, i.p.). *Crassula arborescens* (25 mg/kg and 50 mg/kg, i.p.) protected 12.5% and 37.5% of the mice against PTZ (95 mg/kg, i.p.)-induced tonic convulsion, respectively. Leaf methanol extract of *Crassula arborescens* (100-200 mg/kg, i.p.) significantly delayed the onset and also significantly decreased the incidence of PTZ (95 mg/kg, i.p.)-elicited tonic convulsion in a dose dependent manner. The doses of the leaf methanol extract of *Crassula arborescens* (100 mg/kg and 200 mg/kg, i.p.) protected 62.5% and 75% of the animals respectively against the tonic convulsion produced by PTZ (95 mg/kg, i.p.). Phenobarbitone (12 mg/kg, i.p.) and muscimol (2 mg/kg, i.p.) significantly delayed the onset of PTZ (95 mg/kg, i.p.)-induced tonic convulsion and also significantly decreased the incidence of the tonic convulsion. Phenobarbitone (12 mg/kg, i.p.) and muscimol (2 mg/kg, i.p.) protected 87.5% of the animals against the tonic convulsion respectively. Diazepam (0.5 mg/kg, i.p.) profoundly

antagonised PTZ (95 mg/kg, i.p.)-induced tonic convulsion by protecting 100% of mice against the convulsion. DMSO (0.25 mL, i.p.) or phenytoin (30 mg/kg, i.p.) did not significantly affect the onset or incidence of PTZ (95 mg/kg, i.p.)-induced tonic convulsion (Table 2).

3.2.2 Effect of Leaf Methanol Extract of *Crassula arborescens* on Bicuculline-Induced Convulsion

All the control mice treated with 40 mg/kg (i.p.) of bicuculline exhibited tonic convulsion. Leaf methanol extract of *C. arborescens* (25 mg/kg, i.p.) did not affect the onset or incidence of the tonic convulsion produced by bicuculline (40 mg/kg, i.p.). The dose of 50 mg/kg (i.p.) of the plant extract significantly delayed the onset but did not significantly affect the incidence of the tonic convulsion. It protected 50% of mice against bicuculline (40 mg/kg, i.p.)-induced tonic convulsion. Leaf methanol extract of *C. arborescens* (100-200 mg/kg, i.p.) dose dependently and significantly delayed the onset of bicuculline (40 mg/kg, i.p.)-induced tonic convulsion and also significantly reduced the number of animals convulsing. The doses of 100 mg/kg and 200 mg/kg (i.p.) protected 62.5% and 75% of mice respectively against the tonic convulsion. Phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.) or muscimol (2 mg/kg, i.p.) significantly delayed the onset and the incidence of bicuculline (40 mg/kg, i.p.)-induced tonic convulsion by protecting 87.5% of the animals each, against the convulsion. Phenytoin (30 mg/kg, i.p.) or DMSO (0.25 mL, i.p.) did not affect the tonic convulsion produced by bicuculline (40 mg/kg, i.p.) (Table 3).

3.2.3 Effect of Leaf Methanol Extract of *Crassula arborescens* on Picrotoxin-Induced Convulsion

Picrotoxin (15 mg/kg, i.p.) produced tonic seizures in all the mice used. *C. arborescens* (50-200 mg/kg, i.p.) significantly antagonised picrotoxin (15 mg/kg, i.p.)-induced tonic convulsion by delaying the onset of the convulsion and reducing the number of animals convulsing significantly. These doses of *C. arborescens* protected between 62.5% and 87.5% of mice against the convulsion induced by picrotoxin. *C. arborescens* (25 mg/kg, i.p.) did not affect the onset or incidence of pictotoxin produced tonic convulsion. Phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.) and muscimol (2 mg/kg, i.p.) all significantly delayed the onset and significantly decreased the incidence of the tonic convulsion elicited by picrotoxin (15 mg/kg, i.p.). Phenobarbitone or diazepam protected 100% of the animals while muscimol protected 87.5% of the animals against picrotoxin-induced tonic convulsion. DMSO (0.25 mL, i.p.) or phenytoin (30 mg/kg, i.p.) neither affected the onset nor the incidence of the tonic convulsion produced by picrotoxin (Table 4).

Table 2 Effect of leaf methanol extract of CA (*Crassula arborescens*) on PTZ (pentylenetetrazole)-induced seizures in mice.

PTZ (mg/kg)	CA (mg/kg)	PB (mg/kg)	DZ (mg/kg)	PN (mg/kg)	MS (mg/kg)	DMSO (mL)	No. con/ No. used	Percentage protection (%)	Onset of tonic convulsion (min) Mean ± SEM
95	-	-	-	-	-	-	8/8		4.30 ± 0.62
95	25	-	-	-	-	-	7/8	12.5	4.55 ± 0.85
95	50	-	-	-	-	-	5/8	37.5	18.24^{*} ± 3.63
95	100	-	-	-	-	-	$3/8^{+}$	62.5	21.42^{*} ± 5.10
95	200	-	-	-	-	-	$2/8^{++}$	75	28.15^{*} ± 5.32
95	-	12	-	-	-	-	$1/8^{+++}$	87.5	23.76^{*} ± 1.58
95	-	-	0.5	-	-	-	$0/8^{++++}$	100	0^{*}
95	-	-	-	30	-	-	8/8	0	4.31 ± 0.44
95	-	-	-	-	2	-	$1/8^{+++}$	87.5	28.56^{*} ± 3.16
95	-	-	-	-	-	0.25	8/8	0	4.49 ± 0.30

$^{*}P < 0.001$ compared to PTZ (95 mg/kg, i.p.) control, ANOVA ($n = 8$). $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.005$, $^{++++}P < 0.001$ compared to PTZ (95 mg/kg. i.p.) control, Chi-squared test ($n = 8$). PB: phenobarbitone; DZ: diazepam; PN: phenytoin; MS: muscimol; DMSO: dimethylsulfoxide; No. con/No. used: number convulsed/number used.

Table 3 Effect of leaf methanol extract of CA (*Crassula arborescens*) on BIC (bicuculline)-induced seizures in mice.

BIC (mg/kg)	CA (mg/kg)	PB (mg/kg)	DZ (mg/kg)	PN (mg/kg)	MS (mg/kg)	DMSO (mL)	No. con/ No. used	Percentage protection (%)	Onset of tonic convulsion (min) Mean ± SEM
40	-	-	-	-	-	-	8/8		3.25 ± 0.74
40	25	-	-	-	-	-	8/8	0	5.93 ± 0.63
40	50	-	-	-	-	-	4/8	50	18.25^{*} ± 2.87
40	100	-	-	-	-	-	$3/8^{+}$	62.5	27.46^{**} ± 1.48
40	200	-	-	-	-	-	$2/8^{++}$	75	27.72^{**} ± 1.31
40	-	12	-	-	-	-	$1/8^{+++}$	87.5	26.23^{**} ± 1.06
40	-	-	0.5	-	-	-	$1/8^{+++}$	87.5	27.15^{**} ± 1.11
40	-	-	-	30	-	-	8/8	0	3.31 ± 0.49
40	-	-	-	-	2	-	$1/8^{+++}$	87.5	28.28^{**} ± 1.33
40	-	-	-	-	-	0.25	8/8	0	3.29 ± 0.54

$^{*}P < 0.005$, $^{**}P < 0.001$ compared to bicuculline (40 mg/kg, i.p.) control, ANOVA ($n = 8$). $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.005$ compared to bicuculline (40 mg/kg, i.p.) control, Chi-squared test ($n = 8$). PB: phenobarbitone; DZ: diazepam; PN: phenytoin; MS: muscimol; DMSO: dimethylsulfoxide; No. con/No. used: number convulsed/number used.

Table 4 Effect of leaf methanol extract of CA (*Crassula arborescens*) on PIC (picrotoxin)-induced seizures in mice.

PIC (mg/kg)	CA (mg/kg)	PB (mg/kg)	DZ (mg/kg)	PN (mg/kg)	MS (mg/kg)	DMSO (mL)	No. con/ No. used	Percentage protection (%)	Onset of tonic convulsion (min) Mean ± SEM
15	-	-	-	-	-	-	8/8		10.75 ± 0.75
15	25	-	-	-	-	-	8/8	0	10.88 ± 0.44
15	50	-	-	-	-	-	$3/8^{+}$	62.5	23.50^{*} ± 3.17
15	100	-	-	-	-	-	$2/8^{++}$	75	26.88^{**} ± 2.29
15	200	-	-	-	-	-	$1/8^{+++}$	87.5	27.48^{**} ± 1.98
15	-	12	-	-	-	-	$0/8^{++++}$	100	0^{**}
15	-	-	0.5	-	-	-	$0/8^{++++}$	100	0^{**}
15	-	-	-	30	-	-	8/8	0	11.00 ± 0.65
15	-	-	-	-	2	-	$1/8^{+++}$	87.5	29.13^{**} ± 0.88
15	-	-	-	-	-	0.25	8/8	0	11.13 ± 0.88

$^{*}P < 0.005$, $^{**}P < 0.001$ compared to picrotoxin (15 mg/kg, i.p.) control, ANOVA ($n = 8$). $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.005$, $^{++++}P < 0.001$ compared to picrotoxin (15 mg/kg, i.p.) control, Chi-squared test ($n = 8$). PB: phenobarbitone; DZ: diazepam; PN: phenytoin; MS: muscimol; No. con/No. used: number convulsed/number used; DMSO: dimethylsulfoxide.

3.2.4 Effect of Leaf Methanol Extract of *Crassula arborescens* on N-methyl-DL Aspartic Acid-Induced Tonic Convulsion

The dose of 400 mg/kg (i.p.) of N-methyl-DL aspartic acid produced tonic convulsion in the eight mice used for the assessment. LY233053 (5 mg/kg, i.p.) significantly delayed the onset of N-methyl-DL aspartic acid (400 mg/kg, i.p.)-induced tonic convulsion and significantly reduced the number of animals convulsing. *Crassula arborescens* (100-200 mg/kg, i.p.) significantly delayed the onset of N-methyl-DL aspartic acid (400 mg/kg, i.p.)-produced tonic convulsion but did not significantly affect the incidence of the convulsion. The dose of 200 mg/kg (i.p.) of *C. arborescens* protected only 12.5% of the animals. *C. arborescens* (25-50 mg/kg, i.p.), phenobarbitone (12 mg/kg, i.p.), diazepam (0.5 mg/kg, i.p.), phenytoin (30 mg/kg, i.p.) or DMSO (0.25 mL, i.p.) did not affect the onset of the tonic convulsion induced by N-methyl-DL aspartic acid (400 mg/kg, i.p.) or the number of mice convulsing (Table 5).

3.2.5 Effect of Leaf Methanol Extract of *Crassula arborescens* on Strychnine-Induced Tonic Convulsion

All the mice given strychnine (2 mg/kg, i.p.) exhibited tonic convulsion. Leaf methanol extract of *C.*

Table 5 Effect of leaf methanol extract of CA (*Crassula arborescens*) on NMDLA (N-methyl-DL aspartic acid)-induced seizures in mice.

NMDLA (mg/kg)	CA (mg/kg)	PB (mg/kg)	DZ (mg/kg)	PN (mg/kg)	LY (mg/kg)	DMSO (mL)	No. con/ No. used	Percentage protection (%)	Onset of tonic convulsion (min) Mean ± SEM
400	-	-	-	-	-	-	8/8		2.18 ± 0.52
400	25	-	-	-	-	-	8/8	0	2.39 ± 0.40
400	50	-	-	-	-	-	8/8	0	2.44 ±0.28
400	100	-	-	-	-	-	8/8	0	5.81^{*} ± 0.15
400	200	-	-	-	-	-	7/8	12.5	6.32^{*} ± 0.11
400	-	12	-	-	-	-	8/8	0	2.20 ± 0.41
400	-	-	0.5	-	-	-	8/8	0	2.30 ± 0.73
400	-	-	-	30	-	-	8/8	0	2.19 ± 0.44
400	-	-	-	-	5	-	$1/8^{+}$	87.5	24.91^{**} ± 1.63
400	-	-	-	-	-	0.25	8/8	0	2.15 ± 0.52

$^{*}P < 0.05$, $^{**}P < 0.001$ compared to NMDLA (400 mg/kg, i.p.) control, ANOVA ($n = 8$). $^{+}P < 0.005$ compared to NMDLA (400 mg/kg, i.p.) control, Chi-squared test ($n = 8$). PB: phenobarbitone; DZ: diazepam; PN: phenytoin; LY: LY233053; No. con/No. used: number convulsed/number used; DMSO: dimethylsulfoxide.

Table 6 Effect of leaf methanol extract of CA (*Crassula arborescens*) on SCN (strychnine)-induced seizures in mice.

SCN (mg/kg)	CA (mg/kg)	PB (mg/kg)	DZ (mg/kg)	PN (mg/kg)	DMSO (mL)	No. con/ No. used	Percentage protection (%)	Onset of tonic convulsion (min) Mean ± SEM
2	-	-	-	-	-	8/8		3.18 ± 0.22
2	25	-	-	-	-	8/8	0	3.33 ± 0.31
2	50	-	-	-	-	7/8	12.5	8.78^{*} ± 0.17
2	100	-	-	-	-	$3/8^{+}$	62.5	12.06^{*} ± 0.27
2	200	-	-	-	-	$3/8^{+}$	62.5	16.59^{*} ± 0.18
2	-	12	-	-	-	$2/8^{++}$	75	21.43^{*} ± 0.28
2	-	-	0.5	-	-	8/8	0	3.81 ± 0.60
2	-	-	-	30	-	8/8	0	3.57 ± 0.94
2	-	-	-	-	0.25	8/8	0	3.25 ± 0.75

$^{*}P < 0.001$ compared to strychnine (2 mg/kg, i.p.) control, ANOVA ($n = 8$). $^{+}p < 0.05$, $^{++}P < 0.01$ compared to strychnine (2 mg/kg, i.p.) control, Chi-squared test ($n = 8$). PB: phenobarbitone; DZ: diazepam; PN: phenytoin; No con/No. used: number convulsed/number used; DMSO: dimethylsulfoxide.

arborescens (100-200 mg/kg, i.p.) significantly antagonised strychnine (2 mg/kg, i.p.)-induced tonic convulsion by significantly delaying the onset of the convulsion and significantly reducing the number of animals convulsing. Both doses of 100 mg/kg (i.p.) and 200 mg/kg (i.p.) of *C. arborescens* protected 62.5% of mice against strychnine (2 mg/kg, i.p.)-induced tonic convulsion. *C. arborescens* (25 mg/kg, i.p.) did not significantly affect the tonic convulsion elicited by strychnine (2 mg/kg, i.p.). However, the dose of 50 mg/kg (i.p.) significantly delayed the onset of the tonic convulsion but did not affect the number of animals convulsing. *C. arborescens* (50 mg/kg, i.p.) protected only 12.5% of mice against strychnine (2 mg/kg, i.p.)-induced tonic convulsion. Phenobarbitone (12 mg/kg, i.p.) significantly antagonised the tonic convulsion produced by strychnine (2 mg/kg, i.p.). Phenobarbitone (12 mg/kg, i.p.) protected 75% of mice against strychnine (2 mg/kg, i.p.)-induced tonic convulsion. Diazepam (0.5 mg/kg, i.p.), phenytoin (30 mg/kg, i.p.) or DMSO (0.25 mL, i.p.) did not affect strychnine (2 mg/kg, i.p.)-induced tonic convulsion in any significant manner (Table 6).

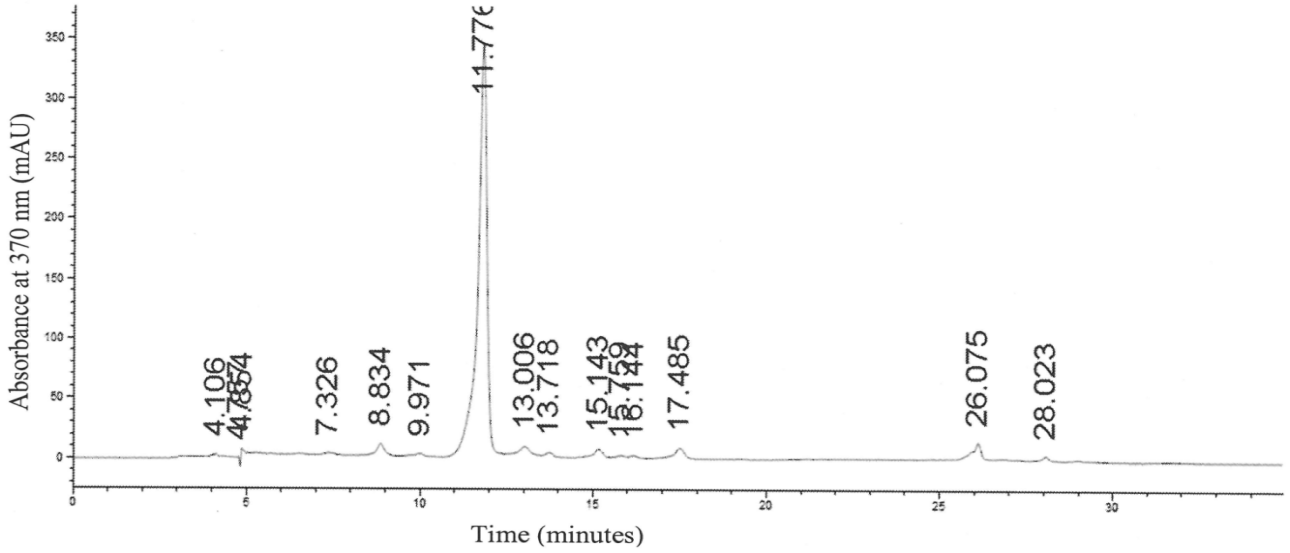

Fig. 1 HPLC fingerprint of methanol extract of *Crassula arborescens*.
Volume of injection: 50 µL; sample concentration: 22 mg/mL; column: Phenomenex Luna (C18); system of elution: A: water (0.01% formic acid), B: acetonitrile (0.01% formic acid), flow rate: 0.8 mL/min, 0 min, solvent B: 18%; 15 min, solvent B: 25%; 20 min, solvent B: 35%; 30 min, solvent B: 90%.

3.3 HPLC Analysis

The HPLC fingerprint of *C. arborescens* obtained showed characteristic peaks at the following retention times (minutes): 8.834, 11.776, 17.485 and 26.075 (Fig. 1).

3.4 Acute Toxicity Study

Leaf methanol extract of *Crassula arborescens* (100-4,000 mg/kg), given orally, did not produce any signs of acute toxicity in or deaths of the mice used. The NOAEL (no-adverse-effect-level) was observed at 4,000 mg/kg (p.o.) which was the highest dose administered during the acute toxicity testing. The LD_{50} of the plant extract when given orally, was therefore, taken to be greater than 4,000 mg/kg. However, when the same dose range (100-4,000 mg/kg) of *Crassula arborescens* was used and the plant extract administered to mice intraperitoneally, signs of acute toxicity, such as piloerection, salivation and hypoactivity were observed at the dose of 400 mg/kg. Two mice also died at this dose. Other signs such as diarrhoea and tremor were observed in higher doses in addition, before death. Five mice died at the dose of 800 mg/kg and from the doses of 1,200-4,000 mg/kg, all the animals died. The LD_{50} of the plant extract when given intraperitoneally, was found to be 781.6 mg/kg.

4. Discussion

According to Rang et al. [11], Waller et al. [12] and Burnham [13], the imbalance between GABA (gamma aminobutyric acid)-mediated inhibition and glutamate-mediated excitation in the brain underpins epilepsy. It has been postulated that the inhibition or enhancement of GABAergic neurotransmission at $GABA_A$ receptors may cause or antagonise convulsion. On the other hand, the inhibition or enhancement of glutamatergic neurotransmission at NMDLA receptors may antagonise or cause convulsion. The data obtained in this study indicate that the leaf methanol extract of *Crassula arborescens* antagonised the tonic convulsion elicited by PTZ, bicuculline, picrotoxin, NMDLA or strychnine. The study also shows that diazepam, phentobarbitone or muscimol antagonised PTZ-, bicuculline- or picrotoxin-induced tonic convulsion but did not affect NMDLA-induced tonic convulsion. Phenytoin did not affect PTZ-,

bicuculline-, picrotoxin-, NMDLA- or strychnine-induced tonic convulsion. LY233053 antagonised NMDLA-induced tonic convulsion. Strychnine-induced tonic convulsion was antagonised by phenobarbitone. Rang et al. [11], De Sarro et al. [14] and Meldrum [15] reported that PTZ may be producing convulsion by inhibiting GABA-mediated inhibition at the $GABA_A$ receptors in the brain. Phenobarbitone and diazepam, standard anticonvulsants, are thought to produce their anticonvulsant effects by acting at their respective receptors on the $GABA_A$ receptor-chloride ionophore complex to enhance GABAergic neurotransmission [11, 15, 16]. Phenobarbitone and diazepam shown in this study to antagonise PTZ-induced tonic convulsion may be doing so by enhancing GABAergic neurotransmission. Phenytoin, another standard antiepileptic drug, known to produce its anticonvulsant effect by blocking sodium ion entry into the brain cells [10], did not affect PTZ-induced tonic convulsion in this study. According to Rang et al. [11] and Lança [17], muscimol, a selective $GABA_A$ receptor agonist, stimulates $GABA_A$ receptors in the brain to produce effects similar to that of naturally occurring GABA. Muscimol was shown in this study to antagonise PTZ-induced tonic convulsion. Leaf methanol extract of *C. arborescens* antagonised PTZ-induced tonic convulsion which may probably implicate GABAergic mechanism in its anticonvulsant activity. Phenobarbitone, diazepam and muscimol, a selective $GABA_A$ receptor agonist, known to enhance or mimic GABA neurotransmission [11, 17], were shown in this study to antagonise tonic convulsion produced by either bicuculline or picrotoxin. Bicuculline, a selective $GABA_A$ receptor antagonist, is thought to produce its convulsant activity by blocking GABAergic neurotransmission at $GABA_A$ receptor in the brain [11]. According to Rang et al. [11], picrotoxin produces its convulsant activity by blocking $GABA_A$ receptor-linked chloride ion channels and preventing chloride ion entry into the brain cells thus inhibiting GABA neurotransmission. Phenytoin, known to produce convulsion by blocking sodium ion entry into the brain cells, did not alter bicuculline- or picrotoxin-induced tonic convulsion. In this study, *C. arborescens* antagonised the tonic convulsion produced by either bicuculline or picrotoxin thus supporting the earlier suggestion that GABAergic mechanism may be involved in the anticonvulsant activity of the plant extract. Rang et al. [11], Besancon et al. [18] and Chapman and Meldrum [19] reported that NMDLA, a specific agonist at the NMDA receptors, produces convulsion by stimulating NMDA receptors in the brain to mimic the effect of the naturally occurring glutamate which is an excitatory neurotransmitter. LY233053, a competitive NMDA receptor antagonist, which acts by blocking the effect of glutamate at NMDA receptors [20, 21], was shown in this study, to antagonise NMDLA-induced tonic convulsion. Phenobarbitone and diazepam, known as enhancers of GABAergic neurotransmission and Phenytoin, known as blocker of sodium ion entry into the brain cells [11], did not affect NMDLA-tonic tonic convulsion. Leaf methanol extract of *C. arborescens* significantly delayed the onset of NMDLA-tonic tonic convulsion indicating that glutamatergic mechanism may be involved in its anticonvulsant activity. According to Rang et al. [11], strychnine produces convulsion by blocking receptors for glycine in the brain. In this study, phenobarbitone and leaf methanol extract of *C. arborescens* antagonised strychnine-induced tonic convulsion. The possibility, therefore, exists that glycinergic mechanism may also be involved in the anticonvulsant activity of *C. arborescens*. It has been reported that different mechanisms may be involved in the anticonvulsant activity of phenobarbitone [11]. The enhancement of glycinergic neurotransmission may probably be one of such mechanisms involved in the anticonvulsant activity of phenobarbitone. Rang et al. [11] reported that benzodiazepines do not alter strychnine-induced convulsion in experimental

animals. It is pertinent to note that Lambert et al. [22] and Boyd et al. [23] reported the use of moderate to high doses of IV phenobarbitone, IV diazepam and IV phenytoin to prevent strychnine convulsion in strychnine poisoning in humans. Phytochemical qualitative analysis of *C. arborescens* carried out in this study revealed the presence of saponins, triterpene steroids, flavonoids, tannins and reducing sugar. It is important to mention that studies by Singh et al. [24], Ibrahim et al. [25], Van Heerden et al. [26] and Chauhan et al. [27] have shown that flavonoids, saponins and triterpene steroids have anticonvulsant activities suggesting that these compounds, also being present in the plant extract, may probably be contributing to the anticonvulsant activity of *C. arborescens*. In this study, a fairly high LD_{50} of probably over 4,000 mg/kg was obtained when leaf methanol extract of *C. arborescens* was given orally suggesting that it is safe in mice at this dose level. On the other hand, the LD_{50} obtained when the plant extract was given intraperitoneally was 781.6 mg/kg. However, only the oral route has been used by traditional medicine practitioners in South Africa to administer the plant extract in the form of infusion or decoction in the treatment of epilepsy and other ailments.

5. Conclusion

The data obtained in this study indicate that *C. arborescens* has anticonvulsant activity which may be underpinned by GABAergic, glutamatergic and glycinergic mechanisms. The HPLC fingerprint of *C. arborescens* obtained showed characteristic peaks at the following retention times (minutes): 8.834, 11.776, 17.485 and 26.075. The results obtained also indicate that the plant extract may be safe in mice when given orally.

Acknowledgments

This study was financially supported by the National Research Foundation (67983), South Africa. The authors wish to thank Mr Franz Weitz and Mr Simthembile Pambuka for their valuable technical assistance.

References

[1] Toelken, H. R. 1997. *A Revision of the Genus Crassula in Southern Africa. Annals of the Bolus Herbarium 8*. Cape Town: University of Cape Town Press, 1-595.

[2] Watt, J. M., and Breyer-Brandwijk, M. G. 1962. *The Medicinal and Poisonous Plants of Southern and Eastern Africa.* 2nd ed. London: E and S Livingstone Limited, 322.

[3] Ikhiri, K., Boureima, D., and Dan-kouloudo, D. 1992. "Chemical Screening of Medicinal Plants Used in the Traditional Pharmacopoeia of Niger." *International Journal of Pharmacognosy* 30: 251-62.

[4] Harborne, J. B. 1984. *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis.* 2nd ed. London: Chapman and Hall Limited.

[5] Vellucci, S. V., and Webster, R. A. 1984. "Antagonism of Caffeine-Induced Seizures in Mice by Ro 15-1788." *European Journal Pharmacology* 97: 289-93.

[6] Amabeoku, G. J., and Chikuni, O. 1993. "Cimetidine-Induced Seizures in Mice: Antagonism by Some GABAergic Agents." *Biochemical Pharmacology* 46: 2171-5.

[7] Lorke, D. 1983. "A New Approach to Practical Acute Toxicity Testing." *Archive of Toxicology* 54: 275-87.

[8] Ojewole, J. A. O. 2006. "Antinociceptive, Anti-inflammatory and Antidiabetic Properties of *Hypoxis hemerocallidea* Fisch and C.A. Mey. (Hypoxidaceae) corm ["African Potato"] Aqueous Extract in Mice and Rats." *Journal of Ethnopharmacology* 103: 126-34.

[9] Hilaly, J., EI Israili, Z. H., and Lyoussi, B. 2004. "Acute and Chronic Toxicological Studies of *Ajuga iva* in Experimental Animals." *Journal of Ethnopharmacology* 91: 43-50.

[10] Bienvenu, E., Amabeoku, G. J., Eagles, P. K., Scott, G., and Springfield, E. P. 2002. "Anticonvulsant Activity of Aqueous Extract of *Leonotis leonurus*." *Phytomedicine* 9: 217-23.

[11] Rang, H. P., Dale, M. M., Ritter, J. M., Flower, R. J., and Henderson, G. 2012. *Rang and Dale's Pharmacology*. 7th ed. Edinburgh: Elsevier Churchill Livingstone, 448-58.

[12] Waller, D. G., Renwick, A. G., and Hillier, K. 2005. *Medicinal Pharmacology and Therapeutics.* 2nd ed. Edinburgh: Elsevier Saunders, 305-17.

[13] Burnham, W. M. 1998. "Antiseizure Drugs." In *Principles of Medical Pharmacology,* 6th ed., edited by Kalant, H., and Roschlau, W. H. E. Toronto: University of Toronto Press, 250-61.

[14] De Sarro, A., Ceccheti, V., Fravolini, V., Naccari, F., Tabarrini, O., and De Sarro, G. 1999. "Effects of Novel 6-desfluoroquinolones and Classic Quinolones on Pentylenetetrazole-Induced Seizures in Mice." *Antimicrobial Agents and Chemotherapy* 43: 1729-36.

[15] Meldrum, B. S. 1975. "Epilepsy and Gamma Aminobutyric Acid Mediated Inhibition." *International Review of Neurobiology* 17: 1-36.

[16] Olsen, R. W. 1981. "GABA-Benzodiazepine-Barbiturate Receptor Interactions." *Journal of Neurochemistry* 37: 1-13.

[17] Lança, A. J. 1998. "Functional Organization of the Central Nervous System." In *Principles of Medical Pharmacology,* 6th ed., edited by Kalant, H., and Roschlau, W. H. E. Toronto: University of Toronto Press, 217-40.

[18] Besancon, E., Guos Lok, J., Tymianski, M., and Lo, E. H. 2008. "Beyond NMDA and AMPA Glutamate Receptors: Emerging Mechanisms for Ionic Imbalance and Death in Stroke." *Trends in Pharmacological Science* 29: 268-75.

[19] Chapman, A. G., and Meldrum, B. S. 1993. "Excitatory Amino Acid Antagonists and Epilepsy." *Biochemical Society Transactions* 21: 106-10.

[20] Borowicz, K. K., Gasior, M., Kleeinrok, Z., and Czuczwar, S. J. 1996. "Competitive NMDA-Receptor Antagonists, LY235959 and LY233053, Enhance the Protective Efficacy of Various Antiepileptic Drugs against Maximal Electroshock-Induced Seizures in Mice." *Epilepsia* 37: 618-24.

[21] Madden, K. P., Clark, W. M., Kochhar, A., and Zivin, A. 1992. "Efficacy of LY233053, a Competitive Glutamate Antagonist in Experimental Central Nervous System Ischemia." *Journal of Neurosurgery* 76: 106-10.

[22] Lambert, J. R., Byrick, R. J., and Hammeke, M. D. 1981. "Management of Acute Strychnine Poisoning." *Canadian Medical Association Journal* 124: 1268-70.

[23] Boyd, R. E., Brennan, P. T., Deng, J-F., Rochester, D. F., and Spyker, D. A. 1983. "Strychnine Poisoning: Recovery from Profound Lactic Acidosis, Hyperthermia and Rhabdomyolysis." *American Journal of Medicine* 74: 507-12.

[24] Singh, D., Singh, B., and Goel, R. K. 2012. "Role of Saponins for the Anticonvulsant Effect of Adventitious Roots of *Ficus religiosa*." *Pharmaceutical Biology* 50 (7): 816-22.

[25] Ibrahim, G., Abdulmumin, S., Musa, K. Y., and Yaro, A. H. 2008. "Anticonvulsant Activities of Crude Flavonoid Fraction of the Stem Bark of *Ficus sycomorus* (Moraceae)." *Journal of Pharmacology and Toxicology* 3: 351-56.

[26] Van Heerden, F. R., Viljoen, A. M., and Mohoto, S. P. A. 2002. "Phytochemical Investigation of *Craterocapsa tarsodes*, a Plant Used for the Treatment of Epilepsy by the Northern Sotho People of South Africa." *South African Journal of Botany* 68: 77-9.

[27] Chauhan, A. K., Dobhal, M. P., and Joshi, B. C. 1988. "A Review of Medicinal Plants Showing Anticonvulsant Activity." *Journal of Ethnopharmacology* 22: 11-23.