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Anxiolytic effects of an aqueous extract of *Crassocephalum bauchiense* Hutch (Asteraceae) in mice with possible GABAergic involvement

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Abstract

Background and Objective: *Crassocephalum bauchiense* Hutch is a species of flowering plant from the Asteraceae family. It's used in traditional medicine for the treatment of brain disorders including epilepsy, depression and anxiety. This study evaluated the anxiolytic effects of *Crassocephalum bauchiense* aqueous extract and its possible mechanism of action.

Materials and Methods: The aqueous extract of *Crassocephalum bauchiense* (25, 50, 100 and 200 mg/kg) were administered orally to mice one hour before the behavioural testing. Elevated plus maze and open field tests were used, respectively for detecting it anxiolytic properties. Immediately after the open field test, animals were sacrificed and the brain GABA content, and the activities of GAD and GABA-T were measured.

Results: *Crassocephalum bauchiense* (100 and 200 mg/kg) significantly increased the number of entries into, percentage of entries into, and percentage of time in open arms, and reduced rearing, head dipping, and the percentage of time in closed arms, in the elevated plus maze. The plant extract significantly increased crossing and reduced rearing and defecation, in the open field test. In biochemical experiments, the concentration of GABA and the activity of GAD were increased; while the GABA-T activity was decreased, respectively, in the brain of *Crassocephalum bauchiense* treated-mice.

Conclusion: These results suggest that *Crassocephalum bauchiense* aqueous extract possess anxiolytic properties in the models employed. The extract might potentially act by GABAergic activation in the central nervous system. These data justify and explain the use of *Crassocephalum bauchiense* to treat anxiety empirically in traditional medicine.

Keywords: *Crassocephalum bauchiense*; anxiolytic; Brain; GABAergic activation.

1. Introduction

Anxiety is one of the mental disorders caused by worries, uncertainties, fears, and tension. Mental disorders can modify physical, psychological or mental health. It can also affect social, family, direct environment of the patient and job responsibilities [1]. Globally an assessed 275 million people experienced an anxiety disorder in 2016, making it the most prevalent mental well-being or neurodegenerative disorder. Around 62% (170 million) were female, relative to 105 million males. In all countries women are more likely to experience anxiety disorders than men [2].

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Approximately two-thirds of the anxious or depressed patients respond to the available anxiolytic drugs but the level of amelioration is still disappointing [3]. Current drugs used for anxiety disorder have failed to alter the course of the illness but only provide symptomatic relief [4]. Then, the medical need for newer, better-tolerated and more effective treatments remains high [3]. An increasing number of herbal medicines have been introduced into neurologic and neuro-psychiatric practice, as alternative or complementary remedies, and also there is a significant number of natural medicines whose therapeutic potential has been assessed in a variety of animal models [5]. Interestingly, these experimental models have contributed to the screening of new neuropharmacological tools and to the understanding of their pharmacological activities [6].

Crassocephalum bauchiense Hutch is a species of flowering plant, belongs to the Asteraceae family. The literature review indicated that *Crassocephalum* genus consists of about 24 species [7]. This plant is found in Cameroon, Nigeria, Congo, Democratic Republic of Congo, and its natural environment is frequently moist savanna. *Crassocephalum bauchiense* Huch is extensively used in traditional medicine in the north of Cameroon. The leaf extract has been used to treat several diseases, including epilepsy, anxiety, depression, inflammation, neuropathic pain, taeniasis and malaria [8-12]. A decoction prepared from the leaves of *Crassocephalum bauchiense* has been reported to be extensively used for the treatment of memory impairment, anxiety and depression. However, there is no detailed study on the alleged anxiolytic properties of this plant species. To provide scientific evidence for its anxiolytic activities known in folk medicine, the overall objective of this work was to evaluate the effects of an aqueous extract of *Crassocephalum bauchiense* leaves on anxiety in mice and assess its implication on GABAergic transmission after exposure of mice to elevated plus maze and open field paradigms, respectively.

2. Material and methods

2.1. Plant material

Fresh leaves of *Crassocephalum bauchiense* used in this study was harvested from the Mawi area of Ngaoundéré, Cameroon in March 2018. The plant was deposited at the National Herbarium, Yaoundé, Cameroun where the Voucher Specimen No.7954/SRF/Cam exists.

2.2. Preparation of the aqueous extract of *Crassocephalum bauchiense*

Fresh leaves of *Crassocephalum bauchiense* were air dried at room temperature under shade for two weeks and ground using an electric blender. A total of 100 g of dried leaves was extracted by sieving. For the preparation of the aqueous extract of *Crassocephalum bauchiense*, 100 g of dried and powdered leaves were soaked in 1000 mL of distilled water for 72 h and filtered. The filtrate was then dried in the oven (Gallenhamp®, England) at 60°C to give a crude extract (7.5 g) with a 7.5% yield (w/w). The extract was prepared 30 minutes to 1 hour before its administration to the mice, and were administered orally (*per os*, *p.o.*) 1 hour before the pharmacological test, at a volume of 10 mL/kg of body weight. The doses were obtained from the main dose used by the traditional practitioner and the concentration (initial concentration; Ci = mass/volume) was obtained from calculation, knowing that the volume of administration is 10 mL/kg. In fact, the following concentrations were used: 2.5, 5, 10 and 20 mg/mL respectively for the doses of 25, 50, 100 and 200 mg/kg. Oral administration of the plant extract was performed using a non-flexible gavage needle with round end, fixed at the extremity of a 1 mL syringe with respect to the volume of administration 10 mL/kg.

2.3. Preliminary phytochemical study

Preliminary qualitative phytochemical analysis of *Crassocephalum bauchiense* aqueous extract were done as previously described by Taiwe et al. [11, 12]. The following family of compounds were identified in the aqueous extract of this plant: for alkaloids, glycosides, tannins, flavonoids, triterpenoids, anthraquinones, saponins, phenols [11, 12].

2.4. Chemicals

Diazepam was purchased from Roche, France. The reagents used for the quantification of brain γ-aminobutyric acid level, glutamic decarboxylase acid and γ-aminobutyric transaminase activities were purchased from Sigma Chemical, USA.

2.5. Experimental animals

Albino mice (20 - 25 g) of both sexes were used in this study. These mice were bred at the Faculty of Science Animal room, University of Buea. All animals were housed in a controlled environment, with free access to food and water and were maintained on a 12 h light-dark cycle. Each animal was used only once. All experiments were performed according to the Guide for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH

publication No. 85-23, revised in 1996) and received an approval from the University of Buea - Institutional Animal Care and Use Committee (UB-IACUC N° 06/2022).

2.6. Behavioural testing for the evaluation of anxiolytic properties of *Crassocephalum bauchiense* aqueous extract

2.6.1. Elevated plus maze paradigm

The elevated plus maze task is a conflict paradigm used by many investigators. In general, the elevated plus maze consists of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 10 cm) with walls approximately 10 cm high and an open roof. The apparatus is configured such that the similar arms are opposite each other. The maze is elevated to an approximate height of 50 cm. Test sessions usually are quite short (approx. 5–10 min) and subjects usually are tested only once. The apparatus that was used is similar to that described by Ngo Bum et al. [13]. Six groups of six mice each were used in our experiment. The negative control group received distilled water (10 mL/kg; orally), the positive control group received diazepam (3 mg/kg; intraperitoneally), and the four test groups received four different doses of *Crassocephalum bauchiense* (25, 50, 100 and 200 mg/kg; orally). One hour after treatment, the mice were individually placed on the elevated plus maze centre platform facing an open arm and observed for 5 minutes [13]. The number of entries by each animal into open or closed arms and the time spent by each animal in either open or closed arms (conventional parameters) were recorded with stopwatches by two trained experimenters. Time on the centre platform and ethological parameters such as rearing and head dipping were also recorded [14].

2.6.2. Open field paradigm

An open field consists of a wall-enclosed area that is of sufficient height to prevent the subject from escaping. The open field used was a wooden square box 40 × 40 × 45 cm; the floor was divided into 16 smaller squares of equal dimensions (10 × 10 cm). This apparatus was similar to that used by Taiwe et al. [12]. One hour after the elevated plus maze test the animals were subjected to the open filed test. Animals placed one by one in the centre of the box could explore the box for 5 minutes, and both exploratory activity and anxiety were evaluated [13]. Hand-operated counters and stopwatches were used to score crossing (number of square floor units entered), rearing (number of times the animal stood on its hind legs), grooming, and defecation. The positive control group received orally diazepam at a dose of 0.3 mg/kg.

2.7. Biochemical evaluation of GABAergic axis in *Crassocephalum bauchiense*-treated mice following the behavioural analyses

2.7.1. Determination of brain GABA content

Following behavioural testing, animals were sacrificed by cervical decapitation and the brains were quickly removed, cleaned with ice-cold 0.90% saline. The measurement of brain GABA level, based on the method of Lowe et al. was carried out as follows [15]. The brains were rapidly removed, blotted, weighed and taken in ice cold 5 mL trichloroacetic acid (10% w/v), homogenized and centrifuged at 10000 g for 10 min at 0°C. A sample (0.1 mL) of tissue extract was taken in 0.2 mL of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.9), was kept in a water bath at 60°C for 30 min then cooled and treated with 5 mL of copper tartrate reagent (0.16% disodium carbonate and 0.03% copper sulphate and 0.0329% tartaric acid). After 10 min, the fluorescence reading was taken at 377/451 nm in a spectrofluorimeter. For GABA standards, different amounts (20, 40, 60, 80, 100 g) mixed with 1.5 M glutamic acid were dissolved in 0.1 mL 10% trichloroacetic acid (w/v). GABA was determined by the measurement of the formed fluorescent product resulting from the reaction of GABA with ninhydrin in an alkaline medium, in the presence of glutamate (Sutton and Simmonds, 1974). The GABA content in brain was expressed in g/g of wet brain tissue.

2.7.2. Determination of brain GAD activity

After incubation of glutamic acid with a substrate, the increase in GABA is proportional to glutamate decarboxylase activity (GAD) [16]. The activity of GAD was measured by using the amount of GABA during incubation with glutamic acid. To 1.0 mL of homogenate and 1.0 mL of 0.05 M glutamic acid (neutralized at pH 6.3-6.7), 0.1 mL of pyridoxal-5-phosphate (25 pg) was added. The mixture was incubated at 37°C for 30 min. After the incubation period, the reaction was stopped by heating at 100°C for 10 min. The increase in GABA was monitored at 30 and 90 s at 455 nm using a spectrophotometer. The GAD activity was expressed in µg/min/mg of tissue.

2.7.3. Determination of brain GABA-T activity

For the determination of GABA-T activity, the brains were removed and immediately submerged in ice-cold artificial cerebrospinal fluid. The brain tissues were then washed to remove blood, blotted to dry and submerged in 5 mL of methanol, homogenized using a glass Teflon homogenizer for 2 min and centrifuged at 10,000 rpm at -10 °C for 15 min. GABA-T activity in the homogenates was measured spectrophotometrically as described by Sytinsky et al. [17]. To a 10 mL volumetric flask, 15 µmol from each of α-oxoglutarate and GABA, 10 µg of pyridoxal phosphate and 1 mL of supernatant of the brain tissue homogenate (10% in sucrose, 0.32 mol/L) were added and the final volume was made up to 3 mL with buffer containing 0.2 M Tris-HCl (pH 8.6). The final mixture was incubated at 37°C for 30 min for reaction in 96-well plates. The reaction was terminated by the addition of 0.5 mL ice-cold 20% trichloroacetic acid. The blank was prepared by replacing the homogenate with methanol from the mixture. The succinic semialdehyde (SSA) produced in the incubation mixture was estimated at 610 nm. The colour complex of SSA and 3- methyl 2-benzothiazolone-2-hydrazone in the presence of 12% $FeCl_3$ was measured against the blank. GABA-T activity was measured in units/mg of protein.

2.8. Statistical analysis

Microsoft excel and GraphPad Prism 6.0 were used to analyse the collected data. Data was expressed as means ± SEM, and percentages was used in the evaluation of the descriptive statistics. Differences between group means were compared using one-way analysis of variance (ANOVA) and individual comparisons of the group mean values was done using Tukey's Multiple Comparison Test. Significant levels were measured at 95% confidence interval (CI) with significant differences set at $P < 0.05$.

3. Results

3.1. Anxiolytic effects of *Crassocephalum bauchiense* aqueous extract on the number of open arm entries, close arm entries, total arm entries, rearing, and the percentage of open arms entries and time in mice placed in the elevated plus maze

Compared with distilled water, *Crassocephalum bauchiense* aqueous extract resulted in a significant increase in the number of entries into open arms of the elevated plus maze. The plant extracts significantly increased this number of entries into open arms from 0.50 ± 0.05 in distilled water-treated mice to 1.33 ± 0.44 ($P<0.05$), 1.50 ± 0.50 ($P<0.05$), and 1.67 ± 0.44 ($p<0.01$) in the groups administered *Crassocephalum bauchiense* aqueous extracts 50, 100 and 200 mg/kg, respectively (Table 1). As expected for a positive control group, diazepam 3 mg/kg administered intraperitoneally also induced an increase in the number of entries into the open arms of the elevated plus maze. In addition, this result show that the aqueous extracts of *Crassocephalum bauchiense* (25 – 200 mg/kg) given systematically produced dose-dependent and equipotent reduction of the number of closed arm entries and total arms entries, respectively. In the negative control animals treated with distilled water, results showed that the number of rearing was 13.00 ± 0.86. The aqueous extracts of *Crassocephalum bauchiense s*ignificantly decreased this level, from 13.00 ± 0.86 in the negative control mice to 1.00 ± 0.67 ($P<0.001$) for the dose 100 mg/kg and from 13.00 ± 0.86 in the negative control mice to 0.50 ± 0.67 ($P<0.001$) for the dose 200 mg/kg, respectively. Similarly, diazepam (3 mg/kg) decreased this number to 0.50 ± 0.50 ($P<0.001$). Statistical analysis showed that, the number of head dipping in the negative control animals was 4.83 ± 4.14. The doses 100 and 200 mg/kg of *Crassocephalum bauchiense* aqueous extracts significantly decrease the number of head dipping to 0.67 ± 0.89 ($P<0.001$) and to 0.50±0.67 ($P<0.001$), respectively.

Table 1 Effects of *Crassocephalum bauchiense* aqueous extract on the number of open arm entries, closed arm entries, total arm entries, rearing, and head dipping in mice placed in the elevated plus maze

	Distilled water	**Doses of *Crassocephalum bauchiense* (mg/kg)**				**Diazepam (mg/kg)**
	---	**25**	**50**	**100**	**200**	**3**
Open arm entries	0.50±0.05	0.67±0.44	1.33±0.44^a	1.50±0.50^a	1.67±0.44 ^{b}	1.83±0.28^b
Closed arm entries	6.17±1.22	2.33±0.78^a	1.17±0.28^b	0.83±0.56^c	0.67±0.89^c	0.67±0.67^c
Total arms entries	6.50±1.33	3.00±0.67^a	2.83±0.56^b	2.17±0.56^c	1.67±0.44^c	2.50±0.83^c
Rearing	13.00±0.86	6.83±0.56^a	1.83±0.56^c	1.00±0.67^c	0.50±0.67^c	0.50±0.50^c
Head dipping	4.83±4.14	1.83±1.50^b	1.17±0.28^c	0.67±0.89^c	0.50±0.67^c	0.17±0.28^c

Data are expressed as mean ± S.E.M., for 6 animals. ap<0.05, bp<0.01, cp<0.001, significantly different compared to the distilled water.

As expected for a positive control group, diazepam 3 mg/kg administered intraperitoneally, *Crassocephalum bauchiense* aqueous extracts also induced an increase in the percentage of entries into and time spent in the open arms of the elevated plus maze (Figure 1).

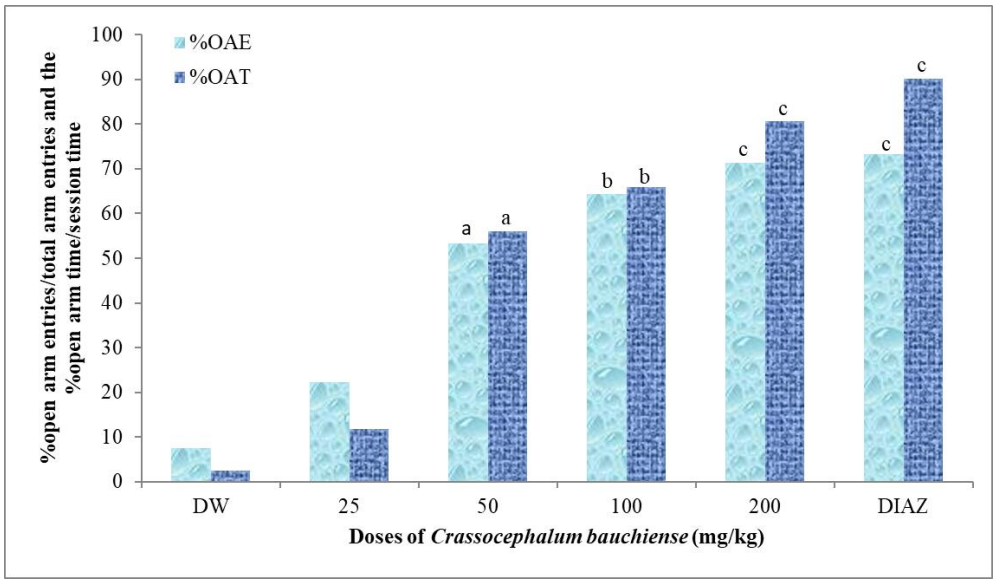


Figure 1 Effects of *Crassocephalum bauchiense* aqueous extract on the percentage of open arm entries/total arm entries and the percentage of open arm time/session time of mice placed the elevated plus maze

Shown are the percentage ± S.E.M. of open arm entries/total arm entries and the percentage of open arm time/session time (5 minutes), for 6 animals. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$, significantly different compared to the distilled water; DW: distilled water; DIAZ: diazepam 3 mg/k; %OAE: percentage of open arm entries; %OAT: percentage of open arm time.

Diazepam also induced a decrease in the percentage of time in closed arms. Like diazepam, *Crassocephalum bauchiense* aqueous extract induced a significant reduction in the percentage of time in closed arms from 55.11% in the control group to 4.16% ($P<0.001$) and 2.77% ($P<0.001$) at the doses of 100 and 200 mg/kg respectively (Figure 2).

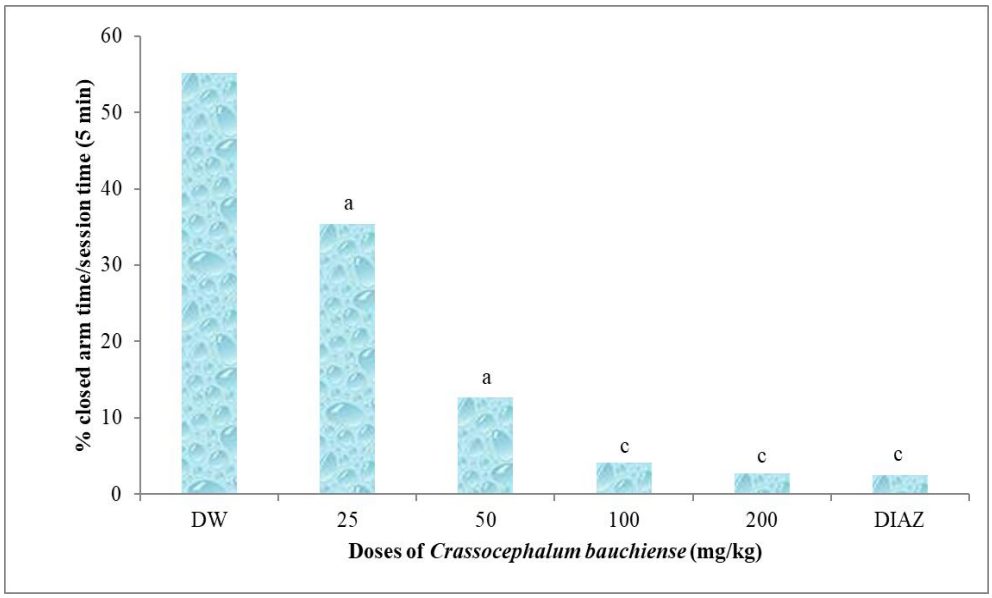


Figure 2 Effects of *Crassocephalum bauchiense* aqueous extract on the percentage of closed arm time/session time of mice placed the elevated plus maze

Shown are the percentage ± S.E.M of closed arm time/session time (5 minutes), for 6 animals. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$, significantly different compared to the distilled water. DW: distilled water; DIAZ: diazepam 3 mg/kg.

3.2. Effects of *Crassocephalum bauchiense* aqueous extract on the behavioural parameters of mice in the open field

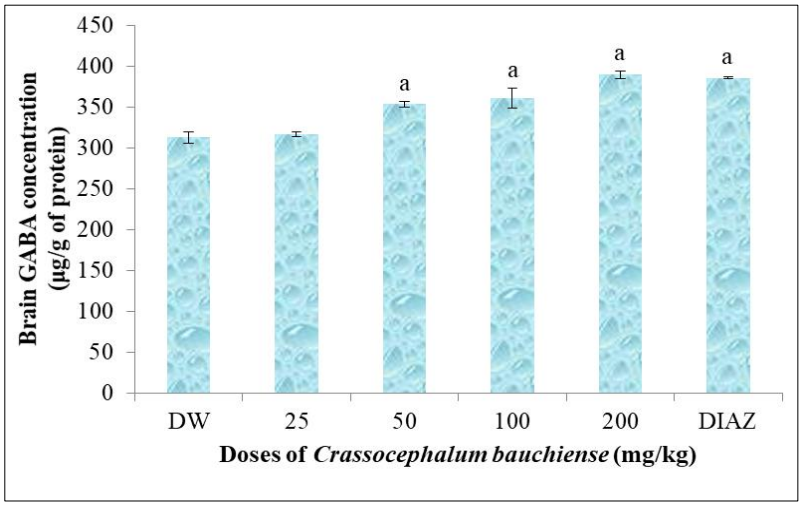
As in the open field test, the number of rearing was significantly decreased by both the *Crassocephalum bauchiense* aqueous extracts and diazepam, respectively (Table 2). Oral administration of *Crassocephalum bauchiense* aqueous extract significantly decreased this number from 10.50 ± 1.17 in the negative control group to 1.33 ± 0.44 (P<0.001) in group treated with 200 mg/kg extract, respectively. By contrast, *Crassocephalum bauchiense*-treated mice manifested significant dose-dependent increases in both the number of crossing and grooming in the open field paradigm at 50, 100 and 200 mg/kg. The number of crossing increased from 12.67 ± 1.56 in the negative control group of mice to 40.33 ± 2.22 (P<0.001) for a dose of 200 mg/kg aqueous extract, respectively. In the same way the number of grooming increased from 1.17 ± 0.56 in the negative control group to 2.33 ± 0.78 and 2.50 ± 0.67 for the doses 100 and 200 mg/kg *Crassocephalum bauchiense* aqueous extracts, respectively. Also, the aqueous extracts significantly reduced in dose-dependent manner the mass of fecal boli in naïve mice placed in open field as compare with the negative control group treated with distilled water. Diazepam produced the same effects as *Crassocephalum bauchiense* aqueous extract. In addition, results showed that the centre time in the negative control group was 3.17 ± 0.56 sec. The aqueous extracts of *Crassocephalum bauchiense* significantly increased this level, from 3.17 ± 0.56 sec in the negative control animals to 27.33 ± 3.33 sec (P<0.001) for the dose 100 mg/kg and from 3.17 ± 0.56 sec in the negative control animals to 32.33 ± 5.67 sec (P<0.001) for the dose 200 mg/kg, respectively (Table 2). These results were comparable to that of diazepam (0.3 mg/kg) where the centre time in the open field 41.67 ± 7.78 sec (P<0.001).

Table 2 Effects of *Crassocephalum bauchiense* aqueous extract on the rearing, crossing, grooming, centre time, and quantity of fecal boli of naïve mice in the open field test

	Distilled water	**Doses of *Crassocephalum bauchiense* (mg/kg)**				**Diazepam (mg/kg)**
		25	**50**	**100**	**200**	**0.3**
Rearing	10.50±1.17	2.33±1.00^{c}	2.17±0.56^{c}	1.50±0.50^{c}	1.33±0.44^{c}	0.83±0.56^{c}
Crossing	12.67±1.56	16.50±1.17	26.33±1.33^{b}	34.33±1.89^{c}	40.33±2.22^{c}	43.17±3.56^{c}
Grooming	1.17±0.56	2.00±0.67^{b}	2.33±0.78^{b}	2.33±0.78^{b}	2.50±0.67^{b}	2.67±1.00^{b}
Fecal boli (g)	0.57±0.18	0.13±0.06^{b}	0.02±0.03^{c}	0.02±0.03^{c}	0.02±0.03^{c}	0.02±0.03^{c}
Centre time (s)	3.17±0.56	10.33±0.89^{a}	19.67±1.67^{c}	27.33±3.33^{c}	32.33±5.67^{c}	41.67±7.78^{c}

Data are expressed as mean ± S.E.M., for 6 animals. ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001, significantly different compared to the distilled water.

3.3. Effects of *Crassocephalum bauchiense* aqueous extract on brain GABA concentration



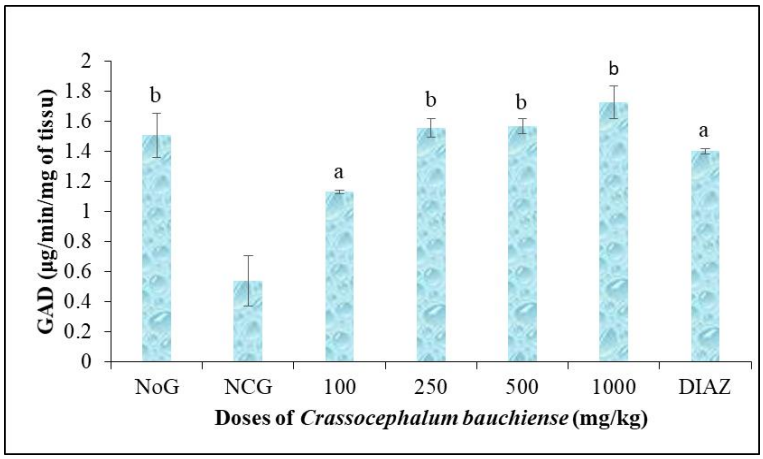
Data are expressed as mean ± S.E.M., for 6 animals. ^{a}p<0.05, significantly different compared to the distilled water. DW: distilled water; DIAZ: diazepam 0.3 mg/kg.

Figure 3 Effects of *Crassocephalum bauchiense* aqueous extracts on brain GABA concentration

A significant increase in the level of brain GABA concentration was observed in animals 1 h after oral administration of *Crassocephalum bauchiense* aqueous extract (Figure 3). *Crassocephalum bauchiense* extracts significantly increased the brain GABA content from 312.83 ± 6.83 µmol/mg tissue in the negative control group to 360.83 ± 11.88 and 389.16 ± 11.89 µmol/mg tissue (P<0.01) at the doses of 100 and 200 mg/kg, respectively. Similarly, diazepam administration caused a significant increase in the level of GABA as compared with distilled water group.

3.4. Effects of *Crassocephalum bauchiense* aqueous extract on brain GAD activities

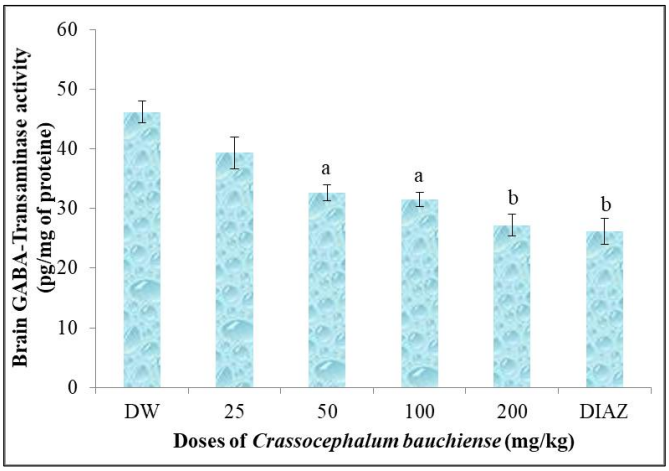
Figure 4 presents the effects of *Crassocephalum bauchiense* extract on GAD activity in the brain of mice. Brain GAD activity increased significantly in distilled-water treated group as compare with the plant extracts-treated animals. GAD activity increased from 0.58 ± 0.16 µg/min/mg of tissue in distilled water-treated group to1.34 ± 0.10 (p<0.01), 1.44 ± 0.05 (p<0.01) and 1.57 ± 0.07 (P<0.01) µg/min/mg of tissue for the groups of mice treated with the respective doses of 50, 100 and 200 mg/kg extracts. Brain GAD activity increased from 2.15 ± 0.50 µmole/g tissue in distilled water-treated group to 4.59 ± 0.59 µmole/g tissue in the group of mice treated with diazepam (Figure 4).



Data are expressed as mean ± S.E.M., for 6 animals. [a]p<0.05, [b]p<0.01, significantly different compared to the distilled water. DW: distilled water; DIAZ: diazepam 0.3 mg/kg.

Figure 4 Effects of *Crassocephalum bauchiense* aqueous extracts on GAD activity

3.5. Effects of *Crassocephalum bauchiense* aqueous extracts on GABA-T activity



Data are expressed as mean ± S.E.M., for 6 animals. [a]p<0.05, [b]p<0.01, significantly different compared to the distilled water. DW: distilled water; DIAZ: diazepam 0.3 mg/kg.

Figure 5 Effects of *Crassocephalum bauchiense* aqueous extract on brain GABA-T activity concentration

A significant difference was observed in GABA-T activity in the brain amongst all the groups of mice (Figure 5). *Crassocephalum bauchiense* aqueous extracts significantly decreased the brain GABA-T activity from 46.17 ± 1.83 pg/mg

of tissue in the negative control group to 31.50 ± 1.17 pg/mg of protein (P<0.05) and to 27.17 ± 1.83 pg/mg of tissue (P<0.01) at the doses of 100 and 200 mg/kg extract, respectively. Obviously, sodium valproate also decreased the brain GABA-T activity level from 46.17 ± 1.83 pg/mg of tissue in the negative control mice to 26.17 ± 22.22 pg/mg of tissue (P<0.01) at a dose 300 mg/kg.

4. Discussion

In our study we used the elevated plus maze paradigm to evaluate the anxiolytic effects of *Crassocephalum bauchiense* aqueous extract. The elevated plus maze has been described as a simple method for assessing anxiety responses of rodents by File and co-workers [18]. Oral administration of an aqueous extract of *Crassocephalum bauchiense* induced a significant reduction of the number of rearing and head dipping in mice. Rearing is a common measure of an increased of general activity, stress, and anxiety of animals placed in the elevated plus maze. The reduction of these behavioural parameters indicated the anti-anxiety like behaviour in mice [13, 14]. The anxiolytic effects of the studied plant extract were also found in the elevated plus maze test where *Crassocephalum bauchiense* aqueous extract increased the number of entries into, the percentage of entries and time in open arms; and *Crassocephalum bauchiense* aqueous extract reduced the percentage of closed arms time [18]. The correlation of the increase in time spent in open arms with the increase in the number of entries in open arms supported the anxiolytic-like activity of the plant extract [14]. With the different concentrations of the plant extract, the fact that the entries into open arms were increasing, while the total entries were decreasing or not changing suggested an increase of exploratory activity not related to the locomotion. The increase of the exploration activity in the open arms, suggested anxiolytic activity as anti-anxiety drugs decrease the stress-induced the inhibition of exploratory behaviour [18-20]. The anxiolytic-like activity of *Crassocephalum bauchiense* aqueous extract could be explained by the presence of some components in the plant extract interacting with the benzodiazepine/$GABA_A$ receptors as agonists, or with the 5-HT_{1A} receptors as agonists, or with the NMDA receptors as antagonists, or with any other mechanisms [20-22].

Developed by Hall and Ballachey, the open field test is an experimental test used to assay general locomotor activity levels, anxiety, and willingness to explore in animals (usually rodents) in scientific research [23]. We also used the open field paradigm to evaluate some parameters related to anxiety-like behaviours in mice. The present data demonstrate that *Crassocephalum bauchiense* aqueous extract has clear and consistent effects on rearing and grooming behaviour in the open field. There was significant reduction in the number of rearing and a significant increase in the number of grooming. Effects of this nature are usually observed during treatment with serotonergic drugs where there was a significant reduction of anxiety of mice placed in the open field [24]. Even the increase centre time and crossing in the open field test suggests the presence of anxiolytic properties that could have induced the increase in exploration activity when compared with normal mice, as closed arm entries and head dipping were reduced in the elevated plus maze [14, 20]. These results demonstrate that *Crassocephalum bauchiense* aqueous extract has a good anxiolytic-like effect without a stimulating head-dipping behaviour and reduction of locomotor activity in the open field. We suggest that the anxiolytic-like effects exhibited herein could be attributed to the interaction of the flavonoids, alkaloids, sterols and/or terpenes present in *Crassocephalum bauchiense* aqueous extract with the serotonergic and/or GABAergic neurotransmission systems [5, 16].

GABA is an important inhibitory neurotransmitter widely distributed in the brain. Its reduction is associated with anxiety [25]. GABAergic neurotransmission in the amygdala is a promising candidate for modulation of anxiety-related responses. A number of lines of research in experimental animals have provided evidence for an important role of GABAergic neurotransmission in the amygdala in modulating anxiety-related behaviours [25]. Administration of *Crassocephalum bauchiense* aqueous extract enhanced the brain GABA levels which suggested an anxiolytic activity of the extract [11, 26, 27]. These properties of *Crassocephalum bauchiense* could be related to the presence of some components in the extract activating the $GABA_A$ receptors complex and their ability to reduce GABA-T activity than with a potent increase of brain GABA content. This result allows us to confirm that *Crassocephalum bauchiense* aqueous extract prevent anxiety by increasing the brain GABA level [22, 25].

In an attempt to find possible mechanisms for the actions of *Crassocephalum bauchiense* aqueous extract, the involvement of the GABA pathway was explored, since this neurotransmitter plays a major function in anxiety disorders [25, 27]. Our data indicated that stress induced by the paradigms decreased GABA levels and GAD activity, while it increased GABA-T activity. This decrease is an obvious manifestation of a loss of GABAergic interneurons and a decrease in $GABA_A$ receptor expression in the brain. The extract of *Crassocephalum bauchiense* increased GABA levels and GAD activity, but decreased that of GABA-T, suggesting an interference with the GABA system.

To confirm our previous results on GABA brain increased by the treatment of mice with the aqueous extract, we evaluated the effects of *Crassocephalum bauchiense* aqueous extract on the activity of GABA-T on the brain of mice

exposed to the elevated plus maze task followed by the open field paradigm. Oral administration of the aqueous extracts significantly attenuated the enzyme activity indicating potent GABA-T inhibitory effects. GABA-T is the primary catabolic enzyme in the mammalian brain that catalyzes the transfer of amino group from GABA to α- ketoglutarate leading to the depletion in the level of GABA [17, 21]. It is well studied that free radicals are generated during anxiety and disturb the balance between GABA and glutamate activity in the brain [17]. These results confirm the increase of brain GABA concentration in pretreated mice with the different doses of *Crassocephalum bauchiense*.

5. Conclusion

This study documents the anxiolytic effects of an aqueous extract of *Crassocephalum bauchiense* using the generalised model of anxiety in mice and examines the components and molecular mechanisms that are likely involved. In conclusion, the results obtained provide the evidence that *Crassocephalum bauchiense* aqueous extract exerts anxiolytic property in naïve mice. It also increased the brain GABA concentration and attenuated the activity of GAD and GABA-transaminase, respectively. These observations lend pharmacological support to the report of the traditional uses of the plant leaves in the treatment of anxiety, insomnia, depression and dementia in some parts of Cameroon.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

All experiments were performed according to the Guide for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH publication No. 85-23, revised in 1996) and received an approval from the University of Buea - Institutional Animal Care and Use Committee (UB-IACUC N° 06/2022).

References

[1] Baldwin, D.S., Clinical experience with paroxetine in social anxiety disorder. International Clinical Psychopharmacology, 2000.

[2] Scott-Hamilton, J., N.S. Schutte, and R.F. Brown, Effects of a mindfulness intervention on sports-anxiety, pessimism, and flow in competitive cyclists. Applied Psychology: Health and Well-Being, 2016. 8(1): p. 85-103.

[3] De La Mora, M.P., et al., Anxiolytic-like effects of the selective metabotropic glutamate receptor 5 antagonist MPEP after its intra-amygdaloid microinjection in three different non-conditioned rat models of anxiety. European Journal of Neuroscience, 2006. 23(10): p. 2749-2759.

[4] Davis, M., Are different parts of the extended amygdala involved in fear versus anxiety? Biological psychiatry, 1998. 44(12): p. 1239-1247.

[5] Zhang, Z.-J., Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life sciences, 2004. 75(14): p. 1659-1699.

[6] Buller, R. and V. Legrand, Novel treatments for anxiety and depression: hurdles in bringing them to the market. Drug discovery today, 2001. 6(23): p. 1220-1230.

[7] Wagner, R.G., G.H. Mohammed, and T.L. Noland, Critical period of interspecific competition for northern conifers associated with herbaceous vegetation. Canadian Journal of Forest Research, 1999. 29(7): p. 890-897.

[8] Arbonnier, M., Trees, shrubs and lianes of the dry zones of West Africa. Trees, shrubs and lianes of the dry zones of West Africa., 2000.

[9] Arbonnier, M., Arbres arbustes et lianes des zones sèches d'Afrique de l'Ouest. Arbres arbustes et lianes des zones sèches d'Afrique de l'Ouest, 2009: p. 1-100.

[10] Mouokeu, R.S., et al., Antibacterial and dermal toxicological profiles of ethyl acetate extract from *Crassocephalum bauchiense* (Hutch.) Milne-Redh (Asteraceae). BMC Complementary and Alternative Medicine, 2011. 11(1): p. 1-7.

[11] Taïwe, G.S., et al., Antipsychotic and sedative effects of the leaf extract of *Crassocephalum bauchiense* (Hutch.) Milne-Redh (Asteraceae) in rodents. Journal of ethnopharmacology, 2012. 143(1): p. 213-220.

[12] Taïwe, G.S., et al., Evaluation of antinociceptive effects of *Crassocephalum bauchiense* Hutch (Asteraceae) leaf extract in rodents. Journal of Ethnopharmacology, 2012. 141(1): p. 234-241.

[13] Bum, E.N., et al., Anticonvulsant, anxiolytic, and sedative properties of the roots of Nauclea latifolia Smith in mice. Epilepsy & Behavior, 2009. 15(4): p. 434-440.

[14] Lister, R.G., The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology, 1987. 92(2): p. 180-185.

[15] Lowe, I.P., E. Robins, and G.S. Eyerman, The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. Journal of neurochemistry, 1958. 3(1): p. 8-18.

[16] de Souza, E.A.P. and P.C.B. Salgado, A psychosocial view of anxiety and depression in epilepsy. Epilepsy & Behavior, 2006. 8(1): p. 232-238.

[17] Sytinsky, I., et al., The gamma-aminobutyric acid (GABA) system in brain during acute and chronic ethanol intoxication. Journal of neurochemistry, 1975. 25(1): p. 43-48.

[18] Pellow, S., et al., Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of neuroscience methods, 1985. 14(3): p. 149-167.

[19] File, S.E. and S. Pellow, The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard. British Journal of Pharmacology, 1985. 86(3): p. 729-735.

[20] Jenck, F., et al., Orphanin FQ acts as an anxiolytic to attenuate behavioral responses to stress. Proceedings of the National Academy of Sciences, 1997. 94(26): p. 14854-14858.

[21] Olivier, J.D., C.H. Vinkers, and B. Olivier, The role of the serotonergic and GABA system in translational approaches in drug discovery for anxiety disorders. Frontiers in pharmacology, 2013. 4: p. 74.

[22] Woode, E., et al., Anxiolytic and antidepressant effects of a leaf extract of Palisota hirsuta K. Schum.(Commelinaceae) in mice. IJP-International Journal of Pharmacology, 2010. 6(1): p. 1-17.

[23] Hall, C. and E.L. Ballachey, A study of the rat's behavior in a field. A contribution to method in comparative psychology. University of California Publications in Psychology, 1932.

[24] Pytka, K., et al., Antidepressant-and anxiolytic-like effects of new dual 5-HT1A and 5-HT7 antagonists in animal models. PLoS One, 2015. 10(11): p. e0142499.

[25] Lydiard, R.B., The role of GABA in anxiety disorders. Journal of Clinical Psychiatry, 2003. 64: p. 21-27.

[26] Evans, A.K. and C.A. Lowry, Pharmacology of the β-Carboline FG-7142, a Partial Inverse Agonist at the Benzodiazepine Allosteric Site of the GABAA Receptor: Neurochemical, Neurophysiological, and Behavioral Effects. CNS drug reviews, 2007. 13(4): p. 475-501.

[27] Savage, K., et al., GABA-modulating phytomedicines for anxiety: A systematic review of preclinical and clinical evidence. Phytotherapy Research, 2018. 32(1): p. 3-18.