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Anxiolytic and antipyretic properties of the root aqueous extract of *Bombax costatum* Pellegr. et Vuillet. (Bombacaceae) in mice: Implication of behavioural and neurochemical approaches

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Abstract

Ethnopharmacological relevance: *Bombax costatum* Pellegr. et Vuillet. (Bombacaceaea) is a plant used in traditional medicine in Cameroon to treat memory impairment, anxiety, insomnia and agitation.

Aim of the study: The aqueous extract of *Bombax costatum* is evaluated for its anxiolytic like effect in mice using experimental models.

Materials and methods: The plant extract is administered orally to mice. They were tested one hour later in the stress-induced hyperthermia, hole board, and open field or elevated plus maze tests, respectively. Finally, the brain Gamma aminobutyric acid [GABA] content and GABA-T were quantified in *Bombax costatum* aqueous extract-treated mice at the end of elevated plus maze test.

Results: *Bombax costatum* aqueous extract showed anxiolytic activity. In stress-induced hyperthermia test, the plant extract significantly antagonised the increase of temperature. There is a significant reduction in the stress-induced hyperthermia from 1.13 ± 0.06°C in the negative control group treated with distilled water to 0.26 ± 0.02°C in the group of mice administered 100 mg/kg aqueous extract. In addition, *Bombax costatum* showed antipyretic activity by reducing the body temperature. In the elevated plus maze test, the aqueous extract increased the number of entries into, percentage of entries into, and percentage of time in open arms. It also reduced the percentage of entries and time in closed arms.

Conclusion: The obtained results suggested that *Bombax costatum* aqueous extracts possess anxiolytic-like and antipyretic activities in mice. This plant could be helpful in the treatment of anxiety and fever in traditional medicine in Cameroon.

Keywords: *Bombax costatum*; Anxiolytic; GABAergic; Animal models; Traditional medicine.

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1. Introduction

Anxiety disorders are the most common mental illnesses with more than 18% of the world's population is affected by anxiety, making it a major problem in psychopharmacology research [1-3]. Anxiety, which is nowadays a true disease of civilization, is frequently accompanied by personality disorders [4]. It is a normal physiological reaction existing in humans. Anxiety is qualified as pathological when it induces suffering in the individual concerned because of its intensity and duration, and it becomes impossible to tolerate or defend oneself against it [4]. Anxiety disorders are associated with a wide range of profound negative sequelae. In low- and middle-income countries, there is a critical shortage of mental health clinicians. Anxiety has an early onset and are associated with significant subsequent morbidity, so intervention deserves attention [2].

The drugs commonly used for anxiety disorders, benzodiazepines, have long-term side effects such as memory impairment, addiction and dependence, hence the urgent need to identify new, less expensive drugs with few side effects [3]. The World Health Organization [5] encourages and supports the use of herbal medicines as a "source of effective and inexpensive medicines" as they are proven to be effective and safe and contribute to achieving the goal of universal access to care. Today, several countries recognize the need for a cohesive and integrative approach to health care. Ethnobotanical studies of African flora have revealed that the root decoction of *Bombax costatum* Pellegr. et Vuillet. (Bombacaceaea) is widely used by traditional healers in Northern Cameroon to treat anxiety disorders, depression and epilepsy [6]. The infusion of the bark and leaves of *Bombax costatum* is used to treat fever, diarrhoea and gonorrhoea [6]. However, its activity on the central nervous system disorders which is a part of the properties attributed to this plant by the traditional medicine, has not yet been studied. Therefore, a study of the decoction of *Bombax costatum* roots has been studied with the hypothesis that it could have anxiolytic effects in mice. In addition, the neurochemical properties were evaluated by measuring the brain γ-aminobutyric acid (GABA) level and γ-aminobutyric transaminase (GABA-T) activity in the brain of *Bombax costatum*-treated animals.

2. Material and methods

2.1. Plant material

2.2. Preparation of *Cymbopogon citratus* aqueous extract

The roots of *Bombax costatum* used in this work were harvested in Garoua (North region of Cameroon) between September and October 2019. The plant was identified by botanists from the Faculty of Sciences, University of Ngaoundéré. Confirmation of the species was performed at the National Herbarium of Cameroon in Yaoundé and a voucher specimen was deposited (4577/HNC). One hundred grams (100 g) of powdered *Bombax costatum* roots was macerated in 1000 mL of distilled water for 1-hour duration, and the mixture was boiled for 20 min. After it cooled, the supernatant (decoction) was collected and filtered with Whatman No. 1 filter paper. The yield of extraction was 5.20%. The decoction, prepared 30 minutes to 1 hour before its administration in mice, was administered intraperitoneally (i.p.) 1 hour before the test. The following doses were used: 10, 25, 50, and 100 mg/kg.

2.3. Preliminary phytochemical study

The decoction of *Bombax costatum* was examined for its phytochemical contains as described previously by Taiwe et al. [7]. It was the case of alkaloids, glycosides, tannins, flavonoids, triterpenoids, anthraquinones, saponins, phenols. A comparative thin layer chromatographic study was also used to evaluate qualitatively the presence of bufadienolides in the decoction of *Bombax costatum* using anisaldehyde sulphuric acid reagent under UV (254 – 365 nm) [7].

2.4. Chemicals

Diazepam was purchased from Roche, France. Phenobarbital and the others reagents used for the quantification of brain γ-aminobutyric acid level and γ-aminobutyric transaminase activity were purchased from Sigma Chemical, USA.

2.5. Animals

Adult male mice (Mus musculus Swiss, weighing 23 - 30 g) were used for all the experiments and each mouse was used only once. Mice were housed in standard cages at 25°C, 12/12 hours light-dark cycle, with free access to food, water. The protocols were performed in concordance with the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee, Yaounde (No. FW-IRB00001954). In addition, the protocols for pharmacological studies were also realised in compliance with the general guidelines for experimental research and screening of traditional medicine as promulgated

by WHO [8] and the recommendations provided in the Animal Research: Reporting of *in vivo* Experiment (ARRIVE) guidelines [9].

2.6. Pharmacological analysis

2.6.1. Stress-induced hyperthermia test

Mice were marked and housed 10 per cage, and they were treated orally with 10 mL/kg distilled water (negative control group), intraperitoneally with 20 mg/kg phenobarbital (positive control group), or orally with the different doses (10, 25, 50 and 100 mg/kg) of the decoction of *Bombax costatum* (test groups). After 60 minutes, mice were consecutively removed from their cage (1-minute intervals each) and their body (rectal) temperature was recorded. This experiment is based on the fact that among animals in the same cage, mice removed later have a higher body temperature than those removed earlier [10, 11]. Stress-induced hyperthermia (SIH) was defined as the difference between the mean temperature of the first three mice and the mean temperature of the last three mice.

2.6.2. Hole-board test

The hole-board apparatus consisted of a grey wooden box (40 cm × 40 cm × 2.2 cm) with 16 equidistant holes 3 cm in diameter in the floor. Animals were treated orally with 10 mL/kg distilled water (negative control group), intraperitoneally with 20 mg/kg phenobarbital (positive control group), or orally with the different doses (10, 25, 50 and 100 mg/kg) of the decoction of *Bombax costatum* (test groups) 1 hour prior to testing. Each animal was placed singly in the centre of the board facing away from the observer and its behaviour (grooming, rearing and head dipping) recorded for 5 minutes duration. The number of lines crossed (crossing) was also noted [12, 13].

2.6.3. Open field test

The open field test was used to evaluate locomotor activity, level of exploration and emotional reaction of animals as described previously [14, 15]. Open field consisted of a surrounding square (40 cm x 40 cm) divided in 16 small square and 1 centre field (10 cm x 10 cm) wall of 19 cm high [16, 17]. The positive control group received intraperitoneally 0.3 mg/kg diazepam and the negative control was treated orally with 10 mL/kg. Test groups were treated orally with the different doses of the plant extract (10, 25, 50 and 100 mg/kg). One hour after the treatment, each mouse was placed individually in the centre of the arena and the number of crossing, grooming, rearing, the time in the centre and faecal boli weight were recorded for 5 minutes duration.

2.6.4. Elevated plus maze test

The Elevated Plus-Maze (EPM) test is one of numerous behavioural test design for the measuring of anxiety behaviour and stress in rodents. This behavioural test is based on the natural aversion of rodents for open and elevated areas, and also on their natural spontaneous exploratory behaviour in new environments [18]. The elevated plus maze was elevated above floor level (50 cm), and consists of two closed arms (16 cm × 5 cm × 10 cm) and two open arms (16 cm × 5 cm), with an extension to a common central platform (5 cm × 5 cm). Animals are grouped and treated as described previously. They were administered orally with the different doses of *Bombax costatum* decoction (10, 25, 50 and 100 mg/kg; test groups), diazepam (3 mg/kg; positive control group) or distilled water (10 mL/kg; normal group). One hour later, each animal was placed individually in the centre of the elevated plus maze and their behaviours were recorded for 5 minutes duration [19-21]. The number of entries by each mouse into open or closed arms, and the time spent by each mouse in either open or closed arms were observed and recorded. In addition, the weight of faecal boli, and the number of grooming and head dipping were also recorded.

2.6.5. Determination of brain GABA concentration and GABA-transaminase activity

The brain GABA level was estimated in groups of mice immediately after the elevated plus maze test. 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 4°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 μg) mixed with 1.5 μM 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 [22]. The GABA content in brain was expressed in μg/g of wet brain tissue.

Activity of GABA-T was also evaluated in the brain of each animal after the elevated plus maze test. Briefly, the brain tissue of each mouse was 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 [23]. GABA-T activity in the homogenates was measured spectrophotometrically as described previously [23] and with few modified [24]. 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, and 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.

2.7. Statistical analysis

Data are shown as means ± Standard Error of the Mean (S.E.M.) or as percentages of entries or time spent for each mouse. Statistical analysis of significance was carried out using one- or two-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests. P values less than 0.05 were considered as significant.

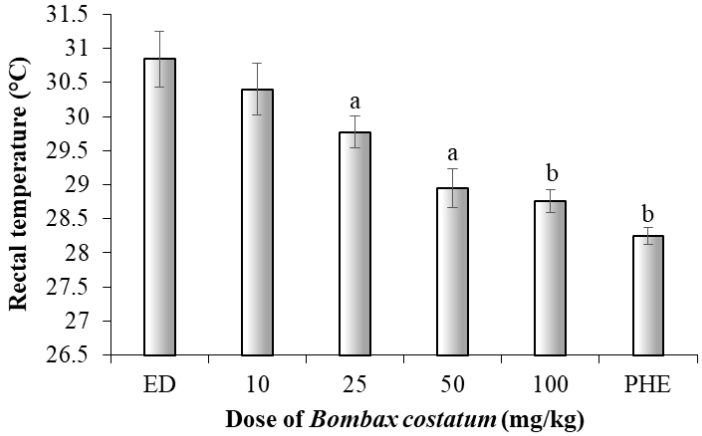
3. Results

3.1. Phytochemical characterization of *Bombax costatum* aqueous extract

The following family of compounds: alkaloids, glycosides, tannins, flavonoids, triterpenoids, anthraquinones, saponins and phenols were tested positive in the preliminary phytochemical study of *Bombax costatum* roots decoction. However, the thin layer chromatography of *Bombax costatum* roots decoction *Bombax costatum* roots decoction indicated that bufadienolides are absent.

3.2. Effects of *Bombax costatum* aqueous extract on stress-induced hyperthermia

Figure 1 shows that phenobarbital produced a significant decrease in rectal temperatures in the stress-induced hyperthermia test which confirmed the sensitivity of the test. Interestingly, the plant extract administered orally to mice produced a significant reduction [F (5, 30) = 12.46, P<0.01] in rectal temperature when compared with the negative control group of animals. The rectal temperature is 30.84 ± 0.41°C in the negative control group treated with distilled water. Oral administration of *Bombax costatum* aqueous extract administered at the doses of 25, 50 and 100 mg/kg induced a significant reduction of the rectal temperature to 29.77 ± 0.23, 28.95 ± 0.28 and 28.25 ± 0.12°C, respectively.

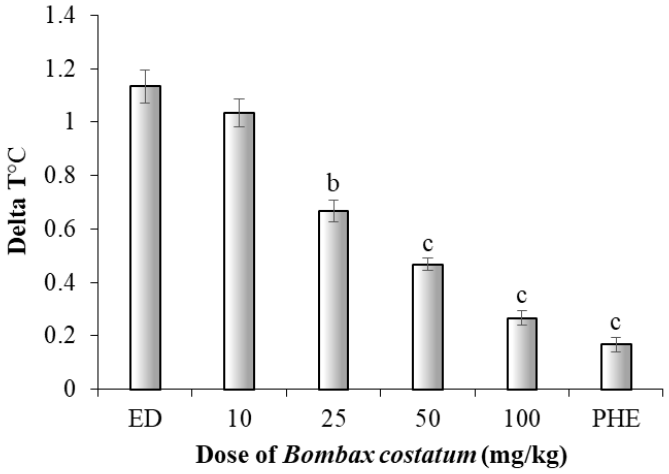


Means + SEM. N = 10 per group. [a]P < 0.05, [b]P < 0.01, ANOVA followed by Tukey's post hoc test. ED, distilled water; PHE, 20 mg/kg phenobarbital

Figure 1 Effects of *Bombax costatum* aqueous extract on body temperature of mice

As expected, phenobarbital produced a significant reduction in temperature between the first three and the last three mice. Oral administration of *Bombax costatum* aqueous extract also produced the same effect in a dose-dependent manner [F (5, 30) = 31.14, P<0.001]. There is a significant reduction in the stress-induced hyperthermia from 1.13 ±

0.06°C from the negative control group treated with distilled water to 0.67 ± 0.04, 0.47 ± 0.02 and 0.26 ± 0.02°C in the group of mice administered respectively 25, 50 and 100 mg/kg *Bombax costatum* aqueous extract (Figure 2).



N = 10 per dose. [b]P < 0.01, [c]P < 0.001, ANOVA followed by Tukey's post hoc test. ED, distilled water; PHE, 20 mg/kg phenobarbital

Figure 2 Effect of *Bombax costatum* aqueous extract on stress-induced hypothermia (expressed as temperature difference (ΔT°C)) in mice

3.3. Effects of *Bombax costatum* aqueous extract on anxiety, locomotor activity and exploratory behaviour in the hole-board test

Bombax costatum-treated mice manifested significant and dose-dependent increases in the number of head-dipping [F(5, 30) = 12.15, p<0.001], and dose-dependent reduction in the latency to the first head-dips [F(5, 30) = 28.42, p<0.001] in the hole-board paradigm at the doses of 25, 50 and 100 mg/kg. Additionally, the plant extract significantly reduced in dose-dependent manner the number of rearing [F(5, 30) = 14.72; p<0.001], and increased the number of grooming [F(5, 30) = 47.56; p<0.001] and crossing [F(5, 30) = 13.81; p<0.001] in the hole board test. Diazepam used as a reference anxiolytic drug produced the same properties *Bombax costatum* aqueous extracts (Table 1).

Table 1 Effects of *Bombax costatum* aqueous extract on the number of grooming, crossing, rearing, head-dipping, and the latency to the first head-dipping in the hole board test

Treatment	Dose (mg/kg)	Number				Onset time (sec)
		Grooming	Crossing	Rearing	Head-dipping	First head-dipping
DW	-	1.00±0.71	4.20±8.70	12.20±7.53	3.40±3.97	28.60±3.28
B. costatum	10	3.60±0.89[a]	7.40±4.84[a]	9.60±4.59[a]	6.13±4.80[a]	19.46±1.12[a]
B. costatum	25	6.20±1.00[b]	11.80±9.43[b]	4.80±2.58[b]	8.28±3.67[c]	14.31±1.65[b]
B. costatum	50	7.80±2.58[c]	13.20±9.97[b]	3.00±5.97[c]	11.80±6.05[c]	5.26±1.21[c]
B. costatum	100	7.00±0.89[c]	16.80±9.75[c]	3.60±3.36[c]	14.00±4.84[c]	6.80±1.40[c]
Diazepam	3	7.20±1.92[c]	14.80±7.69[c]	2.40±2.88[c]	13.20±6.53[c]	5.20±1.30[c]

Data are means ± SEM. N = 6 per dose. [a]P<0.05, [b]P<0.01, [c]P<0.001, ANOVA followed by Tukey's post hoc test. DW: distilled water.

3.4. Effects of *Bombax costatum* aqueous extract on anxiety, locomotor activity and exploratory behaviour in the open field test

As in the hole board test, the number of rears was decreased by both *Bombax costatum* aqueous extracts and the standard anxiolytic diazepam [F (5, 30) = 17.11, p<0.001]. Administration of *Bombax costatum* aqueous extracts to mice significantly increased the number of grooming [F (5, 30) = 14.19, p<0.001], crossing [F(5, 30) = 24.11, p<0.001] and the centre time [F(5, 30) = 22.73, p<0.0001] when compared with the negative control group administered distilled water. Both diazepam and *Bombax costatum* aqueous extracts at doses of 25, 50 and 100 mg/kg, respectively, also

decreased the mass of faecal boli [F(5, 30) = 25.14, P<0.01]. By contrast, the decoction increased crossing [F(6, 30) = 17.01, P < 0.001] and time spent in the centre [F(6, 30) = 11.59, P < 0.001] (Table 2).

Table 2 Effects of *Bombax costatum* aqueous extract on the number of grooming, crossing and rearing, the quantity of faecal boli, and the centre time in the open field test

Treatment	Dose (mg/kg)	Number			Faecal boli (g)	Centre time (sec)
		Grooming	Crossing	Rearing		
DW	-	0.60±0.48	71.80±2.32	36.40±5.00	0.45±0.07	1.60±0.48
B. costatum	10	4.80±1.47^{b}	114.40±7.53^{a}	27.00±8.72	0.38±0.03	9.00±3.58^{c}
B. costatum	25	6.40±2.65^{c}	99.40±2.06^{a}	17.40±3.61^{b}	0.14±0.02^{b}	3.20±1.17^{a}
B. costatum	50	4.80±2.23^{b}	69.60±3.61^{a}	17.20±5.64^{b}	0.08±0.02^{c}	4.00±1.90^{b}
B. costatum	100	4.60±2.940^{b}	106.60±5.31^{a}	9.00±2.10^{c}	0.02±0.01^{c}	8.80±1.72^{c}
Diazepam	0.3	6.20±1.72^{c}	141.20±7.28^{b}	16.80±3.96^{b}	0.02±0.01^{c}	10.00±3.46^{c}

Data are means ± SEM. N = 6 per dose. ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001, ANOVA followed by Tukey's post hoc test. DW, distilled water.

3.5. Effects of *Bombax costatum* aqueous extract on anxiety-like behaviours in the elevated plus maze test

3.5.1. Effects of Bombax costatum aqueous extract on the number of open arm entries, close arm entries, total arm entries, rearing, head dipping and faecal boli

Oral administration of *Bombax costatum* aqueous extracts animals increased the number of entries in the open arms [F(6, 35) = 31.19, p<0.001], the number of total arm entries [F(6, 35) = 54.31, p<0.001] and the ratio open entries/total entries (OE/TE) versus closed entries/total entries (CE/TE) [F(6, 35) = 52.16, p<0.001] in the group treated with the different doses of extract, respectively. The number of open arm entries is 0.67 ± 0.60 in group of mice treated with distilled water. *Bombax costatum* aqueous extract administered at a dose of 100 mg/kg, diazepam 3 mg/kg, or buspirone 10 mg/kg significantly increased this number of open arm entries to 7.61±0.94 (p<0.001), 10.33 ± 0.47 (p<0.001), and 8.50 ± 0.95 (p<0.001) respectively (Table 1).

Table 3 Effects of *Bombax costatum* aqueous extract on the number of open arm entries, close arm entries, total arm entries, rearing, head dipping and faecal boli

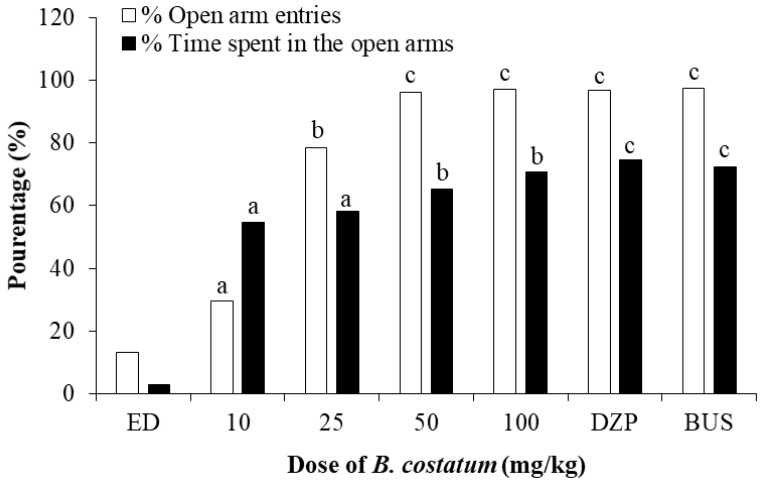
Treatment	Dose (mg/kg)	Open arm entries	Closed arm entries	Total arms entries	Ratio OE/TE vs CE/TE	Rearing	Head dipping	Faecal boli
DW	-	0.67±0.60	4.41±0.57	5.08±1.17	0.15±1.05	16.33±0.47	16.5±0.50	1.24±0.15
B. costatum	10	1.73±0.37^{a}	4.12±0.68	5.85±1.05	0.41±0.54	9.13±0.94^{a}	8.48±0.74^{a}	0.53±0.01^{a}
B. costatum	25	4.81±0.89^{b}	1.33±0.14^{a}	6.14±1.03	3.62±1.31^{a}	8.5±0.50^{a}	8.66±0.47^{a}	0.25±0.06^{a}
B. costatum	50	6.79±0.74^{b}	0.27±0.17^{b}	7.06±0.91^{a}	25.15±2.26^{c}	8.16±0.68^{a}	7.33±0.47^{a}	0.10±0.06^{b}
B. costatum	100	7.61±0.94^{c}	0.22±0.13^{c}	7.83±1.07^{a}	34.60±2.54^{c}	7.5±0.50^{a}	5.50±0.50^{b}	0.02±0.04^{c}
Diazepam	3	10.33±0.47^{c}	0.34±0.05^{c}	10.67±0.52^{b}	30.38±3.13^{c}	4.33±0.47^{a}	5.33±0.47^{b}	0.02±0.03^{c}
Buspirone	10	8.50±0.95^{c}	0.22±0.01^{c}	8.72±0.96^{a}	38.64±2.24^{c}	4.16±0.68^{a}	4.50±0.50^{b}	0.02±0.03^{c}

Data are means ± SEM. N = 6 per dose. ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001, ANOVA followed by Tukey's post hoc test. DW: distilled water.

Interestingly, like diazepam or buspirone, *Bombax costatum* aqueous extract significantly reduced the number of closed arm entries from 4.41 ± 0.57 in the distilled water-treated group to 0.22 ± 0.13 (p<0.001) which is the 100 mg/kg *Bombax costatum*-treated group, and 0.34 ± 0.05 (p<0.001) or 10.22 ± 0.01 (p<0.001) for 3 mg/kg diazepam or 10 mg/kg buspirone respectively. Additionally, the numbers of rearing, head dipping and faecal boli were reduced by the standard anxiolytic drug diazepam or buspirone and the plant extracts (Table 1).

3.5.2. Effects of Bombax costatum aqueous extract on the percentages of open arm entries and time

The percentages of entries and time spent in the open arms increased from 13.19% and 2.89% in the distilled water-treated group, to 96.81% ($p<0.001$) and 74.61% ($p<0.001$) for the group treated with 3 mg/kg diazepam, to 97.48% ($p<0.001$) and 72.39% ($p<0.001$) for the group treated with 10 mg/kg buspirone, and 97.19% ($p<0.001$) and 70.83% ($P<0.001$) for the group administered 100 mg/kg *Bombax costatum* aqueous extracts, respectively (Figure 1).

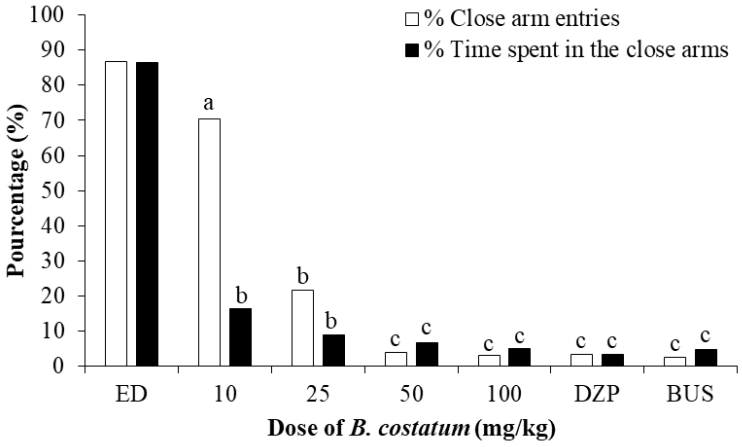


Means + SEM. N = 10 per group. [a]P<0.05, [b]P<0.01, [c]P<0.001 ANOVA followed by Tukey's post hoc test. ED, distilled water; DZP, 3 mg/kg diazepam; BUS, 10 mg/kg buspirone.

Figure 3 Effects of *Bombax costatum* aqueous extract on the percentages of open arm entries and time

3.5.3. Effects of Bombax costatum aqueous extract on the percentage of close arm entries and time

The aqueous extracts of *Bombax costatum* and the positive controls diazepam or buspirone induced a significant reduction in the percentage of entries [$F(6, 35) = 21.14$, $p<0.001$] and time spent in closed arms [$F(6, 35) = 14.27$, $p<0.001$] (Figure 2). Oral administration of *Bombax costatum* aqueous extracts significantly induced a significant reduction in the percentage of entries into closed arms from 86.81% in the distilled water-treated group to 3.82% ($p<0.05$) and 6.56% ($p<0.001$) in the test groups treated with the respective doses of 185 and 370 mg/kg *Dysphania ambrosioides* aqueous extracts.



Means + SEM. N = 10 per group. [a]P<0.05, [b]P<0.01, [c]P<0.001 ANOVA followed by Tukey's post hoc test. ED, distilled water; DZP, 3 mg/kg diazepam; BUS, 10 mg/kg buspirone

Figure 4 Effects of *Bombax costatum* aqueous extract on the percentage of close arm entries and time

3.5.4. Neurochemical effects of Bombax costatum aqueous extract: involvement gamma-aminobutyric acid and gamma-aminobutyric acid transaminase in the elevated plus maze test

Oral administration of *Bombax costatum* aqueous extracts induced one hour later a significant decreased of in the levels of GABA transaminase activity in mice [$F(6, 35) = 13.11$, $p<0.01$] as compared to the normal group (Table 4). *Bombax costatum* aqueous extracts treatment significantly decreased this activity of GABA-transaminase from 386.16 ± 1.85 pg/min/mg of tissue in the distilled water-treated mice to 340.63 ± 6.66 pg/min/mg of tissue ($p<0.001$), and 308.31 ± 0.76 pg/min/mg of tissue ($p<0.001$), respectively in the groups administered *Bombax costatum* 50 and 100 mg/kg, respectively. Diazepam ($p<0.001$) or buspirone ($p<0.001$) also significantly reduced the activity of gamma-aminobutyric acid transaminase.

The results depicted in Table 4 show that *Bombax costatum* aqueous extracts 50 and 100 mg/kg significantly increased the brain GABA concentration from 367.34 ± 5.73 µg/g of tissue to 417.30 ± 1.18 µg/g of tissue ($p<0.05$) and 446.47 ± 2.55µg/g of tissue ($p< 0.05$) respectively, as compared to distilled water-treated mice. Diazepam of buspirone administered at the doses of 3 and 10 mg/kg, respectively exhibited elevated of brain GABA levels ($p<0.001$) as compared to the distilled water-treated mice group.

Table 4 Effect of *Bombax costatum* aqueous extract on brain GABA level and GABA-T activity

Treatment	Dose (mg/kg)	GABA (µg/g)	GABA-T (pg/min/mg)
DW	-	367.34±5.73	386.16±1.85
B. costatum	10	376.70±2.80	380.83±2.42
B. costatum	25	414.20±2.91[b]	303.31±2.72
B. costatum	50	417.30±1.18[b]	340.63±6.66[c]
B. costatum	100	446.47±2.55[c]	308.31±0.76[c]
Diazepam	3	419.04±1.56[c]	275.18±1.51[c]
Buspirone	10	417.72±2.52[c]	261.25±4.35[c]

Values represents mean ± ESM, n = 6, [b]P<0.01, [c]P<0.001, significant difference from the negative control, ANOVA followed by the Turkey test.

4. Discussion

In the present study, we examined the anxiolytic properties of *Bombax costatum* aqueous extracts using different experimental models of anxiety. The stress-induced hyperthermia, hole board, elevated plus maze and open field tests are a well-accepted as experimental animal model typically used to test the effectiveness of anxiolytics drugs [14, 25-27]. The present results indicated that *Bombax costatum* aqueous extracts significantly reduced hyperthermia induced by stress in mice. This reduction is similar to that induced by phenobarbital, suggested the anxiolytic properties of the studied plant extracts, as hyperthermia induced by stress is reversed by anxiolytic drugs [10, 21, 25].

Interestingly, the anxiolytic properties of *Bombax costatum* aqueous extracts were confirmed in the hole board, open field and elevated plus maze tests. In hole-board test, *Bombax costatum* aqueous extracts increased the exploratory behaviour (number of crossing and head-dipping), and decreased the latency to the first head-dips. Since increasing the number of crossing and head-dipping in the hole-board is a sign of the reduction of anxiety in rodent [12, 14], the obtained results also indicated the anxiolytic properties of *Bombax costatum* aqueous extracts could have exerted anxiolytic properties through its flavonoids, alkaloids and tannins contain. For instance, flavonoids selectively bind with high affinity to benzodiazepine receptors and induce a significant anxiolytic activity [21].

Finding of this study in the open field test show that animals treated with graded doses of *Bombax costatum* increased the number of grooming, indicating an anxiolytic-like effect; in addition to the fact that this reduction of an anxiety like behaviour was similar to the one observed following the conventional treatment with diazepam [26, 28]. *Bombax costatum* aqueous extracts and diazepam increased the number of crossing. The increase in the number of crossing (exploratory activity) is a sign of intrinsic inhibition of anxiety induction and not an increase in locomotion since rearing which is a locomotion indicator in this test was reduced [21]. Reduction of faecal boli in mice treated with *Bombax costatum* aqueous extract or diazepam also suggested an anxiolytic activity [10, 14, 21]. As anxiolytic activities could be mediated by different mechanism of action, we hypothesized that the anxiolytic properties of *Bombax costatum* aqueous

extracts in the open field test could be due to the interaction of its contained compounds with receptors in the central nervous system such as benzodiazepine and GABA sites of $GABA_A$ receptor complex as agonists [18, 27, 28].

The elevated plus maze test is based on the observation that the natural behaviour of rodents to display an aversion to novel open spaces and fear of balancing on a relatively narrow, raised platform that can induce anxiety in humans [29]; therefore, avoidance of the open arms is interpreted as an anxiogenic behaviour [28, 30]. The results obtained in the elevated plus maze clearly demonstrated that *Bombax costatum*-treated mice had a strong preference for the open arms compared to those treated with the distilled water. The number of entries and the time spent into open arms and their percentages; the main behavioural parameters evaluated in the elevated plus maze test were significantly increased by the oral administration of *Bombax costatum* aqueous extract. Significant decreases were also recorded in the number of rearing and head dipping associated both to the decrease of closed arms entries and the increase of open arms entries suggested the anxiolytic properties of *Bombax costatum* aqueous extracts [21].

It was found in our study that that *Bombax costatum* aqueous extracts significantly enhanced the brain GABA concentration which again is suggestive of an anxiolytic property of the plant aqueous. Anxiolytics drugs are known to exert their pharmacological action by causing an increase in GABA content in mice brain of animals [31]. Gamma aminobutyric acid (GABA) which is the major inhibitory neurotransmitter of the central nervous system is synthesized at the pre-synaptic neuron by decarboxylation of glutamate, by glutamate decarboxylase [23, 31, 32]. GABA-T is the primary catabolic enzyme in the mammalian brain that catalyses the transfer of amino group from GABA to α-ketoglutarate leading to the depletion in the level of GABA [33]. The involvement of GABA neurotransmission is supported by the inhibition of the activity of GABA-T by *Bombax costatum* aqueous extracts that also explained the increase of brain GABA concentration in administered mice with the different doses of extracts. These results suggest that *Bombax costatum* aqueous extract is able to restore and maintain the balance between neuronal excitation and inhibition through the modulation of GABAergic neurotransmission, and reduce anxiety in mice [21].

5. Conclusion

The present study reveals that *Bombax costatum* aqueous extracts exhibits anxiolytic effects, which validates its folk use in neurological disorders and a step forward toward exploration of evidence-based alternative medicines. Further in-depth advance molecular studies are warranted to elucidate pharmacodynamics basis of the pharmacological actions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest in this study.

Statement of ethical approval

The protocols were performed in concordance with the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee, Yaounde (No. FW-IRB00001954).

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