Behavioural and neurochemical characterisation of the anxiolytic properties of an aqueous extract of *Dysphania ambrosioides* (L.) Mosyakin and Clemants (Chenopodiaceae) in experimental mice

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Abstract

**Ethnopharmacological relevance:** *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Chenopodiaceae) is a medicinal plant known for its anxiolytic, antidepressant and anticonvulsant activities in Cameroonian folk medicine.

**Aim of the study:** The aim of this work is to evaluate the anxiolytic effects of *Dysphania ambrosioides* aqueous extracts and investigate its mechanism of action.

**Materials and methods:** Elevated plus maze test and open field test were used for detecting its anxiolytic properties. The possible mechanism of action of the aqueous extracts were investigated after pretreatment of animals with different antagonists of GABA\(_A\) complex receptors (5 mg/kg N-methyl-\(\beta\)-carboline-3-carboxamide, 4 mg/kg flumazenil or 2 mg/kg bicuculline) 30 minutes prior to the oral administration of 370 mg/kg *Dysphania ambrosioides* aqueous extract.

**Results:** *Dysphania ambrosioides* increased the percentage of entries into and percentage of time in open arms, and reduced rearing, head dipping, and percentage of time in closed arms, in the elevated plus maze. It reduced rearing and defecation, and increased crossing, in the open field. In addition, anxiolytic-like properties of *Dysphania ambrosioides* were blocked by different antagonists of GABA\(_A\) complex receptors (N-methyl-\(\beta\)-carboline-3-carboxamide, flumazenil or bicuculline) as examined in elevated plus maze test. Finally, the activity of GABA-T activity was inhibited and the brain GABA concentration was increased by the extracts, respectively.

**Conclusion:** These results suggest that *Dysphania ambrosioides* possess anxiolytic-like properties in mice that might involve an action on benzodiazepine and/or GABA sites in the GABA\(_A\) receptor complex or by modulating brain GABA concentration in the central nervous system.

**Keywords:** *Dysphania ambrosioides*; Anxiolytic; GABAergic; Brain; Folk medicine
1. Introduction

Behavioural and psychological symptoms of anxiety including agitation, fear, screaming and delusions, occur in about 20-80% of patients with anxiety. It is one of the most frequent and severe mental disorders, with up to 20% of the adult population suffering from these conditions at some time during their life [1]. Despite the availability of current anxiolytic drugs, anxiety disorders and its comorbidities are a global problem. The last decade, anxiety disorders represent one of the biggest challenges for health care systems [2].

The GABAa complex receptors are largely localised in the central nervous system of mammals and represent the most important inhibitory neurotransmitters. After activation of these receptors, they can be potentiated through an allosteric benzodiazepine site and can lead to sedation, muscle relaxant, antagonism of seizure and anxiolytic [3]. However, these effects of benzodiazepine such as diazepam are not exempt to severe memory-impairment representing the limitation in the treatment anxiety in approximatively two-thirds of the anxious patients [3]. Biological abnormalities of anxiolytic disorders are suspected at several levels. A lot of evidences of the central GABAergic system have been demonstrated that certain patients suffering from anxiety were less sedate after administration of curative dose of benzodiazepine, indication a severe deterioration of the efficacy and sensitivity of the central complex GABAa receptors at the benzodiazepine site [4]. However, the improvement level of benzodiazepine receptor agonists is still controverted and disappointing. Then, the medical need for newer, cheaper, better-tolerated and more efficacious anxiolytics remains high, and the use of alternative/complementary medicines to alleviate this disease and its comorbidities are still needed.

*Dysphania ambrosioides* (L.) Mosyakin and Clemants (Chenopodiaceae) is a traditional aromatic herbal medicine used to treat neuropathic pain, inflammatory conditions such as cholecystitis, arthritis, and gastritis as well as neuropsychiatric disorders [5]. The decoction prepared from the aerial part of *Dysphania ambrosioides* have been intensively used by the Cameroonian's traditional healers to treat epilepsy, depression, anxiety, psychoses and infantile convulsions [5-7].

Previous studies performed in our laboratory indicated that the aqueous extracts of *Dysphania ambrosioides* were shown to possess antipyretic and anxiolytic properties, respectively in the stress-induced hypothermia test and the elevated plus maze test [8]; however, there is a substantial lack of mechanistic study of *Dysphania ambrosioides* extracts and the involved signaling pharmacological pathway remains unelucidated. Therefore, the elevated plus maze test was used to examine the possible interactions of *Dysphania ambrosioides* extracts with anxiogenic agents, such as: the partial inverse agonist at the benzodiazepine site of the GABAa receptor complex (N-methyl-$\beta$-carboline-3-carboxamide); the central benzodiazepine receptor antagonist (flumazenil) and the light-sensitive competitive antagonist of GABAa receptors (bicuculline), respectively. In addition, a relationship between the behavioural properties of *Dysphania ambrosioides* extracts and the neurochemical (GABA concentration, GABA transaminase activity) changes of the animals at the end of open field test were examined.

2. Material and methods

2.1. Plant material

The aerial parts of *Dysphania ambrosioides* used for the experiments were harvested between March 2018 and April 2018, in Touboro, area of the North Region of Cameroon. The area of study did not involve endangered or protected species. The collected species was identified by at National Herbarium of Yaoundé (Cameroon), where a voucher was deposited (85040/HNC).

2.2. Preparation of *Dysphania ambrosioides* aqueous extracts

The aerial parts of *Dysphania ambrosioides* was ground, and the obtained powder (100 g) was macerated in 1000 mL of distilled water for 1 hour. The obtained mixture was boiled for 20 minutes duration and the supernatant was filtered using Whatman No 1 filter paper. The resulting aqueous extract (decoction) were then administered orally to mice in a volume of 10 mL/kg. The decoctions of *Dysphania ambrosioides* were prepared daily according to Traditional Healers’s instructions. In another set of experiment the decoction was concentrated using a rotary vacuum evaporator under reduced pressure at 50°C, and from this procedure the yield of extraction (8.7%) was calculated. The stock solution of *Dysphania ambrosioides* extract (decoction, 37 mg/mL), were diluted in distilled water, and three less concentration solutions (3.7, 9.25 and 18.5 mg/mL) were obtained.
2.3. Preliminary phytochemical study
Preliminary qualitative phytochemical screening of *Dysphania ambrosioides* aqueous extract (decoction) were performed as previously described by Taiwe et al. [9]. The following family of compounds were examined: for alkaloids, glycosides, tannins, flavonoids, triterpenoids, anthraquinones, saponins, phenols. A comparative thin layer chromatographic study was made to determine bufadienolides in the decoction of *Dysphania ambrosioides* using anisaldehyde sulphuric acid reagent under UV (254 – 365 nm) [9].

2.4. Chemicals
Diazepam was purchased from Roche, France. N-methyl-β-carboline-3-carboxamide (FG7142), flumazenil (RO151788), and bicuculline and the other reagents used for the quantification of brain γ-amino butyric acid level and γ-aminobutyric transaminase activity were purchased from Sigma Chemical, USA.

2.5. Animals
Adult male Swiss mice weighting 20 – 25 g were obtained from the National Veterinary Laboratory, Garoua, Cameroon, and used throughout these experiments. They were housed in standard plexiglas cages with food and water ad libitum. The animal house was maintained constantly at 25°C on a 12 h light-dark cycle. 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 recommendations provided in the Animal Research: Reporting of In Vivo Experiment (ARRIVE) guidelines published online in PLOS Biology [10], and the general guidelines for experimental research and screening of traditional medicine as promulgated by WHO [11].

2.6. Behavioural testing for the evaluation of anxiolytic properties

2.6.1. Elevated plus maze test
The elevated plus maze 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). The apparatus was elevated above floor level (50 cm). Six groups of six animals each were administered orally with the different doses of *Dysphania ambrosioides* aqueous extracts (37, 92.5, 185 and 370 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 at the centre of the elevated plus maze and their behaviours were recorded for 5 minutes duration [12-14]. 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.

2.6.2. Investigation of possible mechanisms of anxiolytic effects of *Dysphania ambrosioides* aqueous extracts in the elevated plus maze test
To investigate the possible contribution of GABAergic system to the anxiolytic-like effects of *Dysphania ambrosioides* aqueous extract, mice were pretreated intraperitoneally with different antagonists of GABAA complex receptors 30 minutes prior to the oral administration of 370 mg/kg aqueous extract. In each group of animals, mice were injected respectively with: 4 mg/kg flumazenil, competitive antagonist of GABAA complex receptors at the benzodiazepine recognition site, 5 mg/kg N-methyl-β-carboline-3-carboxamide, an inverse partial agonist of GABAA complex receptors at the benzodiazepine recognition site, or 2 mg/kg bicuculline, a light-sensitive competitive antagonist of GABAA complex receptors. Due to implication of GABA complex receptors in anxiety, these GABAergic antagonists were injected intraperitoneally to mice, and 30 min post-treatment, animals were given orally 370 mg/kg *Dysphania ambrosioides* aqueous extract and 1 hour later, animals were subjected to the elevated plus maze test.

2.6.3. Evaluation of locomotion, exploratory behaviour and anxiety in the open field test
The open field used in these experiments consist of a wooden square box (40 × 40 × 45 cm), and the floor of this apparatus was divided into 16 smaller squares (10 × 10 cm) of equal dimensions [15]. Several groups of six mice each were given orally different doses of *Dysphania ambrosioides* aqueous extracts (37, 92.5, 185 and 370 mg/kg; test groups), diazepam (0.3 mg/kg; positive control group) or distilled water (10 mL/kg; normal group). One-hour post-treatment animals were placed individually in the centre of the open field, and they could explore their experimental environment for 5 minutes duration. The number of crossing (number of square floor units entered), grooming, rearing (number of times the animal stood on its hind legs) and the weight of faecal boli (defecation) were recorded for each
animal [14]. At the end of behavioural evaluations, all the animals were euthanized by inhalation of high concentration of compressed carbon dioxide (CO\textsubscript{2}) gas in cylinders, and the whole brain was collected for biochemical analyses.

2.6.4. Biocchemical estimation of GABA concentration and GABA-transaminase activity after the open field test

Concentration of GABA in the brain homogenate was quantified as described previously by Lowe et al. with slight modification [16]. This concentration was expressed in µg/g of wet brain tissue [18].

The brains were removed and submerged in ice-cold artificial cerebrospinal fluid. Briefly, the brain tissue of each mouse was 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 (Nayak and Chatterjee, 2001). Finally, GABA-T activity was quantified in the brain homogenates spectrophotometrically as described previously [18, 19] and modified by Taiwe et al. [20].

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 animal. 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 Dysphania ambrosioides aqueous extracts

The preliminary phytochemical studies demonstrated that the Dysphania ambrosioides aqueous extract contained: alkaloids, glycosides, tannins, flavonoids, triterpenoids, anthraquinones, saponins, phenols. Thin layer Chromatography of Dysphania ambrosioides aqueous extract indicated that bufadienolides are absent.

3.2. Effects of Dysphania ambrosioides aqueous extracts on anxiety-like behaviours in the elevated plus maze test

3.2.1. Effects of Dysphania ambrosioides aqueous extracts on the number of open arm entries, close arm entries, total arm entries, rearing, head dipping and faecal boli

Administration of Dysphania ambrosioides aqueous extracts to mice significantly increased the number of entries in the open arms \( F(5, 28) = 122.11, p<0.001 \), the number of total arm entries \( F(5, 25) = 144.32, p<0.01 \) and the ratio OE/TE versus CE/TE \( F(5, 24) = 101.47, p<0.001 \) in the group treated with the extract at a dose of 370 mg/kg, respectively. Interestingly, Dysphania ambrosioides extracts administered at a dose of 370 mg/kg and diazepam 3 mg/kg significantly increased the number of open arm entries from 0.67 ± 0.44 in the distilled water-treated group to 5.67 ± 1.33 (p<0.001) and 5.33 ± 0.78 (p<0.001), respectively (Table 1). In the results of post hoc analysis, it was also found that, like diazepam, Dysphania ambrosioides extract significantly reduced the number of closed arm entries from 5.00 ± 2.56 in the distilled water-treated group to 1.17 ± 0.28 (p<0.001) which is the 370 mg/kg Dysphania ambrosioides-treated group, and 1.16 ± 0.23 (p<0.001) for 3 mg/kg diazepam, respectively. More so, the numbers of rearing, head dipping and faecal boli were reduced by both diazepam and Dysphania ambrosioides (Table 1).

3.2.2. Effects of Dysphania ambrosioides aqueous extracts on the percentages of open arm entries and time

The percentages of entries and time spent in the open arms increased from 11.76% and 2.89% in the distilled water-treated group, to 82.05% (p<0.001) and 73.72% (p<0.001) for the group treated with 3 mg/kg diazepam, and 82.92% (p<0.001) and 6.78% (P<0.001) for the group administered 370 mg/kg Dysphania ambrosioides aqueous extracts, respectively (Figure 1). Like diazepam, Dysphania ambrosioides aqueous extracts induced a significant increase in these percentages.
Figure 1 Effect of *Dysphania ambrosioides* aqueous extracts on mice placed in the elevated plus maze: percentage of open arm entries and time in open arms.

Shown are the percentage of open arm entries/total arm entries and the percentage of open arm time/session time (5 minutes), for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey’s (HSD) multiple comparison test, *a*p<0.05, *b*p<0.01, *c*p<0.001, significantly different compared to the significantly different compared to the negative control group. DW, distilled water; Da37, 37 mg/kg *Dysphania ambrosioides* aqueous extracts; DZP, 3 mg/kg diazepam.

3.2.3. Effects of *Dysphania ambrosioides* aqueous extracts on the percentage of close arm entries and time

Interestingly, like diazepam, *Dysphania ambrosioides* aqueous extracts significantly induced a significant reduction in the percentage of entries into closed arms from 88.23% in the distilled water-treated group to 30.55% (*p*<0.05) and 17.07% (*p*<0.001) in the test groups treated with the respective doses of 185 and 370 mg/kg *Dysphania ambrosioides* aqueous extracts. The aqueous extracts of *Dysphania ambrosioides* and the positive control diazepam induced a significant reduction in the percentage of time spent in closed arms [F(5, 23) = 92.51, *p*<0.01] (Figure 2).

Figure 2 Effects of *Dysphania ambrosioides* aqueous extracts on mice placed in the elevated plus maze: percentage of close arm entries and time in close arms.

Shown are the percentage of open arm entries/total arm entries and the percentage of open arm time/session time (5 minutes), for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey’s (HSD) multiple comparison test, *a*p<0.05, significantly different compared to the significantly different compared to the negative control group. DW, distilled water; Da37, 37 mg/kg *Dysphania ambrosioides* aqueous extracts; DZP, 3 mg/kg diazepam.
Table 1 Effects of *Dysphania ambrosioides* aqueous extracts on the number of open arm entries, close arm entries, total arm entries, rearing, head dipping and faecal boli, the number of open arm entries, close arm entries, total arm entries, rearing, head dipping and faecal boli in mice placed in the elevated plus maze.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Doses (mg/kg)</th>
<th>Open arm entries</th>
<th>Close arm entries</th>
<th>Total arm entries</th>
<th>Ratio OE/TE vs CE/TE</th>
<th>Rearing</th>
<th>Head dipping</th>
<th>Faecal boli</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>--</td>
<td>0.67 ± 0.44</td>
<td>5.00 ± 2.56</td>
<td>3.83 ± 0.56</td>
<td>13.33 ± 2.23</td>
<td>13.33 ± 0.57</td>
<td>4.67 ± 0.38</td>
<td>1.16 ± 0.28</td>
</tr>
<tr>
<td>Da37</td>
<td>37</td>
<td>1.83 ± 0.28</td>
<td>1.67 ± 0.67c</td>
<td>4.17 ± 0.83</td>
<td>110.00 ± 11.81c</td>
<td>11.50 ± 1.29</td>
<td>3.50 ± 0.83</td>
<td>0.11 ± 0.04c</td>
</tr>
<tr>
<td>Da92.5</td>
<td>92.5</td>
<td>2.67 ± 0.67b</td>
<td>2.17 ± 0.56b</td>
<td>4.83 ± 0.56</td>
<td>123.08 ± 37.34c</td>
<td>10.00 ± 0.57</td>
<td>2.33 ± 0.56b</td>
<td>0.05 ± 0.06c</td>
</tr>
<tr>
<td>Da185</td>
<td>185</td>
<td>4.17 ± 0.56c</td>
<td>1.83 ± 0.89b</td>
<td>6.00 ± 1.33b</td>
<td>227.27 ± 19.62c</td>
<td>5.00 ± 1.43c</td>
<td>1.67 ± 0.78c</td>
<td>0.04 ± 0.06c</td>
</tr>
<tr>
<td>Da370</td>
<td>370</td>
<td>5.67 ± 1.33c</td>
<td>1.17 ± 0.28c</td>
<td>6.83 ± 1.50b</td>
<td>485.71 ± 28.79c</td>
<td>2.17 ± 0.48c</td>
<td>1.83 ± 0.28c</td>
<td>0.02 ± 0.04c</td>
</tr>
<tr>
<td>DZP</td>
<td>3</td>
<td>5.33 ± 0.78c</td>
<td>1.16 ± 0.23c</td>
<td>6.50 ± 1.00b</td>
<td>457.14 ± 24.15c</td>
<td>1.83 ± 0.71c</td>
<td>0.33 ± 0.44c</td>
<td>0.02 ± 0.03c</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey’s (HSD) multiple comparison test, \( P<0.05 \), \( P<0.01 \), \( P<0.001 \), significantly different compared to distilled water; Da37, 37 mg/kg *Dysphania ambrosioides* aqueous extracts; DZP, 3 mg/kg diazepam; OE, open entries; TE: total entries; CE, close entries.

Table 2 Effects of pretreatment with flumazenil, N-methyl-β-carboline-3-carboxamide, or bicuculline on behavioural ameliorations induced by *Dysphania ambrosioides* aqueous extract in the elevated plus maze test.

<table>
<thead>
<tr>
<th>Treatments</th>
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<th>Head dipping</th>
<th>Faecal boli</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>--</td>
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<td>3.83 ± 0.56</td>
<td>13.33 ± 2.23</td>
<td>13.33 ± 0.57</td>
<td>4.67 ± 0.38</td>
<td>1.16 ± 0.28</td>
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<tr>
<td>Da370</td>
<td>370</td>
<td>5.67 ± 1.33c</td>
<td>1.17 ± 0.28c</td>
<td>6.83 ± 1.50a</td>
<td>485.71 ± 28.79c</td>
<td>2.17 ± 0.48c</td>
<td>1.83 ± 0.28c</td>
<td>0.02 ± 0.04a</td>
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<tr>
<td>Da370+Flu</td>
<td>370 + 4</td>
<td>1.83 ± 0.56b</td>
<td>4.17 ± 1.17f</td>
<td>4.00 ± 0.67a</td>
<td>44.00 ± 3.42v</td>
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<td>3.17 ± 0.56a</td>
<td>1.10 ± 0.21a</td>
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<td>370 + 5</td>
<td>2.17 ± 0.56a</td>
<td>4.17 ± 0.89f</td>
<td>3.82 ± 0.89a</td>
<td>52.00 ± 2.58v</td>
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<td>2.83 ± 0.83a</td>
<td>1.12 ± 0.19a</td>
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<td>Da370+Bic</td>
<td>370 + 2</td>
<td>1.83 ± 0.56b</td>
<td>3.33 ± 0.78a</td>
<td>4.00 ± 0.67a</td>
<td>55.00 ± 3.01v</td>
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<td>2.83 ± 0.61a</td>
<td>1.15 ± 0.23a</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey’s (HSD) multiple comparison test, \( P<0.05 \), \( P<0.01 \), \( P<0.001 \), significantly different compared to distilled water-treated group, \( P<0.05 \), \( P<0.01 \), \( P<0.001 \), significantly different compared to 370 mg/kg *Dysphania ambrosioides* aqueous extract-treated group. DW, distilled water; Da370, 370 mg/kg *Dysphania ambrosioides* aqueous extracts; DZP, 3 mg/kg diazepam; Flu, 4 mg/kg flumazenil; Bet, 5 mg/kg N-methyl-β-carboline-3-carboxamide; Bic, 2 mg/kg bicuculline.
3.3. Participation of GABAergic pathway in anxiolytic-like effect of *Dysphania ambrosioides* aqueous extracts

3.3.1. Effects of pretreatment with flumazenil, N-methyl-β-carboline-3-carboxamide, or bicuculline on behavioural ameliorations induced by *Dysphania ambrosioides* aqueous extract in the elevated plus maze test

The anxiolytic-like effects of *Dysphania ambrosioides* aqueous extract administered at a dose of 370 mg/kg was blocked by 4 mg/kg flumazenil, 5 mg/kg N-methyl-β-carboline-3-carboxamide, or 2 mg/kg bicuculline pretreatment as shown in Table 2. Two-way ANOVA revealed a main effect of 370 mg/kg *Dysphania ambrosioides* aqueous extract treatment [F(4, 18) = 78.12, p<0.001], and 4 mg/kg flumazenil pretreatment × 370 mg/kg *Dysphania ambrosioides* aqueous extract treatment interaction [F(4, 18) = 86.31, p<0.01] for the number of open arm entries in the elevated plus maze (Table 2). Interestingly, Table 2 shows that the pretreatment of mice with N-methyl-β-carboline-3-carboxamide, administered intraperitoneally, blocked the increase in the number of open arm entries induced by *Dysphania ambrosioides* aqueous extract in the elevated plus maze test. Two-way ANOVA revealed significant differences of 370 mg/kg *Dysphania ambrosioides* aqueous extract treatment [F(4, 18) = 78.12, p<0.001], and 5 mg/kg N-methyl-β-carboline-3-carboxamide pretreatment × 370 mg/kg *Dysphania ambrosioides* aqueous extract treatment interaction [F(4, 18) = 86.75, p<0.05]. In addition, as shown in Table 2, the two-way ANOVA revealed a main effect of 370 mg/kg *Dysphania ambrosioides* aqueous extract treatment [F(4, 18) = 78.12, p<0.001], and 2 mg/kg bicuculline pretreatment × 370 mg/kg *Dysphania ambrosioides* aqueous extract treatment interaction [F(4, 16) = 49.13, p<0.01] for the number of open arm entries (Table 2).

3.3.2. Effects of pretreatment with flumazenil, N-methyl-β-carboline-3-carboxamide, or bicuculline on ameliorations in the percentages of open arms entries and time-induced by *Dysphania ambrosioides* aqueous extract in the elevated plus maze test

The increase in the percentage of open arm entries and the percentage of open arm entries and time induced by the oral administration of 370 mg/kg *Dysphania ambrosioides* aqueous extract administered at a dose of 370 mg/kg was blocked by 4 mg/kg flumazenil, 5 mg/kg N-methyl-β-carboline-3-carboxamide, or 2 mg/kg bicuculline pretreatment as shown in Figure 3.

![Figure 3](image)

**Figure 3** Effects of pretreatment with flumazenil, N-methyl-β-carboline-3-carboxamide, or bicuculline on ameliorations in the percentages of open arms entries and time-induced by *Dysphania ambrosioides* aqueous extract in the elevated plus maze

Shown are the percentage of open arm entries/total arm entries and the percentage of open arm time/session time (5 minutes), for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey’s (HSD) multiple comparison test, ***P<0.001, significantly different compared to distilled water-treated group, *P<0.05, ##P<0.01, significantly different compared to 370 mg/kg *Dysphania ambrosioides* aqueous extract-treated group. DW, distilled water; Da370, 370 mg/kg *Dysphania ambrosioides* aqueous extracts; DZP, 3 mg/kg diazepam; Flu, 4 mg/kg flumazenil; Bet, 5 mg/kg N-methyl-β-carboline-3-carboxamide; Bic, 2 mg/kg bicuculline.

3.3.3. Effects of pretreatment with flumazenil, N-methyl-β-carboline-3-carboxamide, or bicuculline on ameliorations in the percentages of close arms entries and time-induced by *Dysphania ambrosioides* aqueous extract in the elevated plus maze test

The decrease in the percentage of close arms entries and time induced by the oral administration of 370 mg/kg *Dysphania ambrosioides* aqueous extract administered at a dose of 370 mg/kg was antagonised by 4 mg/kg flumazenil, 5 mg/kg N-methyl-β-carboline-3-carboxamide, or 2 mg/kg bicuculline pretreatment as shown in Figure 4.
Figure 4 Effects of pretreatment with flumazenil, N-methyl-β-carboline-3-carboxamide, or bicuculline on ameliorations in the percentages of close arms entries and time-induced by Dysphania ambrosioides aqueous extract in the elevated plus maze

Shown are the percentage of open arm entries/total arm entries and the percentage of open arm time/session time (5 minutes), for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey’s (HSD) multiple comparison test, ***P<0.001, significantly different compared to distilled water-treated group, #P<0.05, ##P<0.01, significantly different compared to 370 mg/kg Dysphania ambrosioides aqueous extract-treated group. DW, distilled water; Da370, 370 mg/kg Dysphania ambrosioides aqueous extracts; DZP, 3 mg/kg diazepam; Flu, 4 mg/kg flumazenil; Bet, 5 mg/kg N-methyl-β-carboline-3-carboxamide; Bic, 2 mg/kg bicuculline.

3.4. Effects of Dysphania ambrosioides aqueous extracts on exploratory behaviour and locomotion in the open field test

As shown in Table 3, oral administration of Dysphania ambrosioides aqueous extracts produced a significant effect on mice spontaneous locomotor activities. One way ANOVA revealed a significant changes in the number of rearing [F(5, 25) = 7.45, p<0.001], crossing [F(4, 25) = 34.17, P<0.05], grooming [F(5, 25) = 5.43, P<0.01], quantity of faecal boli [F(5, 25) = 2.43, P<0.05] and the centre time [F(4,25) = 12.73, P<0.001] the of the animals in the open field test. Similarly, diazepam administration significantly ameliorates the exploratory activity of the animals in the open field test. Like in the elevated plus maze test, the number of rearing was decreased (p<0.001) respectively by both 370 mg/kg Dysphania ambrosioides or 0.3 mg/kg diazepam. They also significantly decreased the mass of faecal boli (p<0.001). Controversially, 370 mg/kg Dysphania ambrosioides aqueous extracts increased the number of crossing (p<0.001), grooming (p<0.001), and the time spent by animals in the centre (p<0.001) (Table 2).

Table 3 Effects of Dysphania ambrosioides aqueous extracts on the number of rearing, crossing, grooming, the centre time and the quantity of faecal boli in the open field test.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Doses (mg/kg)</th>
<th>Rearing</th>
<th>Crossing</th>
<th>Grooming</th>
<th>Faecal boli</th>
<th>Centre time</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>--</td>
<td>10.33 ± 2.00</td>
<td>13.83 ± 4.83</td>
<td>2.17 ± 0.56</td>
<td>0.40 ± 0.28</td>
<td>2.17 ± 0.83</td>
</tr>
<tr>
<td>Da37</td>
<td>37</td>
<td>5.33 ± 1.67b</td>
<td>16.17 ± 3.11</td>
<td>4.33 ± 0.44a</td>
<td>0.14 ± 0.03</td>
<td>9.33 ± 1.11c</td>
</tr>
<tr>
<td>Da92.5</td>
<td>92.5</td>
<td>4.17 ± 0.56c</td>
<td>16.83 ± 2.17</td>
<td>4.67 ± 1.00a</td>
<td>0.10 ± 0.07a</td>
<td>14.67 ± 2.33c</td>
</tr>
<tr>
<td>Da185</td>
<td>185</td>
<td>2.33 ± 0.78c</td>
<td>19.33 ± 2.33a</td>
<td>5.17 ± 0.56b</td>
<td>0.06 ± 0.07c</td>
<td>26.83 ± 1.89c</td>
</tr>
<tr>
<td>Da370</td>
<td>370</td>
<td>2.17 ± 0.56c</td>
<td>20.67 ± 3.11a</td>
<td>5.33 ± 0.67b</td>
<td>0.05 ± 0.05c</td>
<td>29.17 ± 1.56c</td>
</tr>
<tr>
<td>DZP</td>
<td>3</td>
<td>1.33 ± 0.44c</td>
<td>22.83 ± 6.17a</td>
<td>5.17 ± 0.56b</td>
<td>0.03 ± 0.04c</td>
<td>37.33 ± 4.11c</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey’s (HSD) multiple comparison test, bP<0.01, cP<0.001, significantly different compared to the significantly different compared to the negative control group. DW, distilled water; Da37, 37 mg/kg Dysphania ambrosioides aqueous extracts; DZP, 0.3 mg/kg diazepam.
3.5. Effects of *Dysphania ambrosioides* aqueous extracts on the activity of gamma-aminobutyric acid transaminase (GABA-T) and the level of gamma-aminobutyric acid (GABA) after the open field test

There was a remarkable variation in the levels of GABA transaminase activity in *Dysphania ambrosioides* aqueous extracts-treated mice \([F(5, 32) = 18.71, p<0.01]\) as compared to the normal group (Table 3). *Dysphania ambrosioides* aqueous extracts treatment significantly decreased this activity of GABA-transaminase from 50.29 ± 5.47 pg/min/mg of tissue in the distilled water-treated mice to 29.85 ± 2.28 pg/min/mg of tissue \((p<0.05)\), and 23.89 ± 5.12 pg/min/mg of tissue \((p<0.05)\), respectively in the groups administered *Dysphania ambrosioides* 187 and 370 mg/kg, respectively. Diazepam also significantly reduced the activity of gamma-aminobutyric acid transaminase \((p<0.05)\). The results depicted in Figure 5 show that the oral administration of *Dysphania ambrosioides* aqueous extracts at the doses of 187 and 370 mg/kg significantly increased the brain GABA concentration from 377.19 ± 11.38 μg/g of tissue to 440.62 ± 5.64 μg/g of tissue \((p<0.05)\) and 473.05 ± 11.28 μg/g of tissue \((p<0.05)\) respectively, as compared to distilled water-treated mice. Sodium valproate administered at a dose of 0.3 mg/kg exhibited elevated of brain GABA levels \((p<0.05)\) as compared to the normal group.

![Figure 5](image-url)  
*Figure 5* Effects of *Dysphania ambrosioides* aqueous extracts on the activity of gamma-aminobutyric acid transaminase (a) and the level of gamma-aminobutyric acid (b)

Results are expressed as mean ± S.E.M. for 6 animals. Data were analysed by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, \(^*p<0.05\), significantly different compared to distilled water-treated group. DW, distilled water; Da370, 370 mg/kg *Dysphania ambrosioides* aqueous extracts; DZP, 0.3 mg/kg diazepam.
4. Discussion

One of the most popular behavioural tests for research on anxiety and frequently used mouse models of anxiety is the elevated plus maze [13]. The measures of anxiety are the number of open-arm entries and the number of closed-arm entries expressed as a percentage of the total number of arm entries and the amount of time spent on the open arms [21, 22]. The elevated plus maze test was used in the present study to determine the effect of pretreatment with aqueous extracts of Dysphania ambrosioides on anxiety-related parameters. The elevated plus maze was based on the natural aversion of rodents to open spaces. The elevated plus maze test is the most popular method for measuring anxiety, as it is based on the natural aversion of rodents to open spaces. The elevated plus maze test is based on the natural aversion of rodents to open spaces. The elevated plus maze test is based on the natural aversion of rodents to open spaces. The elevated plus maze test is based on the natural aversion of rodents to open spaces.

In the elevated plus maze test, the total number of arm entries and the percentage of time spent in the open arms were significantly increased by the aqueous extracts of Dysphania ambrosioides. This suggests that the aqueous extract of Dysphania ambrosioides has anxiolytic properties. The increase in the number of open-arm entries and time spent in the open arms reflects a reduction in stress [8, 14, 24]. This suggests that Dysphania ambrosioides aqueous extracts have anxiolytic properties. The decrease in the number of rearing and head dipping in the maze also indicates a decrease in anxiety [14, 25].

The relative contribution of GABA complex receptors to the anxiolytic-like effects of Dysphania ambrosioides aqueous extracts was investigated through pretreatment of mice with antagonists of GABA_A complex receptors (N-methyl-β-carboline-3-carboxamide, flumazenil and bicuculline) before oral administration of the aqueous extract. The significant reduction in the number of entries in the open arms of the treated group with the respective antagonist of GABA_A complex receptors, N-methyl-β-carboline-3-carboxamide, flumazenil or bicuculline and the aqueous extract at a dose of 370 mg/kg, indicated the participation of the GABAergic neurotransmission in the anxiolytic effects of Dysphania ambrosioides aqueous extracts. Results obtained from this study showed that the pretreatment of mice with the respective antagonist of GABA_A complex receptors abolished the anxiolytic effects of Dysphania ambrosioides. These results indicate that the effects are mainly mediated via the GABAergic system [17, 26].

In the open field paradigm, Administration of Dysphania ambrosioides aqueous extracts increased the number of crossings, the number of grooming, and the time spent in the centre of the open field. These modifications suggest the increase of locomotor and explorative activities in mice [8, 23]. This is can be explain by the fact that, the total and closed arms entries and rearing, in the elevated plus maze test, and rearing in the open field test, respectively were significantly reduced by Dysphania ambrosioides, and justify the increase of exploratory behaviour and the reduction of anxiety in mice [14]. More so, the decrease in faecal boli produced by the aqueous extracts-treated animals suggests the reduction of stress in the open field test and the presence of anxiolytic effects [25].

More so, the level Gamma aminobutyric acid (GABA) which is the major inhibitory neurotransmitter of the central nervous system was determined. GABA is synthesized at the pre-synaptic neuron by decarboxylation of glutamate, by glutamate decarboxylase [27]. Anxiolytics (e.g. diazepam) are known to exert their pharmacological action by causing an increase in GABA content in mice brain of animals [28, 29]. It was found that Dysphania ambrosioides significantly enhanced the brain GABA concentration which again is suggestive of an anxiolytic property of the plant. The involvement of GABA neurotransmission is supported by the inhibition of the activity of GABA-T by the aqueous extract of Dysphania ambrosioides that also explained the increase of brain GABA concentration in pretreated mice with the aqueous extract. 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 [30]. These results suggest that Dysphania ambrosioides is able to restore and maintain the balance between neuronal excitation and inhibition and thus have anxiolytic activities through the modulation of GABAergic neurotransmission.

5. Conclusion

In conclusion, our results provide the evidence that Dysphania ambrosioides aqueous extract exerts anxiolytic property in mice. It also increased the brain GABA concentration and attenuated the activity of GABA-transaminase. These findings demonstrate that Dysphania ambrosioides has anxiolytic properties that might involve an action on benzodiazepine and/or GABA sites in the GABA_A receptors complex, or through the modulation of the GABA concentration.
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

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, Yaoundé (No. FW-IRB00001954).

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