Pharmacological evaluation of the anxiolytic-like effects of an aqueous extract of the *Raphanus sativus* L. sprouts in mice

Laura Yunuen Hernández-Sánchez\(^{a,b,1,2}\), María Eva González-Trujano\(^{b,*,3}\), Diego A. Moreno\(^{c,*,4}\), Heike Vibrans\(^{a,5}\), Israel Castillo-Juárez\(^{2,6}\), Alejandro Dorazco-González\(^{d,7}\), Marcos Soto-Hernández\(^{8,8}\)

\(^a\) Posgrado en Botánica, Colegio de Postgraduados Campus Montecillo, Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco 56220, Estado de México, Mexico

\(^b\) Laboratorio de Neurofarmacología de Productos Naturales, Instituto Nacional de Psiquiatría Ramón de la Fuente Muniz, Calz México-Xochimilco 101, Colonia, Huixquilucan, Tlalpan 14370, Ciudad de México, Mexico

\(^c\) Grupo Laboratorio de Fitoquímica y Alimentos Saludables (LabFAS), Departamento de Ciencia y Tecnología de Alimentos, CEBAS, CSIC Campus Universitario de Espinardo, 25. Espinardo, Murcia E-30100, Spain

\(^d\) Departamento de Química Inorgánica, Instituto de Química, Circuito exterior s/n, Ciudad Universitaria, Coyoacán 04510, Ciudad de México, Mexico

**Corresponding author.**

**E-mail addresses:** lauyunuen41@gmail.com (L.Y. Hernández-Sánchez), evag@imp.edu.mx, evagontru@yahoo.com.mx (M.E. González-Trujano), dmoreno@cebas.csic.es (D.A. Moreno), heike@colpos.mx (H. Vibrans), israel.castillo@colpos.mx (I. Castillo-Juárez), adg@unam.mx (A. Dorazco-González), msoto@colpos.mx (M. Soto-Hernández).

\(^1\) Taken in part from the PhD research of Laura Yunuen Hernández-Sánchez

\(^2\) ORCID: 0000-0003-4823-5928

\(^3\) ORCID: 0000-0002-1508

\(^4\) ORCID: 0000-0002-6547-8764

\(^5\) ORCID: 0000-0002-1800-4320

\(^6\) ORCID: 0000-0001-6983-5565

\(^7\) ORCID: 0000-0002-7056-4237

\(^8\) ORCID: 0000-0001-8577-7991

---

**ARTICLE INFO**

**Keywords:** Anxiety

**Brassicaceae**

**Glucosinolates**

**Mexican traditional medicine**

**Sulforaphane**

---

**ABSTRACT**

*Raphanus sativus* L. (Brassicaceae), commonly known as radish, is consumed worldwide as a vegetable. However, its benefits on mental health are unknown. The aim of this study was to evaluate its anxiolytic-like effects and safety using different experimental models. An aqueous extract of *R. sativus* sprouts (AERSs) was pharmacologically evaluated by intraperitoneal route (i.p.) at 10, 30, and 100 mg/kg and orally (p.o.) at 500 mg/kg on behavior by using open-field and plus-maze tests. In addition, its acute toxicity (LD\(_{50}\)) was determined by the Lorke’s method. Diazepam (1 mg/kg, i.p.) and buspirone (4 mg/kg, i.p.) were the reference drugs. A significant and anxiolytic-like dosage of AERSs (30 mg/kg, i.p.) resembling the effects of reference drugs was chosen to explore the involvement of GABA\(_A\)/BDZs site (flumazenil, 5 mg/kg, i.p.) and serotonin 5-HT\(_1A\) receptors (WAY100635, 1 mg/kg, i.p.) as a possible mechanism of action. A 500 mg/kg, p.o. dosage of AERSs produced an anxiolytic-like response equivalent to 100 mg/kg, i.p. No acute toxicity was observed since a LD\(_{50}\) > 2000 mg/kg, i.p. The phytochemical analysis allowed the identification and quantification of major presence of sulforaphene (2500 µM), sulforaphane (15 µM), Iberin (0.75 µM), and indol-3-carbinol (0.75 µM), as major constituents. Both the GABA\(_A\)/BDZs site and serotonin 5-HT\(_1A\) receptors were involved in the anxiolytic-like activity of AERSs, depending on the pharmacological parameter or the experimental assay tested. Our results demonstrate that the anxiolytic activity of *R. sativus* sprouts involves GABA\(_A\)/BDZs site and serotonin 5-HT\(_1A\) receptors supporting its health benefits in the treatment of anxiety beyond the satisfaction of basic nutritional needs.

---


Received 24 January 2023; Received in revised form 17 March 2023; Accepted 21 March 2023

Available online 28 March 2023

© 2023 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Diseases of the central nervous system (CNS) are a primary global health concern, with an increased incidence of psychiatric disorders such as anxiety. Anxiety is a normal emotional state in certain situations to increase the appropriate response capacity of the human being. However, when it becomes a constant and long-term condition (at least six months), it is considered pathological. It may cause significant discomfort and misaligned in the presence of physical, psychological, and behavioral patterns, which in turn generate an economic and social burden [1].

Current pharmacological therapeutic options are not entirely effective and are accompanied by several adverse side effects. With medications such as selective serotonin (SSRIs) or serotonin-norepinephrine (SNRIs) reuptake inhibitors, patients may exhibit symptoms such as nausea, diarrhea, headache, insomnia, nervousness, restlessness, and decreased sexual interest [2]. Benzodiazepines (BDZs) are useful for acute events but are not suitable for long-term administration as they are associated with physiological dependence, psychomotor deterioration, and rebound anxiety after withdrawal [3]. The group of azapirones is better tolerated and without dependence problems, but generally, the latency for the anxiolytic response is 2 or 4 weeks, causing patients to discontinue treatment [2]. The search for new therapies has become necessary, and the use of plants not only as nutritious food but also as medicinal or functional food is a suitable option and a source of potential therapy for chronic CNS diseases [4].

*Raphanus sativus* L. (Brassicaceae), commonly known as radish [5], is a widely used and highly diverse vegetable, probably domesticated several times in Europe and Asia. The main variety groups are those of the Mediterranean and Eastern and Southern Asia. Roots, hypocotyls, stems, leaves, and sprouts have been the target of divergent selection and their consumption has increased worldwide [6–9]. The plant was introduced to Mexico by the Spaniards, and was adopted by various local populations, integrating it into the concept of “*quelite*”, which are edible tender vegetables. It is consumed as a root/hypocotyl vegetable, and for its edible leaves, as well as sprouts that can be produced by hydroponic strategy by enriching bioactive compounds through the application of stress conditions in order to achieve a natural food with added nutritional value to exert more efficient physiological action on the consumer’s health [10]. Local populations also use the plant for its medicinal properties [11], as in other regions of the world, for example, in China, Japan, India, Israel, and Greece. It is used as an antiinflammatory, antitussive, anticancer, anti-inflammatory, antioxidative, as a treatment for anemia, and in dysfunctions of both the female and male reproductive system [12]. Its efficacy as an antihypertensive, anti-lipase, against insulin resistance, gastroprotective, antitussive [13], and neuroprotective agent has also been described [14,15].

Currently, there is considerable interest in studies focused on germinated seeds (sprouts) due to health benefits that may counteract chronic noncommunicable diseases [11,16] better than other parts of the plant [17,18]. Moreover, germination is a simple process with a fast and high-yielding production cycle. Sprouts often contain numerous plant-produced nutrients, including high concentrations of antioxidants [19,20]. Radish sprouts are also rich in bioactive compounds such as glucosinolates and their cognate bioactive isothiocyanates including sulforaphene (from parental glucoraphenone) and other phytochemicals [21].

In a preliminary study using a hydroalcoholic extract (250 and 500 mg/kg) of pods by oral administration, *Raphanus sativus* var. caudatus was shown to have anxiolytic-like effect in mice [22]. However, to our knowledge, there are no preclinical reports of the CNS activity of radish sprouts. Thus, we investigated anxiolytic-like effects of an aqueous extract of the *R. sativus* sprouts using a parental and enteral administration route in murine anxiety models. The main components and a possible mechanism of action were also explored. The results reinforce the use of these plant food extracts as health promoters for managing mental conditions.

2. Materials and methods

2.1. Plant material

Red radish for seed sprouts (*Raphanus sativus* cv. Sango), certified untreated, were obtained from Intersemillas, S.A. (Valencia, Spain). The seeds were decontaminated using 0.5% sodium hypochlorite for 30 min, then left under aeration overnight with 0.5% sodium hypochlorite for inhibition and germination. Then, they were sown on a layer of cellulose (CN Seeds, U.K.) in 30 × 25 cm trays and kept under dark conditions for 48 h in a growth chamber. After this point, the sprouts were allowed to grow until day 8 (PAR radiation 200 μmol/m²/s; light/dark cycle 16 h/8 h, air temperature 22°C/18°C, relative humidity 60%/80%). After eight days, the sprouts were weighed, frozen in liquid nitrogen, and freeze-dried to prepare a fine powder.

2.2. Extract preparation

An aqueous extract was prepared from the sprouts powder by constant agitation in distilled water at a 1:15 p/v ratio at 150 rpm for two hours and then filtered, eliminating the excess water by lyophilization.

2.3. Phytochemical analysis

The red radish extract was qualitatively filtered, collected, and freeze-dried to store the material until experiments with animal models. To quantify the isothiocyanates and indoles present in the extract, we followed the protocol as previously detailed [10] by using a Ultra-High Performance Liquid Chromatograph (UHPLC) coupled with a 6460-triple quadrupole-MS/MS (Agilent Technologies, Waldbronn, Germany) and a Zorbax Eclipse Plus C18 column (2.1 ×50 mm, 1.8 μm). Sulfurophane (SFN), indole-3-carbinol (13C), and 3,3-diiodolymethane (DIM) from Santa Cruz Biotech (California, USA) were used as standards.

2.4. Pharmacological evaluation

2.4.1. Reagents and drugs

Diazepam (DZP) (Psicopharma®, Mexico), busiprone, (±)–8-Hydroxy-2-(dipropylamino) tetralin hydrobromide (8-OH-DPAT), Fluazenil (FMZ), and WAY100635 (WAY) were purchased from Sigma-Aldrich, (St. Louis, MO). Drugs were freshly prepared on the day of the experiments and administered by intraperitoneal (i.p.) or esophageal (p.o.) route using a volume of 1 mL/100 g body weight. Busipron (BUS), 8-OH-DPAT, FMZ, and WAY were dissolved in saline solution (s.s., 0.9% NaCl), while DZP was dissolved in tween 80 (0.2% in s.s). The aqueous extract was suspended in the vehicle (distilled water).

2.4.2. Animals

Swiss Webster mice of 25–30 g body weight were placed in groups of 6 animals in acrylic boxes maintained with unrestricted food and water, at a controlled temperature of 22 ± 2°C with a 12 h light/dark cycle. All experiments followed the recommendations of the bioethical and scientific research committees, the protocols CONBIOETICA-09-CEI-010–20170316 (in March 2017) and INP-NC123280.0 of the Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, as well as the national (NOM-062-ZOO–1999) and international guidelines for the use of laboratory animals.

2.4.3. Experimental design

The activity of the aqueous extract was explored by parenteral administration using four single doses (3, 10, 30, and 100 mg/kg, i.p.), which were compared to one dose by enteral administration (500 mg/kg, p.o.). The treatment groups of six animals were compared with mice receiving the vehicle (distilled water, 1 mL/100 g). Anxiolytic drugs
such as DZP1 (1 mg/kg, i.p.) and BUS4 (4 mg/kg, i.p.) were used as reference drugs. The anxiolytic-like effects were evaluated 30 min after administration of the treatments. After every individual experiment, each apparatus was cleaned with a 70% ethanol solution (See timeline).

2.4.4. Animal models of anxiety

2.4.4.1. The open field test. Each mouse was placed in an acrylic box divided into 12 squares (6 × 6 cm). The number of squares explored in a period of 2 min was considered ambulatory activity [23]. In addition, the number of times the mice stood on their two hind limbs, placing their two forelimbs on the cylinder wall (rearings), was counted for 5 min [24]. A significant reduction in the rearing events was considered an anxiolytic-like response, and a significant reduction in ambulatory activity a sedative effect [25].

2.4.4.2. The elevated plus-maze test. To corroborate the anxiolytic-like activity of treatments, the plus maze test [26] was also included, as it is well-known and widely validated. It consisted of a wooden cross, elevated 50 cm from a base, with two open arms (30 × 5 cm) and two closed ones (30 × 15 × 5 cm), and a central open space (5 × 5 cm) where each mouse was placed. Then, the latency, number of entries in each closed or opened arm, and the time remaining in each of them was registered over a period of 5 min. The percentage of number of entries into open arms was also included as: [entries into open arms / (entries into open arms + entries into close arms)] x 100.

2.4.5. Mechanism of action

Since the dose-response anxiolytic-like effects of the aqueous extract of R. sativus were not observed in a dose dependent manner in all the tests, the dosage of 30 mg/kg, i.p. was chosen as the best to explore a possible mechanism of action, since it was the most consistent dosage showing significant response in the assessed tests. Independent groups of mice were treated with either a GABA<sub>A</sub>/BDZs site receptor antagonist using FMZ (5 mg/kg, i.p.) or a serotonin 5-HT<sub>1A</sub> receptor antagonist in the presence of WAY (0.3 mg/kg, i.p.). Fifteen minutes after administration of the antagonist, mice received a significant dose of the aqueous extract (30 mg/kg, i.p.) or the reference drug. Then, the anxiety tests were assayed.

2.4.6. Statistical analysis

Data are expressed as the mean ± SEM of 6 replicates. Statistical differences were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett’s or Tukey’s test for comparison versus vehicle or among the groups, respectively. Differences were considered significant at p < 0.05. The data were analyzed using GraphPad Prism software (Prism 6 for Windows Version 6.04, GraphPad Software, 2012).

3. Results

The phytochemical analysis allowed the identification and quantification of major presence of sulforaphene (2500 µM), sulforaphane (15 µM), iberin (0.75 µM), and indole-3-carbinol (0.75 µM) as main constituents of the aqueous extract of R. sativus sprouts, corresponding to 0.001, 0.029, 5.037, and 0.001 mg/g dry weight (Fig. 1).

After pharmacological evaluation, treatment with DZP1 (1 mg/kg, i.p.) or BUS4 (4 mg/kg, i.p.) significantly reduced the ambulatory activity of mice (F<sub>7,40</sub> = 3.505, p = 0.0049) in the open-field assay (Fig. 2A). In contrast, mice that received the extract of R. sativus, by enteral or oral route, showed no significant differences compared to the vehicle group (Fig. 2B).

![Fig. 1. Chemical structures of the main constituents identified in the aqueous extract of Raphanus sativus cv. Sango sprouts.](image1)

![Fig. 2. Anxiolytic-like effects of an aqueous extract of Raphanus sativus cv. Sango sprouts, using intraperitoneal (i.p.) or oral (p.o.) route of administration, and the reference drugs diazepam (DZP1, 1 mg/kg, i.p.) or buspirone (BUS4, 4 mg/kg, i.p.), compared to the vehicle (control group) in the open-field test in mice. A) ambulatory behavior, and B) rearing behavior. Data are shown as the mean ± S.E.M. of six replications. One-way ANOVA followed by Dunnett’s test, *p < 0.05 and **p < 0.01.](image2)
parenteral administration, did not change ambulatory exploration compared to the control group (Fig. 2A). Interestingly, the escape behavior of the mice expressed as vertical stretching (rearing) was significantly reduced in mice receiving the extract at all doses tested ($F_{7,40} = 2.767, p = 0.0196$), but not in animals administered with the reference drugs (Fig. 2B).

In the maze test, both anxiolytic drugs and the highest dose of the extract (100 mg/kg, i.p.) significantly delayed the first entry of mice into the closed arms. A dose of 500 mg/kg, p.o. achieved a similar response to BUS4, but with greater biological variability than parenteral administration ($F_{7,40} = 8.655, p < 0.0001$) (Fig. 3A). The average time that mice spent in open arms increased significantly in the presence of both, the reference drugs, and the extract at doses of 30 and 100 mg/kg, i.p., with an equivalent response in mice receiving 500 mg/kg, p.o. ($F_{7,40} = 9.696, p < 0.0001$) (Fig. 3B). This average obtained from an individual increase in open-arm frequency ($F_{7,40} = 15.00, p < 0.001$) (Fig. 3C) was associated with a significant compensatory decrease in the frequency of closed-arm entries ($F_{7,40} = 13.97, p < 0.0001$) (Fig. 3D). Significant increase and dose-dependent response was observed from a dosage of 10 mg/kg, i.p. in the time that mice spent in open arms. This effect was improved when doses of the extract were increased at 30 mg/kg or 100 mg/kg, i.p., and at 500 mg/kg, p.o. as observed in the plus-maze test (Fig. 3C and 3D). The largest effect was observed in the presence of DZP1, followed by doses of 30–100 mg/kg, i.p., and 500 mg/kg, p.o., of the extract. At the same time, 10 mg/kg, i.p. of the extract resembled the response of BUS4 (Fig. 3C and 3D). A similar frequency in the number of entries was observed in the individual open ($F_{7,40} = 2.958, p = 0.0138$) (Fig. 3E) or closed arms ($F_{7,40} = 3.187, p = 0.0091$) (Fig. 3F) from 10 to 100 mg/kg, i.p. and in 500 mg/kg, p.o. resembling the significant frequency obtained with DZP1 alone (Fig. 3E and 3F). As a complement of the frequency of entries in both arms, the percentage of entries in open arms respect to the sum of open and closed arms entries allowed us observing that the mice receiving the lowest doses of extract showed 28% (3 mg/kg, i.p.) and 21% (10 mg/kg, i.p.) more exploration in closed arms than open arms, similar to the exploration of the control group with 22%. This percentage was reduced in the presence of DZP1.
and the extract at 30 mg/kg, i.p. and 500 mg/kg, p.o. to 7%, 5%, and 6%, respectively. In contrast, the extract at the highest dosage (100 mg/kg, i.p.) and BUS4 showed 4% and 10% more exploration in open arms.

Regarding the mechanism of action in the open field test, the antagonists and the extract alone did not modify the ambulatory activity of mice (F(3, 20) = 1.830, p = 0.1743) (Fig. 4A). In contrast, the observed significant changes in rearing behavior after treatment with the R. sativus extract (30 mg/kg, i.p.) were inhibited in the presence of the serotonin 5-HT1A receptor antagonist (WAY1, 1 mg/kg, i.p.) but not in combination with the GABA_A/BDZs site receptor antagonist (FMZ5, 5 mg/kg, i.p.) (F(3, 20) = 4.490, p = 0.0145) (Fig. 4B).

In the maze test, the GABA_A/BDZs site receptor antagonist did not modify the response of the extract, whereas the serotonin 5-HT1A receptor antagonist facilitated a significant delay in the closed arm entries at a 30 mg/kg, i.p. of the extract that was non-significant when given alone (F(3, 20) = 12.38, p < 0.0001) (Fig. 5A). As for the time spent in open arms, the significant increase in the average produced by the extract alone was inhibited in the presence of both antagonists (F(3, 20) = 18.35, p < 0.0001) (Fig. 5B). This result was mainly dependent on the blockage of both antagonists in the individual time mice spent in the open arms (F(3, 20) = 20.82, p < 0.0001) (Fig. 5C) compared to the blockage of the antagonists on the significant decrease in the time mice spent in closed arms in the plus maze (F(3, 20) = 23.57, p < 0.0001) (Fig. 5D). The significant difference produced by the extract in the number of entries in the open (F(3, 20) = 17.57, p < 0.0001) or closed (F(3, 20) = 15.10, p < 0.0001) arms was not modified by both antagonists (Fig. 5E and F, respectively). Mice receiving the extract (30 mg/kg, i.p.) alone or combined with the antagonists WAY1 or FMZ5 did not show statistical difference (F(2, 15) = 1.003, p < 0.39), since these groups demonstrated 5%, 1% or 5% more or less exploration in open arms, respectively, compared to 22% more exploration in closed arms in the case of the control group.

4. Discussion

In this work, the anxiolytic-like activity of an aqueous extract of R. sativus sprouts was evaluated in different tests performed in mice. Its parental administration in four doses was compared with an enteral dose, and the acute toxicity by i.p. route of administration was calculated. A preliminary analysis identified the main chemical constituents, as well as the involvement of the GABA_A/BDZs site and serotonin receptors of the 5HT1A subtype, as CNS inhibitory targets.

Open-field and plus-maze tests were used as complementary experimental models to understand the tranquilizing effects, anxiolytic or sedative, among the most common assays [27,28]. The plus-maze assay is considered the first choice in the search for anxiolytic drugs from medicinal plants [28-31] but also from food [4], as they involve exploration and/or flight behavior of mice [32]. In our study, R. sativus extract demonstrated anxiolytic-like activity in mice receiving a single dose of an aqueous extract at a dose range of 30–100 mg/kg, i.p. These effects remained at a dose of 500 mg/kg using the enteral route. The tranquilizing effects of the aqueous extract of R. sativus sprouts were not associated with a sedative response, as observed in the presence of clinical drugs. To our knowledge, this is the first time that the anxiolytic-like effects of R. sativus sprouts have been reported. Nevertheless, a preliminary study by [22] reported that an ethanolic extract of R. sativus var. caudatus fruits produced anxiolytic-like effects without modifying the ambulatory capacity of the mice, this study supports our results and suggests that R. sativus possesses constituents that moderately inhibit the CNS.

Brassica and R. sativus species belong to Brassicaceae family, they are economically valuable crops but also sources of health-beneficial phytochemicals, even for the CNS, such as glucosinolates and isothiocyanates [33,34]. However, it is a fact that species of the genus Raphanus have been scarcely investigated compared to Brassica species, and there is insufficient research on their beneficial effects on the CNS of both genera. Regarding their properties for anxiety disorders, B. oleracea has been reported to produce significant anxiolytic-like effects administered at 200 mg/kg, p.o. in mice assayed in the hole-board and plus-maze tests [35]. This report is consistent with our results using 500 mg/kg, p.o. of the extract of R. sativus, suggesting that food intake allows the bioactive compounds to be orally reached [36]. Parenteral administration produced similar effect at lower doses suggesting major bioavailability of the bioactive compounds. Enzymatic activity (for example, myrosinase) and microbiota involvement have been reported to play an important role in the biotransformation of metabolites of Brassicaceae species, such as glucosinolates converted to isothiocyanates [37], like the well-known bioactive metabolite sulforaphane [34]. In this study, the anxiolytic-like effects of R. sativus sprouts after acute enteral or parenteral administration were investigated for the first time. However, anxiolytic-like responses were preliminarily reported for an aqueous or a methanol extract of R. sativus var. caudatus pods and Brassica juncea leaves using oral route and chronic administration (250–500 mg/kg) for 60 days [22] and 10 days, respectively [38]. Similarly, the anxiolytic-like effects of B. oleracea inflorescences (200 mg/kg, p.o.) and B. nigra seeds (100 and 200 mg/kg, p.o.) were
investigated for a hydroalcoholic extract in the plus-maze and Y-maze tests in mice [35,39]. All these results together support the functional activity of Brassicaceae family species as a source of dietary alternatives for supporting mental health due to their calming properties.

The chemical composition of *R. sativus* sprouts has been shown to include flavonoids and glucosinolates [40]. The latter are generally transformed into isothiocyanates as corroborated in the aqueous extract of *R. sativus* cv. Sango that contains mainly the presence of sulforaphene followed by sulforaphane, and an equivalent amount of iberin and indole-3-carbinol. The content of sulforaphene and sulforaphane was higher in *R. sativus* cv. Sango compared to *R. sativus* L. var. caudatus with sulforaphene at 2.253 mg/g and sulforaphane at 0.111 mg/g of the crude extract [41]. In general, isothiocyanates are currently valued due to their effects on the CNS, as there is evidence of their potent antioxidant activity mediated by activation of the Nrf2/ARE pathway [42]. Neuronal dysfunction and inflammation may increase through the cumulative effects of free radicals, but these two isothiocyanates may blunt their effects. Indole-3-carbinol, which reduces neuroinflammation and oxidative-stressive state and increases brain BDNF levels, has recently been reported to produce antidepressant but not anxiolytic-like effects under repeated administration of 10, 30, and 60 mg/kg for 10 days [43]. This suggests more relevance for the therapy of depression than anxiety. There is little information on the effect of iberin isothiocyanate on the CNS, but it has been described as an interesting metabolite to produce concentration- and time-dependent inhibition of neuroblastoma cells growth [44] and cytotoxic effects in different kinds of cancer. Sulforaphene was the most abundant constituent found in the aqueous extract of *R. sativus*, which has been shown to be a CDK5 inhibitor that attenuates cognitive deficit in transgenic mice from an experimental Alzheimer’s model [45].

Clinical therapy for anxiety is known to involve the use of drugs with GABAergic action, such as BDZs, mainly for acute attacks of generalized anxiety [46]. In comparison, serotonin 5-HT<sub>1A</sub> receptor agonists are more common for controlling chronic anxiety [47]. Significant changes in the serotonin 5-HT<sub>1A</sub> receptor blockade were observed in the open-field test, while it and GABA<sub>A</sub>/BDZ site receptor blockade were suggested in the anxiolytic-like effects of the aqueous extract of *R. sativus* sprouts in the plus-maze. Sulforaphane, as one of the main metabolites identified in the extract, has already been reported as an anxiolytic for its depressant properties on the CNS by involving the Nrf2 pathway as a
possible mechanism of action [48,49]. These results together suggest that more than one bioactive metabolite could be influencing the effects of the R. sativus aqueous extract, acting synergistically to enhance its functionality and tranquilizing medicinal properties. It is a preliminary study exploring the anxiolytic-like pharmacological effects of R. sativus and the most common inhibitory receptors involved in anxiety therapy. Therefore, further pharmacological evidence should be explored in the future to confirm the involvement of these and other receptors and their signal transduction pathways. Scientific evidence is important not only for medicinal plants, but also for food used as a functional diet for anxiety therapy. R. sativus as a functional food may be within the reach of the world’s population, as its cultivation is not difficult, and it can be obtained from various crops and techniques throughout the year [18]. As food and as a part of a traditional medicine, it could help and prevent or reduce anxiety disorders are currently on the rise in people around the world.

In conclusion, we found evidence of the calming properties of the aqueous extract of R. sativus sprouts. Two critical inhibitory neurotransmitter systems of anxiety therapy appear to be involved. This pharmacological activity was corroborated not only in parenteral but also in enteral administration, without acute toxicity. The results suggest isothiocyanates as bioactive constituents and a source of alternative therapy for anxiety disorders.

Institutional review board statement
The study was conducted according to the guidelines for care and handling animals at international, national (NOM-062-ZOO-1999) and local statements approved by our institutional ethics committee (CON- BIOETICA-09-CEI-010–20170316, in March 2017) and research protocol INPRFM-NC123280.0.

Funding
This work was partially supported by CONACyT (Grant number 20855/PI/18, D.A.M.).

Data Availability
Data will be made available on request.

Acknowledgments
Laura Yunuen is grateful for the PhD fellowship financed by CONACyT No. 733222. We also thank to Psych. Aide Gonzalez for proof-reading the manuscript, and Paula García-Ibañez, funded by a grant from the Fundación Séneca-CARM, Spain (21273/FPI/19), for the valuable help in the preparation and processing of extracts.

References


